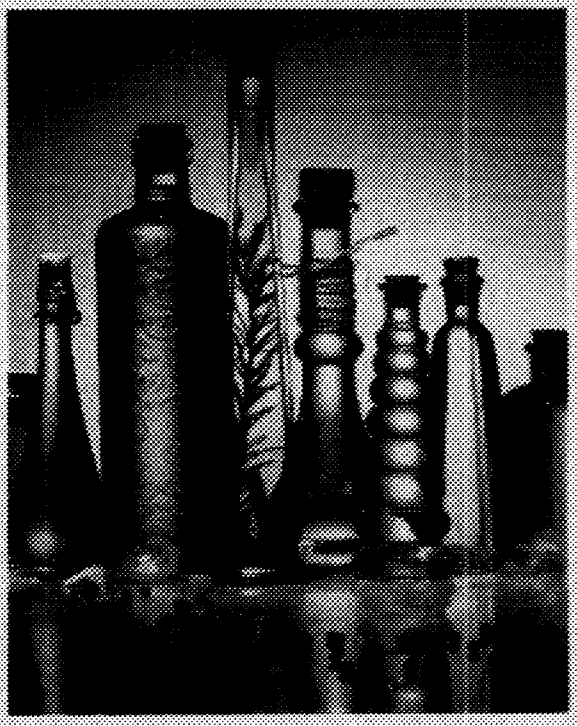
PN-ADC-907

PROCESSING OF EDIBLE OILS: PROCESSING, QUALITY & SAFETY



APRIL 2, 2001

Mr. Monoj K. Gupta
MG Edible Oils
Agriculture Led Export Businesses
12 Dokki Street, 6th Floor
Dokki, Cairo
TEL 02-338-1445
FAX 02-748-0729

USAID PROJECT NO. 263-0264

Abt

Curriculum Vitae

Name:	**Monoj Kumar Gupta.**
Address:	**MG Edible Oil Consulting, Int'l. Inc.** **9 Lundy's Lane, Richardson, TX – 75080** **monojgupta@worldnet.att.net**
Born:	**January 6, 1937**
Education:	**Undergraduate in Chemical Engineering** **Jadavpur University, Calcutta, India, 1962** **Graduate In Chemical Engineering** **University of Florida, 1965**
Experience:	**Group Leader In Food R&D, Procter & Gamble Co., 1965-74. Manufacturing Manager, Procter & Gamble Co., 1974-81** **Senior Process Engineer & Process Specialist, Anderson Clayton Co., 1981-87** **Section Manager and Principal Scientist at Frito Lay, Inc., As an advisor on all oil related R&D activity for the worldwide operation, 1987-98**
Currently	**President, MG Edible Oil Consulting Int'l. Inc.**
Professional Affiliation:	**Associated with the Am. Oil Chemists Society as:** **1. Current member of the Special Publications Committee.** **2. Chairperson of the Lipid Oxidation & Quality Division, 1995-97** **4. President of the South Central Section, 1987-95** **5. Member of Institute Of Food Technology**
Presently	**Serving as a member of the Governing Board of the American Oil Chemists Society**

2

Consulting:	**Both in US and overseas on oil processing, process engineering, product formulation and application of lipids in food products. Design and construction of oil refineries** **Promoting Nu-Sun oil from its very conception.** **Promoting soybean oil utilization overseas through the American Soybean Association and the Food & Agricultural Service (FAS)**
Advisory Board:	**Served as a Steering Committee member of the Long Term Research Planning Committee of the USDA, Peoria, IL, January 1999.**
Publications & Teaching	**Numerous publications in technical journals and chapters in technical books published in the U.S., UK, South America and Asia.** **Teaching short courses at Texas A&M University, University of California, Am. Oil Chemists Society. Directed short courses jointly sponsored by the Institute of Food technology and Am. Association of Cereal Chemists.**

MGVitae

REGISTRATION INFORMATION
EDIBLE OILS

Name _____

Title _____

Company _____

Address _____

_____

TEL _____

FAX _____

Email _____

Attendees may register by calling in or faxing this registration to ALEB. They may also register at the door.

Registration Fee: There will be a 80 LE fees for this program. Registrants will receive a course notebook, coffee breaks morning and afternoon and a certificate of participation following completion of evaluation forms.

For further details, contact:

Agriculture Led Export Businesses (ALEB)
12 Dokki Street
Dokki, Cairo, EGYPT
TEL 202-348-0728/338-1445 (6 Lines)
FAX 202-348-0729

PROCESSING OF EDIBLE OILS: PROCESSING, QUALITY & SAFETY

A SHORT COURSE
SPONSORED
BY THE
AGRICULTURE LED EXPORT
BUSINESSES PROJECT (ALEB)
CAIRO, EGYPT
THE CENTER FOR ADVANCED FOOD
TECHNOLOGY
PISCATAWAY, NJ, USA

USAID Project No. 263-0264

April 2, 2001 at 9:00 at the ALEB Offices, Dokki, Cairo

PROCESSING OF EDIBLE OILS: PROCESSING, QUALITY AND SAFETY

INTRODUCTION

Vegetable oils are obtained from various oil seeds, palm fruit, palm kernal, olives and other seeds and fruits. The most common seed oils are derived from soybean, cottonseed, sunflower, edible rapeseed (Canola), and safflower. Soybean oil and palm oil are the two largest volume oils in the world. Palm oil production is expanding and is expected to surpass soybean oil by 2005. In the Mediterranean Basin, olive oil is the most commonly used oil. It is gaining market share throughout the world because of its flavor and association with a healthy lifestyle. Egypt is making an effort to penetrate the world olive oil market. To do this, they will need to assure that they manufacture high quality, safe and wholesome products.

This program will provide attendees with information on oil processing, handling, storage, and distribution. Quality issues and the development and maintenance of standards will also be discussed.

COURSE OUTLINE

9:00 - Registration

9:30 - History of Oils Usage - R. Stier

10:30 - Handling Crude Oils - M. Gupta
This session will review methods for handling and storage of crude oils.

11:30 - Crude Oil Unloading & Storage - M. Gupta
This discussion will review proper procedures for unloading and storage of crude oils.

12:30 - Refining of Edible Oils - M. Gupta
This lecture will discuss refining of edible oils, including bleaching, hydrogenation and deodorization.

14:00 - Lunch

15:00 - Storage of Refined Oils - M. Gupta
This session will review proper storage techniques for refined and deodorized oils.

15:30 - Quality Control Tests for Edible Oils - M. Gupta

16:00 - Packaging & Distribution - M. Gupta
Review of critical control points in oil packaging; factors that affect shelf-life

FACULTY

Mr. Monoj Gupta (MG Edible Oils International) - Mr. Gupta is the president of MG Edible Oils International. He is a chemical engineer by education who has built and managed refineries in Mexico and around the world while working with Anderson Clayton. He has also helped develop products using fats and oils. Before becoming a consultant, he helped Frito-Lay save millions of dollars by developing programs to assure the quality of oils used in their products and for managing oils during production.

FOR FURTHER INFORMATION

Mr. Richard F. Stier
Director, Technical Services
Mr. Morad S. Ahmed
Co-Director, Technical Services
Agricultural Led-Export Businesses
12 Dokki Street, 6th Floor
Giza, Egypt
TEL 02-338-1445 (6 Lines)
FAX 02-748-0729
EMAIL morad@egyptonline.com
aleb@aleb.org

COURSE INFORMATION

Monday April 2, 2001 @ 9:00 at the ALEB Training Center, 5th Floor, 12 Dokki Street

FATS AND OILS REFERENCE SITES

GOVERNMENT	
Canadian Food Inspection Agency	www.cfia-acia.agr.ca
European Union on Line	http://europa.eu.int
Food and Agriculture Organization	http://www.fao.org
Foreign Agricultural Service – Attache Report	http://www.fas.usda.gov/scriptsw/attacherep/default.asp
United States Food & Drug	www.fda.gov
US FDA - Trans fatty Acids Labeling	http://www.cfsan.fda.gov/~lrd/hhtfacid.html
United States Department of Agriculture	http://www.usda.gov
United States Grade Standards Olive Oil	http://www.ams.usda.gov/standards/oliveoil.pdf
ASSOCIATIONS	
Aceite de Oliva de Espana	http://www.asoliva.com
African Oil Chemists Society	http://www.geocities.com/CapeCanaveral/Lab/1669
American Association of Cereal Chemists	www.scisoc.org/aacc
American Oil Chemists Society	www.aocs.org
American Soybean Association	http://www.amsoy.org
Arrow Group	http://arrowgroup.com/industry_regulations.htm
California Olive Industry	www.calolive.org
California Olive Oil Council	http://www.cooc.com/home.html
Canola Council of Canada	http://www.canola-council.org
Codex Alimentarius Standards On-Line	http://www.codexalimentarius.net/STANDARD/standard.htm
Codex Standard for Olive Oil	ftp://ftp.fao.org/codex/standard/volume8/en/CX_033e.pdf
International Olive Oil Council	http://www.internationaloliveoil.org/

Institute of Food Technologists	www.ift.org
National Cottonseed Products Association	http://www.cottonseed.com
National Institute of Oilseed Products	http://www.oilseed.org/index.html
National Oil Seed Processors Association	http://www.nopa.org
National Sunflower Association	www.sunflowernsa.com
North American Olive Oil Council	http://www.naooa.org
Olive Growers Council	http://www.olivecouncil.com
Palm Oil Research Institute of Malaysia	www.porim.gov.my
University of California Olive Site	http://fruitsandnuts.ucdavis.edu/olive2.html
METHODS	
American Oil Chemists Society	www.aocs.org
Association of Official Analytical Chemists	www.aoac.org
Fri-Check Oil Tester	www.fri-check.de
International union of Pure and Applied Chemistry	http://iupac.chemsoc.org

Search Results

Your search for "Standards for Olive Oil" matched 14 of 1078752 documents.
14 are presented, ranked by relevance.

Return to search form Refine your search

Rank	Score	Title/Information	Date	Size
1	0.82	**Community legislation in force: Document 399R0640** ▷ http://europa.eu.int/eur-lex/en/lif/dat/1999/en_399R0640.html *399R0640 Commission Regulation (EC) No 640/1999 of 25 March 1999 amending Regulation (EC) No 2815/98 concerning marketing standards for olive oil Official Journal L 082 , 26/03/1999 p. COMMISSION REGULATION (EC) No 640/1999 of 25 March 1999 amending Regu ...*	21-02-01	7 K
2	0.80	**PROCEEDINGS OF THE COURT OF JUSTICE AND THE COURT OF FIRST INSTANCE OF THE EUROPEAN COMMUNITIES** ▷ http://curia.eu.int/en/act/act00/0034en.htm *The jurisdiction of the Court of Justice to interpret Article 50 of TRIPs is thus not restricted solely to situations covered by trade-mark law. By order of 16 June 1998, the Supreme Court referred to the Court of Justice for a preliminary ruling three qu ...*	13-02-01	218 K
3	0.80	**Community legislation in force: Document 398R2815** ▷ http://europa.eu.int/eur-lex/en/lif/dat/1998/en_398R2815.html *398R2815 Commission Regulation (EC) No 2815/98 of 22 December 1998 concerning marketing standards for olive oil Official Journal L 349 , 24/12/1998 p. 3. In the case of an extra virgin or virgin olive oil imported from a third country, the de ...*	15-03-01	17 K
4	0.80	**Microsoft Word - en 855.doc** ▷ http://europa.eu.int/eur-lex/en/com/pdf/2000/en_500PC0855.pdf *2 COMMISSION REPORT TO THE COUNCIL AND THE EUROPEAN PARLIAMENT THE QUALITY STRATEGY FOR OLIVE OIL TABLE OF CONTENTS INTRODUCTION..... 14 Box 1 : A ...*	16-01-01	209 K
5	0.80	**Commission Regulation (EC) No 2815/98 of 22 December 1998 concerning marketing standards for olive oil** ▷ http://europa.eu.int/eur-lex/en/consleg/main/1998/en_1998R2815_index.html *Disclaimer: Consolidation entails the integration of basic instruments of Community legislation, their amendments and corrections in single, non-official documents. Each document is intended for use as a documentation tool and the Institutions do not ass ...*	19-10-00	8 K
6	0.77	**PROCEEDINGS OF THE COURT OF JUSTICE AND THE COURT OF FIRST INSTANCE OF THE EUROPEAN COMMUNITIES** ▷ http://curia.eu.int/en/act/act00/0024en.htm *By its sixth plea, the German Government challenges the Commission's finding that Paragraph 52(8) of the ESiG infringes Article 52 of the Treaty. By the questions which they have referred to the Court, both national courts essentially request the Court to ...*	14-02-01	148 K
7	0.77	**PROCEEDINGS OF THE COURT OF JUSTICE AND THE COURT OF FIRST INSTANCE OF THE EUROPEAN COMMUNITIES** ▷ http://curia.eu.int/en/act/act00/0025en.htm *Those questions were raised in proceedings between Mr Engelbrecht and the Rijksdienst voor Pensioenen (National Pensions Office), the Belgian social security agency, concerning his retirement pension. The first question referred by the national court was ...*	14-02-01	222 K
8	0.77	**PROCEEDINGS OF THE COURT OF JUSTICE AND THE COURT OF FIRST INSTANCE OF THE EUROPEAN COMMUNITIES** ▷ http://curia.eu.int/en/act/0025en.htm *Those questions were raised in proceedings between Mr Engelbrecht and the Rijksdienst voor Pensioenen (National Pensions Office), the Belgian social security agency, concerning his retirement pension. The first question referred by the national court was ...*	06-12-00	222 K
9	0.77	**PROCEEDINGS OF THE COURT OF JUSTICE AND THE COURT OF FIRST INSTANCE OF THE EUROPEAN COMMUNITIES** ▷ http://curia.eu.int/en/act/0024en.htm *By its sixth plea, the German Government challenges the Commission's finding that Paragraph 52(8) of the ESiG infringes Article 52 of the Treaty. By the questions which they have referred to the Court, both national courts essentially request the Court to ...*	27-10-00	148 K
10	0.77	**PROCEEDINGS OF THE COURT OF JUSTICE AND THE COURT OF FIRST INSTANCE OF THE EUROPEAN COMMUNITIES** ▷ http://curia.eu.int/en/act/act99/9912en.htm *By those questions, which may conveniently be considered together, the national court is essentially asking in what circumstances Article 3(1)(c) of the Directive precludes registration of a trade mark which consists exclusively of a geographical name. P ...*	18-01-00	70 K

8

11	0.77	**EUR-Lex: Consolidated Legislation - Analytical structure - 03.60.59**	28-03-01	14 K

http://europa.eu.int/eur-lex/en/consleg/reg/en_register_036059.html

Council Regulation (EEC) No 2261/84 of 17 July 1984 laying down general rules on the granting of aid for the production of olive oil and of aid to olive oil producer organizations Commission Regulation (EEC) No 27/85 of 4 January 1985 laying down detaile ...

12	0.77	**EUR-Lex: Consolidated Legislation - Index Page - 1998**	28-03-01	37 K

http://europa.eu.int/eur-lex/en/consleg/index_1998.html

98/20/EC: Council Decision of 19 December 1997 authorising the Kingdom of the Netherlands to extend the application of a measure derogating from Article 21 of the Sixth Council Directive 77/388/EEC on the harmonisation of the laws of the Member States rel ...

13	0.77	**Directory of Community legislation in force: 03.60.59 - Oils and fats**	22-03-01	79 K

http://europa.eu.int/eur-lex/en/lif/reg/en_register_036059.html

OJ 172 30.09.1966 p.3025 (SE SER1 (65-66) p.221) Amended by 368R2146 (OJ L 314 31.12.1968 p.1) (SE SER1 68 (II) p.599) Amended by 370R1253 (OJ L 143 01.07.1970 p.1) (SE SER1 70(II) p.405) Amended by 172B Amended by 372R1547 (OJ ...

14	0.77	**http://europa.eu.int/eur-lex/en/com/rpt/2000/com2000_0429en01.pdf**	08-12-00	295 K

2 CONTENTS REPORT FROM THE COMMISSION TO THE COUNCIL, THE EUROPEAN PARLIAMENT AND THE ECONOMIC AND SOCIAL COMMITTEE: THE OPERATION OF DIRECTIVE 98/ 34/ EC FROM 1995 TO 1998 ..... 16 4. PART II: ...

Refine your search:

Search Reset

PROCESSING OF EDIBLE OILS

WORKSHOP TOPICS

1. A Short History of Fats & Oils
2. Introduction to Fats & Oils - Applications
3. Basic Oil Chemistry
4. Decomposition of Oils
5. Protection of Oil
6. Handling Crude Oils
7. Processing Vegetable Oils
8. Refining Vegetable Oils
9. Bleaching
10. Hydrogenation
11. Deodorization
12. Protecting Oils After Processing
13. Quality Control
14. Packaging & Distribution

APPENDIX

- Sample Costs to Establish an Olive Orchard
- Spanish Varietals
- Grade Standards and Quality
- AOAC Methods
- IUPAC Methods
- AOCS Methods
- Rapid Methods
- Consumer Guide to Fats

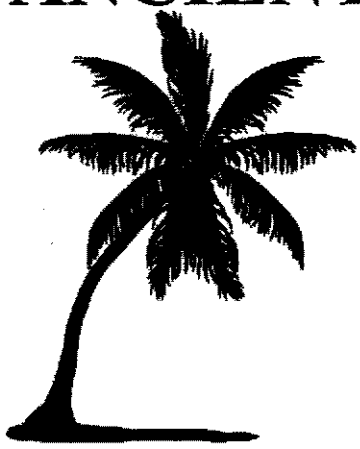
A SHORT HISTORY OF FATS AND OILS

RICHARD F. STIER
TECHNICAL DIRECTOR
ALEB PROJECT
CAIRO, EGYPT

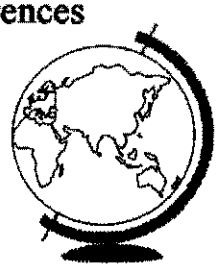
نبذة قصيرة عن تاريخ الدهون والزيوت

ريتشارد فلمنج ستير
المدير الفني
مشروع دعم الاعمال الزراعية الموجهة للتصدير (ALEB)
القاهرة، ج.م.ع

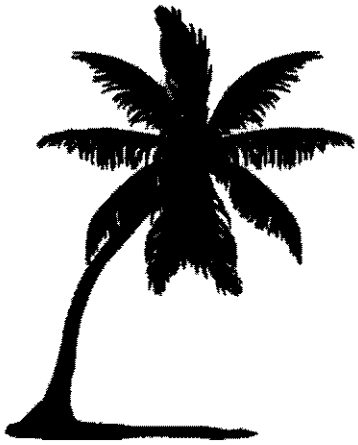
FRYING: AN ANCIENT PROCESS



- Egypt - As evidenced by wall paintings
- China - 5,000 years ago to pre-cook meat prior to roasting
- Biblical references



القلي: عملية قديمة



- مصر – يتضح من النقوش والرسومات على جدران المعابد المصرية
- الصين – منذ ٥,٠٠٠ عاما مضت كان يتم طهي اللحوم مسبقا قبل شويها
- هناك بعض الاشارات في الانجيل في هذا الصدد

12

"And unleavened bread, and cakes leavened temper with oil and wafers anointed with oil; of wheaten flours though shalt make."

Exodus 29:1

"وخبز فطير وأقراص فطير ملتوتة بزيت ورقاق فطير مدهونة بزيت من دقيق حنطة تصنعها."

سفر الخروج، الاصحاح ٢٩، العدد ١

EDIBLE OILS

- Early days - Used what was available
- Olive oil in Mediterranean
- Animal fats in Northern Europe
- Palm & soy in Asia and Africa
- Hydrogenated oils came into use in early 1900's following invention
- Improved refining/blending techniques
- Egypt today - Olive oil & others produced from imported crudes

الزيوت القابلة للأكل

- في العقود القديمة كان يتم استخدام كل ما هو متاح
- كـــان يـــتم استخدام زيت الزيتون في مناطق البحر المتوسط
- كانت الدهون الحيوانية تستخدم في شمال أوروبا
- زيت النخيل والصويا في آسيا وأفريقيا
- بـــدأ اســـتخدام الـــزيوت المهدرجة في اوائل القرن العشرين بعد بداية الاختراعات مباشرة
- كانت هناك تقنيات محسنة للتكرير / الخلط
- في مصر اليوم – يتم انتاج زيت الزيتون وغيره من الزيوت من الزيوت الخام المستوردة

EDIBLE OILS

- Flavor carriers
- Energy
- Processing - Food flavor & texture drive the process
- Heating efficiency quick cooking

الزيوت القابلة للأكل

- الطاقة
- التصنيع – نكهة الطعام وقوامه هما من العوامل الهامة في العملية التصنيعية

فعالية التسخين هي التي تؤدي الى الطهي السريع

COOKING OILS

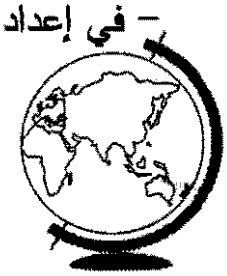
A LOCAL PROCESS FOR CENTURIES

- Bakers
- Cooks
- Restaurants/vendors
- Home preparation

زيوت الطهي

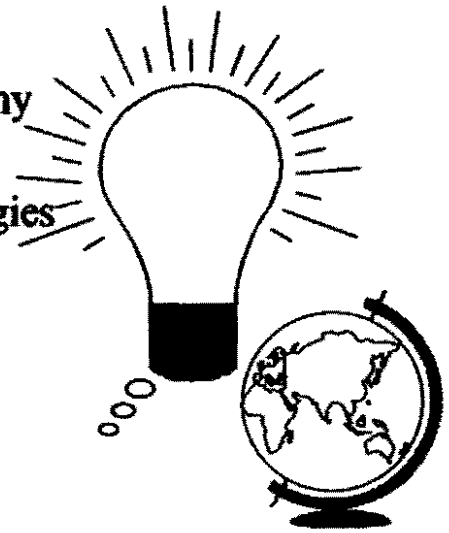
عملية محلية منذ العصور يستخدمها

- الخبازون
- الطهاة
- المطاعم / البائعون
- في إعداد الطعام المنزلي



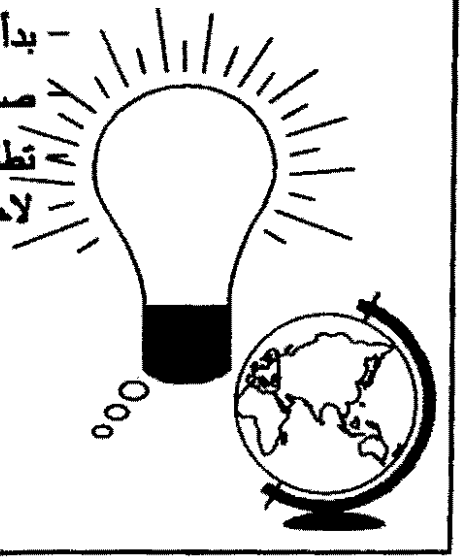
INDUSTRIAL FRYING

- Began in the 1890's
- Local industry for many years
- Needed new technologies to move forward



القلي الصناعي

- بدأ عام ١٨٩٠
- صناعة محلية للعدة سنوات
- تطلب الامر تكونولجيا جديدة لاحراز مزيد من التقدم



GROWTH OF FROZEN POTATO INDUSTRY

UNITED STATES FIGURES

YEAR	POUNDS OF POTATOES
1947	3.5 Million
1972	2.7 Billion
1988	5.2 Billion
1994	7.9 Billion

نمو وتطور صناعة البطاطس المجمدة

أرقام من الولايات المتحدة الامريكية

العام	أرطال من البطاطس
١٩٤٧	٣,٥ مليون
١٩٧٢	٢,٧ بليون
١٩٨٨	٥,٢ بليون
١٩٩٤	٧,٩ بليون

Growth of the frozen potato industry corresponded with the development of the oil-blanched or par-fried potato by researchers at J.R. Simplot in 1953.

إن نمو صناعة البطاطس المجمدة وتطورها يتوافق مع تطوير البطاطس المقلية مسبقا من قبل الباحثين لدى J.R. Simplot عام ١٩٥٣

EQUIPMENT

- Kettle Systems
- First continuous fryer developed by Freeman McBeth in 1929
- The system was installed at Ross Potato Chip Company in Richland, PA
- Development slowed by World War II

المعدات

- أنظمة الغلايات
- تم صنع أول غلاية تعمل بشكل متواصل من قبل فريمان ماكبث عام ١٩٢٩
- تم تركيب الجهاز بشركة Ross Potato Chip ريتشلاند، بنسلفانيا
- أدت الحرب العالمية الثانية الى ابطاء هذا التطوير

ADVANCES IN FOODSERVICE FRYING

- Open pots to computers
- Automation
- Built in filters
- Safety
- Ease of use

أوجه التقدم في خدمات الطعام

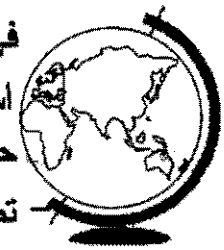
- تطورت العملية من استخدام القدور المفتوحة الى استخدام الكمبيوتر
- التشغيل الأوتوماتيكى
- المرشحات المبيتة داخل الاجهزة
- السلامة
- سهولة الاستخدام

PACKAGING ADVANCES

- Amphora in ancient times
- Glass used and still used
- Fried snacks originally distributed locally
- Sold door-to-door or in stores in barrels
- Cans used early & still in use
- Systems have evolved for different products

أوجه التقدم في التعبئة

- كـــان يـــتم استخدام الأمفورة (قارورة ضيقة العنق ذات ذراعين صغيرين) في الازمنة القديمة
- كان وما زال الزجاج يستخدم حتى الآن
- كان يتم توزيع الوجبات الخفيفة المقلية محليا
- كـــان يتم البيع عن طريق المرور على المستهلين في منازلهم او في مخازن وذلك في براميل كبيرة
- اســـتخدمت العلب منذ القدم لكنها لا تزال تستخدم حتى الآن
- تطورت العديد من الانظمة لمنتجات مختلفة



22

EDIBLE OILS

- Suppliers manufacture a range of oil products
- Customer service is increasingly important
- Product/process development
- Customer driven - clear bottles

الزيوت القابلة للأكل

- يقوم الموردون بتصنيع مجموعة كبيرة من منتجات الزيوت
- إن خدمة العملاء من الامور شديدة الاهمية
- تطوير المنتج / عملية التصنيع
- زجاجات شفافة ونقية تقدم للمستهلكين

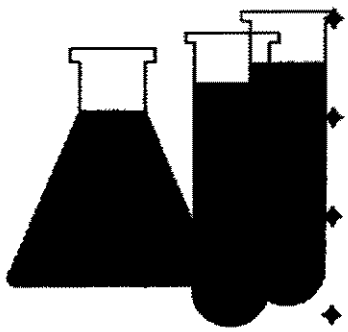
"Oil processors have always handed down the product (oil) to the snack food industry. The oil has been judged to be good by criteria chosen by the oil processors."

Gupta, M., 1994, Inform, 5:5, 647-649

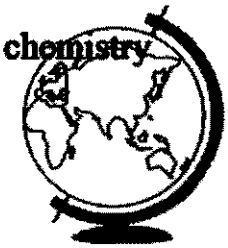
"دائما ما يقوم مصنعو الزيوت بتسليم منتجاتهم من الزيوت الى صناعة الوجبات الخفيفة. ويتم الحكم على جودة الزيوت طبقا للمعايير التي يضعها مصنعو الزيوت."

Gupta, M., 1994, Inform, 5:5, 647-649

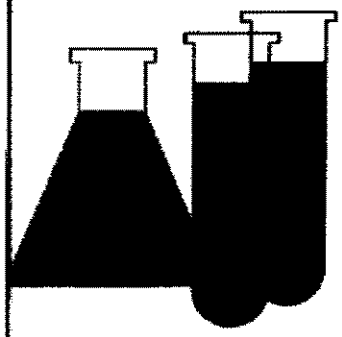
FRYING AS A SCIENCE



- Frying research has been in progress for almost 90 years
- Early work was on oil chemistry (Academia)
- Industrial research on the process
- Relationships of oil chemistry to process ignored



القلي كعلم من العلوم



- لقد تقدمت الابحاث الخاصة بالقلي على مدى ٩٠ عاما
- كان العمل الاول يتناول كيمياء الزيوت (أكاديميا)
- أجريت بحوث صناعية على العملية
- تم إغفال أوجه العلاقة بين كيمياء الزيوت وعملية التصنيع

EDIBLE OILS USE

- Thousands of years old
- Used extensively - great heating medium
- Cooking - Yields delicious & desirable products
- Usage - Expanded with technology
- Greatest growth since 1950
- Continues to grow despite anti-fat people
- Fat and fatty foods taste good

استخدام الزيوت القابلة للأكل

- منذ آلاف من الاعوام
- تم استخدام الزيوت بشكل واسع النطاق – وسط تسخين كبير
- الطهي – ينتج عنه منتجات شهية ومرغوبة
- الاستخدام – توسع مع التطور التكنولوجي
- بدأ التطور الكبير منذ عام ١٩٥٠
- استمر التطور والنمو برغم المناهضين للدهون
- الدهون والاغذية الدهنية مذاقها جيد

HISTORICAL PERSPECTIVES ON FRYING or HOW WE GOT TO WHERE WE ARE TODAY

Richard F. Stier, Consulting Food Scientist

INTRODUCTION

At the LIPIDFORUM held in Helsinki, Finland in 1994, Dr. Christian Gertz of Hagen, Germany, whimsically observed that "everybody believes that deep-fat frying had its beginnings in the early 1950's". In reality, frying is one of the oldest means for preparing food known to man. Frying supposedly had its origins in China, where foods were pre-cooked prior to roasting. Egyptian wall paintings show dough being fried in oil indicating that Europe and North Africa were using hot oil to cook foods before the time of Christ. Even the Bible has references which could be construed as an early attempt at frying. In Exodus 29:3, Moses tells the people of Israel,

> *"And unleavened bread, and cakes unleavened temper with oil and wafers anointed with oil; of wheaten flour thou shalt make them."*

Sounds like a fried snack, doesn't it? Little did we know that such foods were once part of religious ritual.

How frying was discovered is unknown, but it may have been accidental, as many of early man's findings undoubtedly were. Perhaps fat from a roasting piece of meat was collected in a container and someone dropped a piece of food into that container. When the food was salvaged, they discovered that it was not only cooked but tasted good. It is this feature, good taste, which has driven frying throughout the years. In the United States, where foods containing fat are reviled and condemned, people crave high fat foods, be they fried snacks, buttery cookies or rich ice creams, for their flavors, textures and the ultimate satisfaction they provide. I do not know if fat is a "bad word" in this country, but with rising affluence and greater awareness regarding diet and health, it may well become so.

For many years, frying was a process that cooks, bakers or consumers used to prepare foods for local consumption. Chefs prepare fried pastries, such as the delicate and delicious beignets of New Orleans or pomme frites from fresh potatoes, a staple of many meals in Northern Europe; local bakers made doughnuts or other fried dough products; and housewives deep-fry chicken, fish and other foods for their families. The coatings that these cooks used were very

basic; flour, eggs, bread crumbs and perhaps some spices. Remember, these foods were fried and usually eaten very shortly thereafter. Fried foods were not a product that could be stored or transported very well..

In a strange way, Dr. Gertz's comments that frying was discovered in the early 1950's are not so far off, however. Frying as a commercial operation needed a few technological advances to "get going". These included but are not limited to the development of industrial freezing, which required a frozen distribution network, including the mass production of home and foodservice freezers, the development of packaging as a real science, the development and expansion of extrusion technology and the continued improvement of fryers from what were simply kettles with low capacity to the continuous high volume systems in use today. In support of Dr. Gertz's comment, all one needs to do is look at how frozen potato processing has grown. In 1947, the amount of frozen potatoes processed in the United States was only 3.5 million pounds. Twenty-five years later that figure had grown 770 fold to 2.7 billion pounds; the great bulk of the product going to the fast food and restaurant industries. By 1988, this figure had risen to 5.2 billion pounds, 4.5 billion pounds of which were French fries. !994 saw figures of 7.9 and 6.7 billion pounds. respectively. This massive growth corresponded to the development of oil-blanched or par-fried potato by researchers at J.R. Simplot in 1953. It was also be related to the explosion in the growth of chain restaurants. Today, the figure exceeds eight billion pounds. Of course, there was really no frozen vegetable industry prior to 1929, the year that Clarence Birdseye developed the first commercial freezing systems.

Let's use the potato chip as an example of how technology has contributed to the evolution of an industry. The potato chip had its origins at the Cary Moon's Lake House, an upscale restaurant, in Saratoga Springs, New York during the horse racing season in 1853. A very wealthy customer, Commodore Cornelius Vanderbilt, wanted very crisp fried potatoes with his dinner. After refusing several offerings, the chef, an Indian named George Crum, sliced the potatoes very thin, fried and salted them and presented them to the patron. The customer loved the crunchy fried potatoes and the potato chip was born. It's rather ironic when one considers that a product that came from what was probably considered a gourmet kitchen has evolved into a universal and inexpensive snack. For the potato chip to evolve as it did, however, there needed to be several major advances in technology, including developments in packaging, oil refining and industrial frying. Since potato chips are subject to both rancidity and staling, the early markets were true cottage industries. The first chip makers sold their products door-to-door on the day they were made or they were sold from barrels at local markets. Therefore, for the market to grow, packaging systems that protected the product and provided some shelf life were an absolute necessity. When one thinks about it, today's packages, and the technology involved in their development, that are used for chips and other fried snacks are quite a marvel. They literally utilize "space age" technology to

provide consumers with a wide range of flavors, textures and sensations. So....let's look at how some of these technologies evolved to create the industry we have today.

Equipment

The equipment used in deep-fat frying has changed radically over the years. The original potato chips prepared by Mr. Crum so long ago were probably cooked over an open fire in a black iron pot filled with oil. Since this was upstate New York in 1853, the oil was in all probability tallow or lard. The fry kettle really changed little for almost 80 years. Up until the late 1940's and early 1950's, most industrial fryers were really nothing more than large kettles heated directly by gas or oil burners. These fryers did their job, but compared to today's systems were extremely energy inefficient and quite dangerous; dangerous from a worker safety standpoint and as a potential fire hazard. The first continuous system was developed by Freeman McBeth in 1929 and installed at Ross Potato Chip Company in Richland, Pennsylvania. The depression and the onset of World War II slowed adoption of this new technology, so most operators continued kettle frying. With the need to expand production, fryer design began to change radically in the early 1950's. Manufacturers began to rely more on stainless steel and less on black iron. The kettle or batch systems were replaced by continuous fryers which allowed production volumes to increase from a few hundred pounds to thousands of pounds per hour.

The same held true for the foodservice industry. When Harland Sanders began franchising his Kentucky Fried Chicken, the franchise arrangement included not only the Colonel's spices and recipe, but his pots. The original Kentucky Fried Chicken was fried in pressure cookers on the stove top. Today, the same product is cooked in commercially manufactured fryers designed specifically for the industry. The only place where one finds people frying in pots today is the occasional street vendor. I have seen such operations on the streets of China and in Egypt where dough and other products are fried and sold directly.

Many companies have made contributions to the development of the frying industry, but one company has been a pioneer in fryer development since 1950's. This company is called Heat and Control and they have remained at the forefront for the past 45 years. With apologies to others such as Vandenburg, Flo-Mech Limited, Casa Herrara and others, this presentation was prepared with input from Mr. Andy Coridis, one of the founders of H & C. I wanted to get Mr. Coridis' insights on development of the industrial fryers. Mr. Coridis has witnessed the many changes which have occurred in this industry. They include improvements in materials, control systems, safety systems, heating systems, cleaning and accessibility, waste and exhaust systems and overall system designs. Some of the changes have been as a result of good engineering,

whereas others due to trial and error. When Mr. Coridis first became involved with H&C, the group consisted of five people, plus a secretary. Everyone got their hands dirty when it came to design and actual manufacture of fryer systems.

A new type of continuous fryers was developed in the late 1940's for Crispy Potato Chips of Stockton, California; a project with which Mr. Coridis and Mr. Clark Benson were intimately involved. The fryer was a double zoned immersion tube fired system with a capacity in excess of 600 pounds per hour. As time went on and engineers continued to learn, the capacity of these continuous systems increased. By 1968, volumes increased to 3200 pounds per hour. Today capacities are 5,000 pounds plus per hour. 1986 saw the introduction of the first three zone fryer. The multizone systems allow processors greater control of the process and therefore, better control of quality.

Heating systems have evolved from direct fired kettles in which a flame was directed onto the kettle to ones in which the heating elements were immersed in the kettle below the product zone. Indirect systems utilize a thermal fluid which is circulated through heater tubes immersed in the fryer, similar to those in the direct systems. The latest systems use external heating systems in which the oil itself is heated by passage through a heat exchanger. There are advantages and disadvantages with each system, but one distinct advantage has been increased safety to the workers and from fire.

Another major change which has occurred over the years involves level control in fryers. In early systems, operators watched the level and would manually add oil. This was obviously rather cumbersome. With the large volumes of oil consumed in continuous systems, designers looked for other means to assure that oil levels were maintained. Float valves were used, but they would stick on occasion. If they did stick, oil levels could drop exposing heater tubes which would create a potential fire hazard. Designers then turned to air actuated valves which provided higher margins of safety. Today, infrared eyes monitor oil levels and tell controllers that it is time to add oil.

Ironically, with all the effort that has gone into the development of new systems, the batch or kettle system has made a comeback recently. The kettle-style potato chip has a unique texture which has found favor amongst potato chip connoisseurs. These products may be produced in batch kettles, but the advances in fryer design and the greater understanding of how food fries has allowed processors to utilize continuous systems (multi-zone frying) to produce products with batch-style characteristics.

Another frying system that is relatively new in the marketplace is vend frying. Inventors have been trying to develop a machine that can be used to sell vended foods for some ten to fifteen years with limited success. The early systems failed for a variety of reasons. These included poor quality oil, lack of

maintenance and a failure to properly clean the units. It appears that people have "worked the bugs out, so to speak. Vending systems for fried foods are now being introduced throughout Europe. There were four companies who exhibited such units at the recent Anuga Show in Cologne. One company has been operating for six years, another for 1 ½ years and the other two are brand new players in the market. In speaking with these operators, it appears that they have learned some lessons from the previous failures.

Packaging

As fryer capacity and the technology needed to make these systems has expanded to meet a growing demand for fried snacks, par-fried potatoes, microwavable foods, and other fried foods, packaging technology has had to grow along with the industry. The first commercial potato chips were produced for local consumption. They were distributed or sold in paper bags, which obviously was quite messy, or sold from barrels in the markets. As the industry grew, changes in packaging became a necessity to accommodate growth. Tins were used by many at this time. The first wax paper bags were hand-made by Laura Scudders in 1926. Shortly thereafter, some processors began using cellophane. With fried snacks, processors needed packages which would protect the product (chips are prone to breakage) from both physical and chemical degradation. Cans and glass jars were and are still used in some cases. These provide an oxygen barrier to prevent rancidity, a vapor barrier to exclude water to prevent staling, a barrier to light to prevent light induced rancidity (cans only) and will protect the products from crushing. These kind of packages are heavy and relatively expensive, however. Today, most snacks are packaged in films of some sort. The films used for snacks today may customized to include any one of the following characteristics; light barriers, water vapor barriers, resistance to oil pickup, easy open features, attractive graphics, and ability to support a gas flush. These packages are generally multilaminates which may include plastics and aluminized layers.

Of course, there are many other fried bakery and snack products for which different packages have evolved. Doughnuts and fried pies are generally fried in hard fats which do not weep. They are packaged in boxes or bags which resist oil soakage. The latest advances for some par-fried foods are the microwave packages. Unlike traditional packages which protect and contain a product, these include a susceptor to help focus the heat and assure proper cooking of the food, be it a pizza, pocket or pastry. Studies have also indicated that oils used for frying have a tendency to "focus" microwaves helping to assure proper cooking of the product.

Frying Oils

The types of oils used for frying have changed little over the years, but the quality of these oils has changed drastically over that time. In the early days,

chefs and processors used what was available, and learned by trial and error what worked and what did not. There was a reason that doughnut bakers favored tallows, lards and tropical oils. These products provided them with the finished products they wanted. A doughnut fried in a liquid oil does not hold its coating and will "*weep*" oil. The industry has changed in what they use, however. In the United States and in other places around the world, tallows and lards have been dropped over time in favor of vegetable oils. Today, soybean oil is the most common cooking oil in the United States, but many other products are also employed.

In this day and age, most processors demand that their suppliers provide them with oils that specifically meet the needs of their products. For example, McDonald's requires that the par-fried potatoes prepared for their restaurants be fried in a designated "Mac Oil". The change in oil quality and industry attitude towards using oils that meet their needs was very succinctly stated by Monoj Gupta of Frito-Lay in a letter to the editor of the American Oil Chemists Society journal Inform (1994),

> "*Oil processors have always handed down the product (oil) to the snack food industry. The oil has always been judged to be good by criteria chosen by the oil processors.*"

Gupta was basically telling the suppliers to listen to the needs of their buyers.

As we will discuss in greater detail later, advances in processing and packaging technology have increased what is available to the food processor. As an example, it has been over 100 years since the technology to hydrogenate oils was developed. This technology has allowed oil refiners to modify and stabilize oils to meet a range of needs.

Frying as a Science

It is only recently that engineers and chemists have begun to conduct research to better understand the science of frying. In fact, at a frying short course held in 1991 at the University of California at Davis, Jim Grob of Frymaster referred to frying as an art. It is no longer an art. There has been a great deal of work in recent years which has provided new insights into how and why food fries. This is not to say that researchers have ignored frying in the past. In the early 1900's, researchers, most often women in the home economics departments in mid-western universities conducted some very elaborate studies on frying oils and fried foods. Their research was limited by the tools which they had at their disposal. The great majority of the research which was done over the years focused on the how the oils changed over time. Researchers isolated and identified thousands of compounds in degrading oils. Using these data, oil chemists developed a fairly good understanding of how oil degrades and what compounds could be used as indices of oil quality. What was not done,

however, was to relate degrading oil to its functionality as a heat transfer medium.

This changed in 1987, when Dr. Michael Blumenthal published a monograph first proposing his surfactant theory of frying. This paradigm made an effort to explain the relationship between oil chemistry and its ability to produce quality fried food. The theory made several basic assumptions about food and what was needed for frying to occur. These seven assumptions may be seen in Table 1. Dr. Blumenthal's theory has been closely scrutinized by his colleagues for the past ten years and has found greater favor as time has passed. Another researcher doing work on how food fries is Dr. Paul Singh at the University of California at Davis. Dr. Singh and his students have been conducted extensive investigations on oil as a heating medium. They have shown that the heat capacity and ability to transfer heat changes in frying oils as they are used. This greater understanding of the heating medium, plus the development of new technologies and materials from which fryers can be made may be a step on the road towards making deep-fat frying even more efficient. One of Dr. Singh's students has continued to work with frying oils in his new position in academia. Dr. Brian Farkas is now at North Carolina State and is working to better understand the basic engineering principles behind why food fries.

TABLE 1 SURFACTANT THEORY OF FRYING

1)	Frying is a dehydration process. When food is fried, water and any material in that water are pumped out of the food into the surrounding oil
2)	The heat transfer medium, oil, is non-aqueous and the product being prepared is for all intents and purposes almost all water. Oil and water are immiscible.
3)	For frying to occur, heat must be transferred from the non-aqueous medium, oil, to the mostly aqueous medium, the food.
4)	Any changes in the heat transfer or cooking ability of the oil must result from the degradation products or chemicals formed as a result of the breakdown of the oil.
5)	The food materials leaching into the oil itself and oxygen adsorption at the oil-air interface all contribute to changing the makeup of the oil from a pure triglyceride into a mixture of literally hundreds of compounds.
6)	Those materials which affect the heat transfer at the oil-food interface must act to reduce the initially high surface tension between the two immiscible materials.
7)	As oil degrades, more surfactant materials are formed causing increased contact between oil and food.

SUMMARY

Although frying has been used for thousands of years, it is only in the last fifty years or that it has become a mainstay of both industrial and foodservice food preparation. It is used because it is fast, easy to control and yields products that have excellent flavor, aroma and textural properties. The changes that have occurred in the industry have been a marriage of the rapid changes which have come about in a variety of fields including equipment design, product development, ingredient technology, packaging, oil refining, and changing demographics. The work being conducted on oil as a heat transfer medium by Dr. Singh and others could have far reaching effects on fryer design and how oils are monitored, and could eventually make frying a more efficient operation.

Maybe Dr. Gertz was not too far off when he said that "*everybody believes that deep-fat frying had its beginnings in the early 1950's*".

REFERENCES

1) Anonymous, (1987), "50 Years: A foundation for the Future", Snack Food Association, McLean, Virginia

2) Anonymous, (1995), "The Almanac of the Canning, Freezing, Preserving Industries", Joseph E. Judge and Sons, Westminster, Maryland

3) Blumenthal, M.M., (1987), "Optimum Frying: Theory and Practice", Libra Laboratories Monograph Series.

4) Blumenthal, M.M., (1991), "A New Look at the Chemistry and Technology of Deep-Fat Frying", Food Technol., 45:2, 68-71, 94.

5) Blumenthal, M.M. and Stier, R.F., (1991), "Optimization of Deep-Fat Frying Operations", Trends in Food Science and Technology, Vol.2:6, 144-148.

6) Desrosier, N.W. and Tressler, D.K., (1977), "Fundamentals of Food Freezing", Avi Publishing, Westport, CT.

7) Gertz, C., (1994), "Deep-Fat Frying, History, Introduction", LIPIDFORUM, Helsinki, Finland.

8) Grob, J.T., (1990), "Shortening Degradation and Frying Systems", Presented at Short Course Sponsored by the University of California at Davis entitled Understanding Deep-Frying of Foods: Science and Practice, May 16-18, Davis, CA.

9) Gupta, M., (1994), ""Letter to the Editor", Inform, 5:5, 647-648.

10) Hughes, M.S., (1991), "Potayto, Potahto - Either Way You Say It, They A'Peel", Smithsonian, Vol.22:7, 138-149.

11) Jones, L.C., (1953), "Simplot Potato Research Leads to New Quick Frozen Products", Western Canner Packer, 45:7, 13-16.

12) Morris, D., (1990), "Packaging Considerations for Fried Foods", Presented at Short Course Sponsored by the University of California at Davis entitled Understanding Deep-Frying of Foods: Science and Practice, May 16-18, Davis, CA.

13) Ore-Ida Foods, (1996), "The History of the French Fry", Ore-Ida Marketing Group, Boise, Idaho

14) Rhoades, R.E., (1982), "The Incredible Potato", National Geographic, May, p. 675-694.

15) Sacharow, S. and Griffin, R.C., (1980), "Principles of Food Packaging, 2nd Edition", Avi Publishing, Westport, CT

16) Singh, R.P., (1994), "Oil as a Heat Transfer Medium", Presented at Short Course Sponsored by the Institute of Food Technologists entitled Understanding Deep-Frying of Foods, June 24-25, Atlanta, GA.

17) Stier, R.F., (1996), "Understanding High Volume Frying", Baking & Snack, 18:2, 70-76.

18) Stier, R.F., (1996), "Understanding High Volume Frying:Part 2", Baking & Snack, 18:3, 51-54.

19) Stier, R.F., (1996), "Understanding High Volume Frying: Part 3", Baking & Snack, 18:4, 41-44.

20) Stier, R.F. and Blumenthal, M.M., (1990), "Heat Transfer in Frying", Baking & Snack Systems, 12:9, 15-19

21) Stier, R.F. and Blumenthal, M.M., (1994), "Quality in Frying", Baking & Snack, 16:11, 38-42.

22) Talburt, W.F. and Smith, O., (1975), "Potato Processing", 3rd Edition, Avi Publishing Company, Westport, CT

23) Tannahill, R.. (1988), "Food in History", Crown Publishers, New York, NY

24) Toussaint-Samat, M., (1992), "History Of Food", Blackwell Publishers, Cambridge, MA

25) Viola, H.J. and Margolis, C., (1991), "Seeds of Change: A Quincentennial Commemoration", Smithsonian Institution Press.

26) Waller, G., (1966), "Saratoga: Saga of An Impious Era"

Time Line for Deep-Fat Frying

YEAR	EVENT
3,000 BC	Chinese fry meat as prelude to roasting
1,300 BC	Hebrews fry flat breads
1537	Potatoes introduced to Europe
1600-1700	French fried potatoes is created
1853	Potato chip invented in Saratoga Springs, NY by George Crum
1890's	Potato chip industry begins in United States
1897	Hydrogenation of edible oils invented
1906	Commercial oil roasting of shelled peanuts by Planters
1908	JP Dushesues founds Leominster Potato Chip Company
1926	Laura Scudder's developed first potato chip bag of waxed paper
1927-30	Cellophane begins to be used for potato chip bags
1929	Clarence Birdseye develops new commercial freezing technologies
1932	First tortilla chips (Doolin and Filler) in San Antonio, Texas
1933	Dixie wax paper introduces pre-printed glassine bag. Cracker barrel marketing of chips comes to an end
1930-1935	National Potato Chip Institute warns consumers that chips are not fattening if eaten in small amounts
1938	H.W. Lay Company founds Lay's Potato Chip Company in Atlanta
1946	First automatic packaging machine for chips developed
1945-1950	Extruded snacks introduced; MacBeth introduces continuous immersion cookers
1950	Under pan fired cookers introduced
1950-52	Fryers with external heat exchangers with oil circulation introduced; Pork rinds introduced
1950-1955	Laminate bags with polypropylene/cellophane and polypropylene glassine introduced
1953	Simplot scientists develop technique for par-frying potato slices
1957	Heat & Control introduces "Big Goose, a 1,600 lb. capacity continuous system
1958	Urshel develops new slicers for potato chip manufacture
1961	Frito-Lay merger
1969	Potato chip controversy develops with introduction of Pringles and Chippos
1970-1975	7,000 lb. capacity fryers introduced
1973	DGF in Germany proposes regulations based on oxidized fatty acids for restaurant frying oils
1987	Blumenthal publishes surfactant theory of frying

Some chapters follow,taken from the book "The olive tree, the oil, the olive", published by the International Olive Oil Council. These excerpts narrate the history, expansio and geographical distribution of the olive tree and its produce, and show how they have influenced Mediterranean culture throught a number of signature dishes from various Mediterranean countries.

38

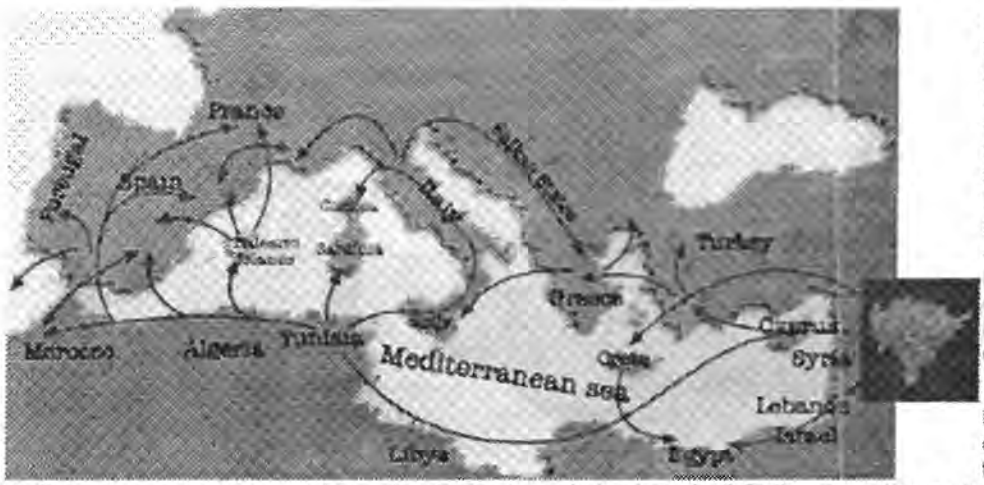
the olive tree
The origin and expansion of the olive tree

The origin of the olive tree is lost in time, coinciding and mingling with the expansion of the Mediterranean civilizations which for centuries governed the destiny of mankind and left their imprint on Western culture.

Olive leaf fossils have been found in Pliocene deposits at Mongardino in Italy. Fossilised remains have been discovered in strata from the Upper Paleolithic at the Relilai snail hatchery in North Africa, and pieces of wild olive trees and stones have been uncovered in excavations of the Chalcolithic period and the Bronze Age in Spain. The existence of the olive tree therefore dates back to the twelfth millennium BC.

The wild olive tree originated in Asia Minor where it is extremely abundant and grows in thick forests. It appears to have spread from Syria to Greece via Anatolia (De Candolle, 1883) although other hypotheses point to lower Egypt, Nubia, Ethiopia, the Atlas Mountains or certain areas of Europe as its source area. Caruso for that reason believes it to be indigenous to the entire Mediterranean Basin and considers Asia Minor to have been the birthplace of the cultivated olive some six millennia ago. The Assyrians and Babylonians were the only ancient civilisations in the area who were not familiar with the olive tree.

Taking the area that extends from the southern Caucasus to the Iranion plateau and the Mediterranean coasts of Syria and Palestine (Acerbo) to be the original home of the olive tree, its cultivation developed considerably in these last two regions, spreading from there to the island of Cyprus and on towards Anatolia or from the island of Crete towards Egypt.



In the 16th century BC the Phoenicians started disseminating the olive throughout the Greek isles, later introducing it to the Greek mainland between the 14th and 12th centuries BC where its cultivation increased and gained great importance in the 4th century BC when Solon issued decrees regulating olive planting.

From the 6th century BC onwards, the olive spread throughout the Mediterranean countries reaching Tripoli, Tunis and the island of Sicily. From there, it moved to southern Italy. Presto, however, maintained that the olive tree in Italy dates back to three centuries before the fall of Troy (1200 BC). Another Roman annalist (Penestrello) defends the traditional view that the first olive tree was brought to Italy during the reign of Lucius Tarquinius Priscus the Elder (616 - 578 BC), possibly from Tripoli or Gabes (Tunisia). Cultivation moved upwards from south to north, from Calabria to Liguria. When the Romans arrived in North Africa, the Berbers knew how to graft wild olives and had really developed its cultivation throughout the territories they occupied.

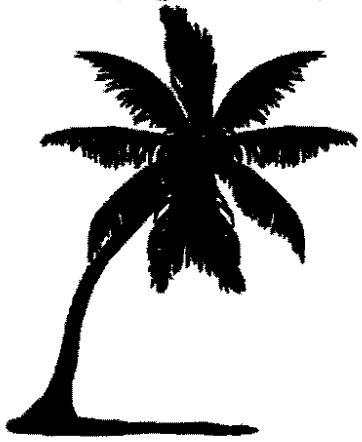
The Romans continued the expansion of the olive tree to the countries bordering the Mediterranean, using it as a peaceful weapon in their conquests to settle the people. It was introduced in Marseilles around 600 BC and spread from there to the whole of Gaul. The olive tree made its appearance in Sardinia in Roman times, while in Corsica it is said to have been brought by the Genoese after the fall of the Roman Empire.

Olive growing was introduced into Spain during the maritime domination of the Phoenicians (1050 BC) but did not develop to a noteworthy extent until the arrival of Scipio (212 BC) and Roman rule (45 BC). After the third Punic

A SHORT HISTORY OF FATS AND OILS

RICHARD F. STIER
TECHNICAL DIRECTOR
ALEB PROJECT
CAIRO, EGYPT

FRYING: AN ANCIENT PROCESS



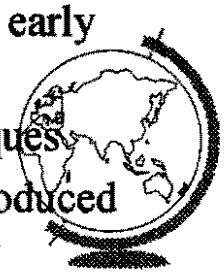
- Egypt - As evidenced by wall paintings
- China - 5,000 years ago to pre-cook meat prior to roasting
- Biblical references

"And unleavened bread, and cakes leavened temper with oil and wafers anointed with oil; of wheaten flours though shalt make."

Exodus 29:1

EDIBLE OILS

- Early days - Used what was available
- Olive oil in Mediterranean
- Animal fats in Northern Europe
- Palm & soy in Asia and Africa
- Hydrogenated oils came into use in early 1900's following invention
- Improved refining/blending techniques
- Egypt today - Olive oil & others produced from imported crudes



42

EDIBLE OILS

- Flavor carriers
- Energy
- Processing - Food flavor & texture drive the process
- Heating efficiency, quick cooking

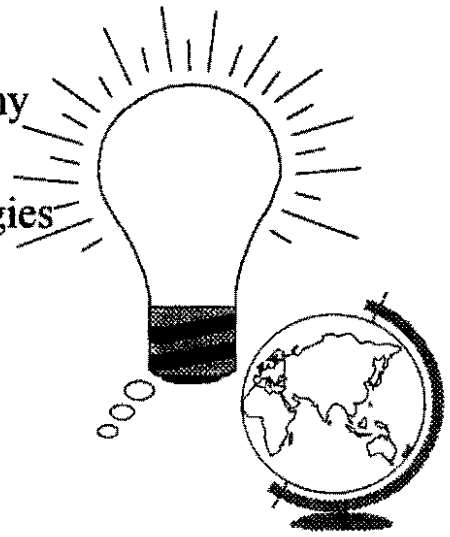
COOKING OILS

A LOCAL PROCESS FOR CENTURIES

- Bakers
- Cooks
- Restaurants/vendors
- Home preparation

INDUSTRIAL FRYING

- Began in the 1890's
- Local industry for many years
- Needed new technologies to move forward



GROWTH OF FROZEN POTATO INDUSTRY

UNITED STATES FIGURES

YEAR	POUNDS OF POTATOES
1947	3.5 Million
1972	2.7 Billion
1988	5.2 Billion
1994	7.9 Billion

Growth of the frozen potato industry corresponded with the development of the oil-blanched or par-fried potato by researchers at J.R. Simplot in 1953.

EQUIPMENT

- Kettle Systems
- First continuous fryer developed by Freeman McBeth in 1929
- The system was installed at Ross Potato Chip Company in Richland, PA
- Development slowed by World War II

ADVANCES IN FOODSERVICE FRYING

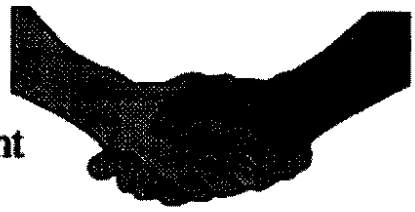
- Open pots to computers
- Automation
- Built in filters
- Safety
- Ease of use

PACKAGING ADVANCES

- Amphora in ancient times
- Glass used and still used
- Fried snacks originally distributed locally
- Sold door-to-door or in stores in barrels
- Cans used early & still in use
- Systems have evolved for different products

EDIBLE OILS

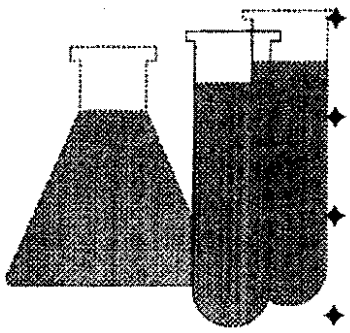
- Suppliers manufacture a range of oil products
- Customer service is increasingly important
- Product/process development
- Customer driven - clear bottles



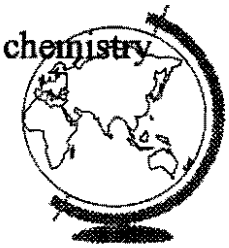
"Oil processors have always handed down the product (oil) to the snack food industry. The oil has been judged to be good by criteria chosen by the oil processors."

Gupta, M., 1994, Inform, 5:5, 647-649

FRYING AS A SCIENCE



- Frying research has been in progress for almost 90 years
- Early work was on oil chemistry (Academia)
- Industrial research on the process
- Relationships of oil chemistry to process ignored



EDIBLE OILS USE

- Thousands of years old
- Used extensively - great heating medium
- Cooking - Yields delicious & desirable products
- Usage - Expanded with technology
- Greatest growth since 1950
- Continues to grow despite anti-fat people
- Fat and fatty foods taste good

Processing of Edible Oils
Processing, Quality & Safety

By
Monoj K Gupta
MG Edible Oil Consulting, Int'l
9 Lundy's Lane, Richardson, Texas 75080
Telephone; 972-234-1634, Fax: 972-234-5973
E-Mail: monoj.gupta@worldnet.att.net

تصنيع الزيوت القابلة للأكل
التصنيع وضمان الجودة والسلامة

Monoj K Gupta
MG Edible Oil Consulting, Int'l
9 Lundy's Lane, Richardson, Texas 75080
Telephone; 972-234-1634, Fax: 972-234-5973
E-Mail: monoj.gupta@worldnet.att.net

MAJOR SOURCES OF VEGETABLE OILS

- **SEEDS**
  - **SOYBEAN**
  - **EDIBLE RAPE SEED**
  - **SUNFLOWER**
  - **COTTONSEED**
  - **SAFFLOWER**
  - **GROUNDNUT**
  - **CORN**
- **FRUITS**
  - **PALM**
  - **OLIVE**

المصادر الرئيسية للزيوت النباتية

- **البذور**
  - بذور فول الصويا
  - بذور اللفت القابلة للاكل
  - بذور عباد الشمس
  - بذور القطن
  - بذور العصفر
  - بذور فول السوداني
  - بذور الذرة
- **الفاكهة**
  - النخيل
  - الزيتون

TYPICAL OIL APPLICATIONS

- WESTERN HEMISPHERE
  - BAKING
  - INDUSTRIAL FRYING
  - RESTAURANT & FOOD SERVICES
  - COOKING & SALAD DRESSING
  - MAYONNAISE
  - MARGARINE
  - SOUPS
  - INFANT FORMULA
  - COFFEE CREAMER
- ASIA, AFRICA, MIDDLE EAST
  - COOKING
  - INDUSTRIAL FRYING
  - BAKING
  - MAYONNAISE
  - MARGARINE

الاستخدامات الشائعة للزيوت

- العالم الغربي
  - الخبز
  - القلي الصناعي
  - المطاعم وخدمات تقديم الاغذية
  - الطهي وتوابل السلاطات
  - المايونيز
  - السمن الصناعي النباتي
  - الحساء
  - اغذية الاطفال
  - كريمة القهوة
- آسيا وافريقيا والشرق الاوسط
  - الطهي
  - القلي الصناعي
  - الخبز
  - المايونيز
  - السمن النباتي الصناعي

FUNCTIONS OF OILS IN FOOD APPLICATIONS

- **BAKING**
  - PROVIDES TEXTURE & MOISNESS TO THE PRODUCT
  - ACTS AS A VEHICLE TO INCORPORATE EMULSIFIERS
  - PROVIDES DESIRED MOUTH FEEL TO THE PRODUCT
- **TOPICAL APPLICATION AS SPRAY OIL**
  - ENHANCES FLAVOR OF THE PRODUCT
  - ADDS SMOOTHNESS TO PRODUCT TEXTURE
- **FRIED FOODS**
  - PROVIDES FRIED FOOD FLAVOR
  - PROVIDES THE DESIRED TEXTURE TO THE FRIED FOOD

وظيفة الزيوت في الاستخدامات الغذائية

- الخبز:
  - يعطي قواما وطراوة للمنتج
  - يعمل كاداة لادخال المستحلبات
  - يعطي مذاقا جيدا للمنتج
- الاستخدام الموضعي للزيت
  - يقوي من نكهة المنتج
  - يزيد من طراوة قوام المنتج
- الاغذية المقلية
  - يعطي نكهة جيدة للاغذية المقلية
  - يعطي القوام المطلوب للاغذية المقلية

FUNCTIONS OF OILS IN FOOD APPLICATIONS (CONT)

- **INSTANT OR PREPARED DRINKS**
  - ACTS AS A WETTING AGENT
  - ACTS AS A FLAVOR RELEASING AGENT
  - ACTS AS A MOUTH FEEL ENHANCER
- **SALAD DRESSING / SOUPS**
  - ADDS SMOOTHNESS TO THE DRESSING
  - ACTS AS A FLAVOR RELEASER
- **CANDY/ CHOCOLATE**
  - ACTS AS A FLAVOR RELEASING AGENT
  - PROVIDES SMOOTHNESS TO THE PRODUCT
- **INFANT FORMULA**
  - SUPPLIES ESSENTIAL FATTY ACIDS TO THE FORMULA

وظيفة الزيوت في الاستخدامات الغذائية (تابع)

المشروبات السريعة او الجاهزة

يعمل كعامل مرطب

- يعمل كعامل باعث للنكهة
- يساعد على تحسين المذاق

توابل السلاطات/الحساء

- يضيف طراوة للتوابل
- يعمل كباعث للنكهة

الحلوى/الشكولاته

- يعمل كعامل باعث للنكهة
- يعطي طراوة للمنتج

اغذية الاطفال الرضع

- مصدر للاحماض الدهنية الاساسية للاغذية

OTHER USES OF OILS

- **FOOD APPLICATIONS**
  - EMULSIFIERS
  - CANDY COATING
  - PREPARED AND INSTANT DRINKS
  - PREPARED MEALS
  - RELEASING AGENT IN CANDY AND BAKING PROCESS
  - SPRAY ON CEREAL PRODUCTS
- NON-FOOD APPLICATIONS
  - PAINT (DRYING AGENT)
  - LUBRICANT
  - INDUSTRIAL SOAPS
  - FATTY ACIDS
  - FATTY ALCOHOLS
  - TOILET SOAP
  - DETERGENTS
  - NEWSPRINT
  - CROP DUSTING

استخدامات أخرى للزيوت

الاستخدامات الغذائية

- المستحلبات
- تغطية الحلوى
- المشروبات السريعة والجاهزة
- الوجبات الجاهزة
- عامل مانع لالتصاق الحلوى والخبز
- يتم رشه على منتجات الحبوب

الاستخدامات غير الغذائية

- الطلاء (عامل مجفف)
- يستخدم كمادة تشحيم
- الصابون الصناعي
- الاحماض الدهنية
- الكحوليات الدهنية
- صابون التواليت
- المنظفات
- طباعة الصحف
- تعطير المحاصيل

OLIVE OIL

EXPORT OPPORTUNITIES

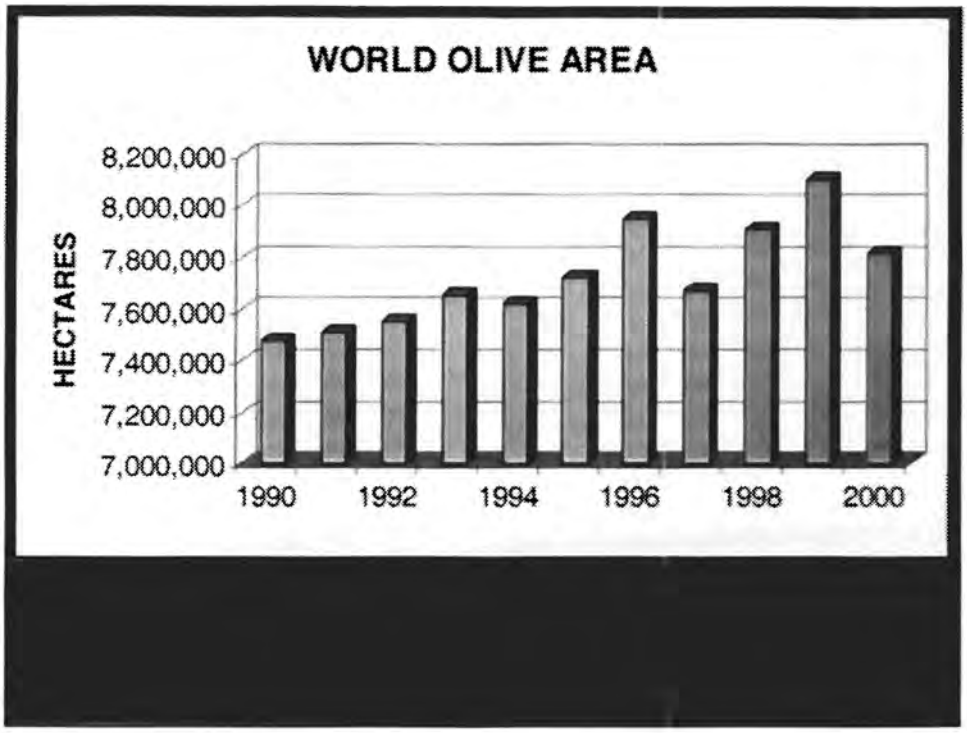
APRIL 2, 2001

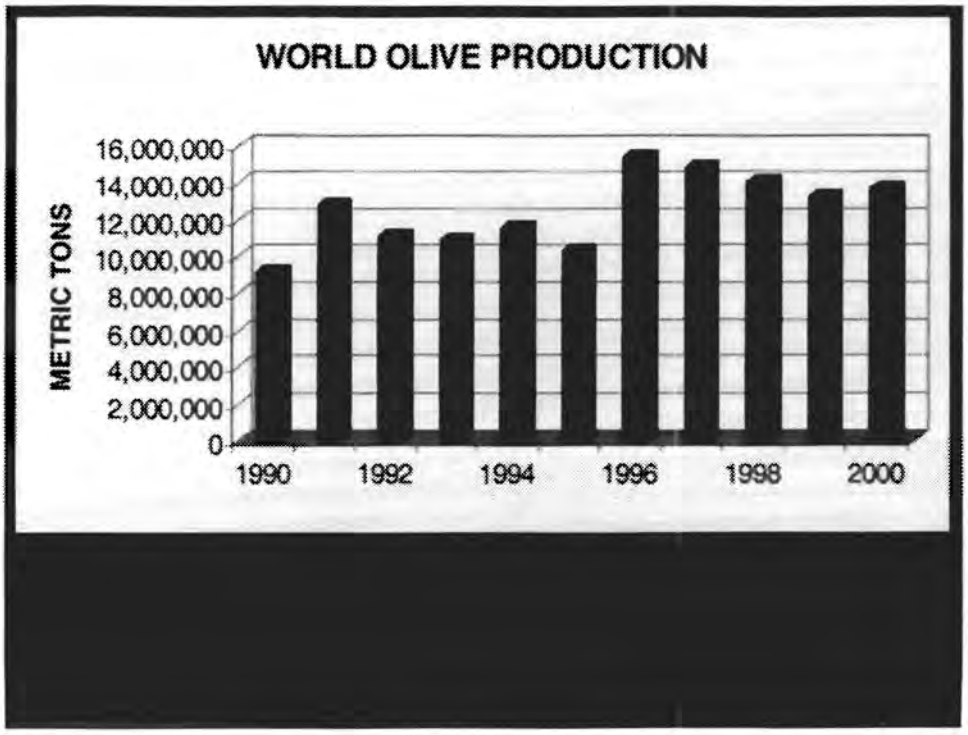
TERRY DUNN

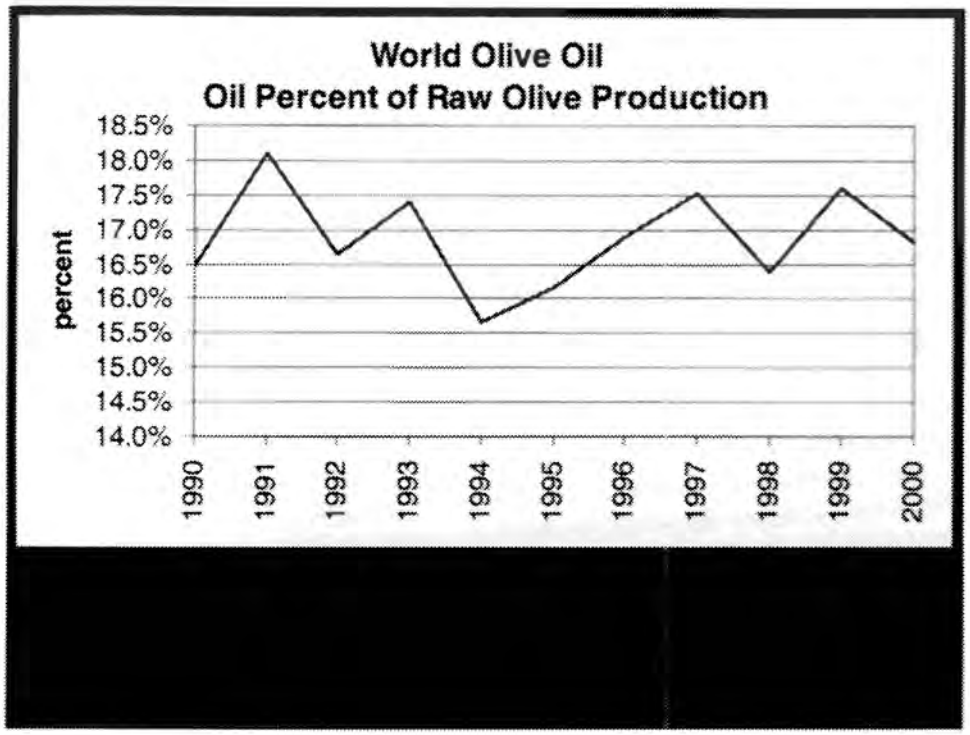
DIRECTOR

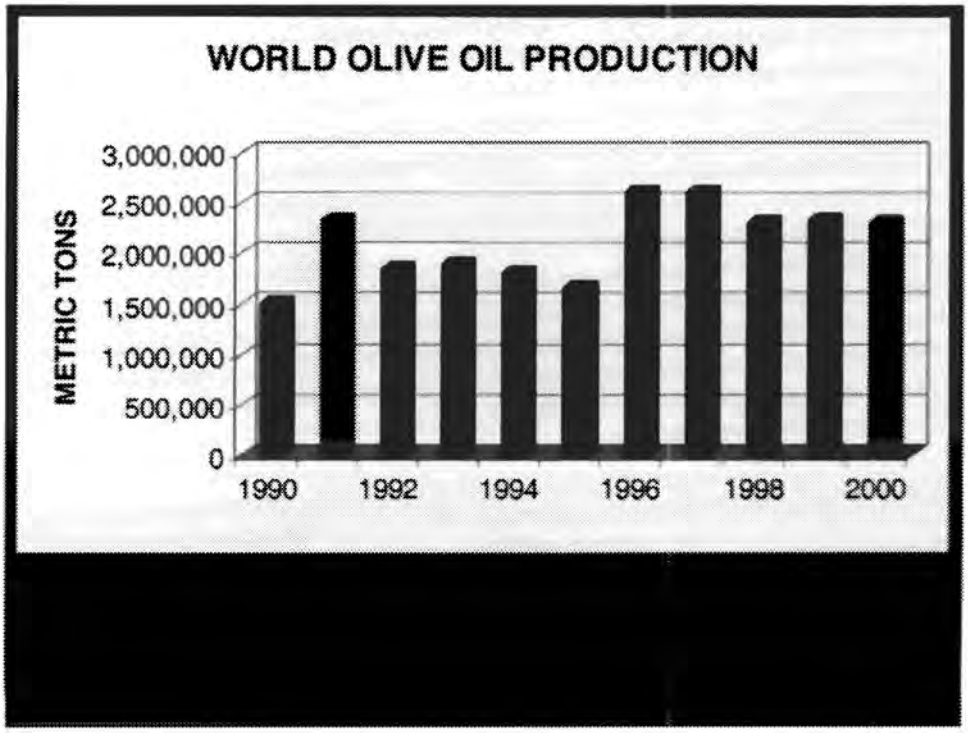
ALEB MARKET INFORMATION SERVICES

55

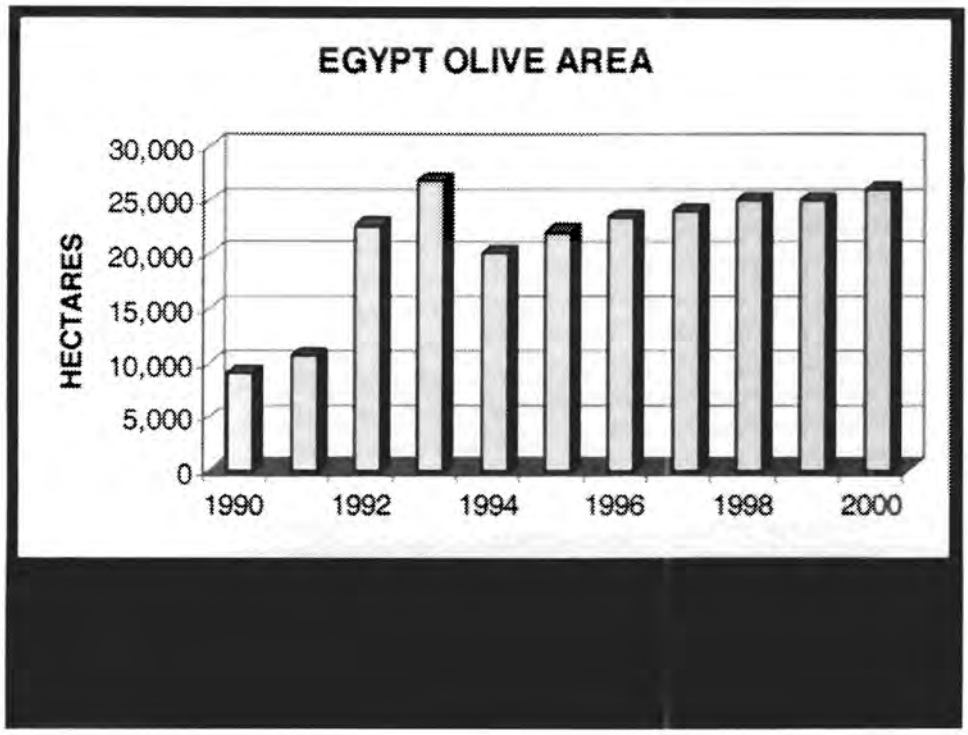

WORLD OLIVE AREA
HECTARES
8,200,000
8,000,000
7,800,000
7,600,000
7,400,000
7,200,000
7,000,000
1990
1992
1994
1996
1998
2000

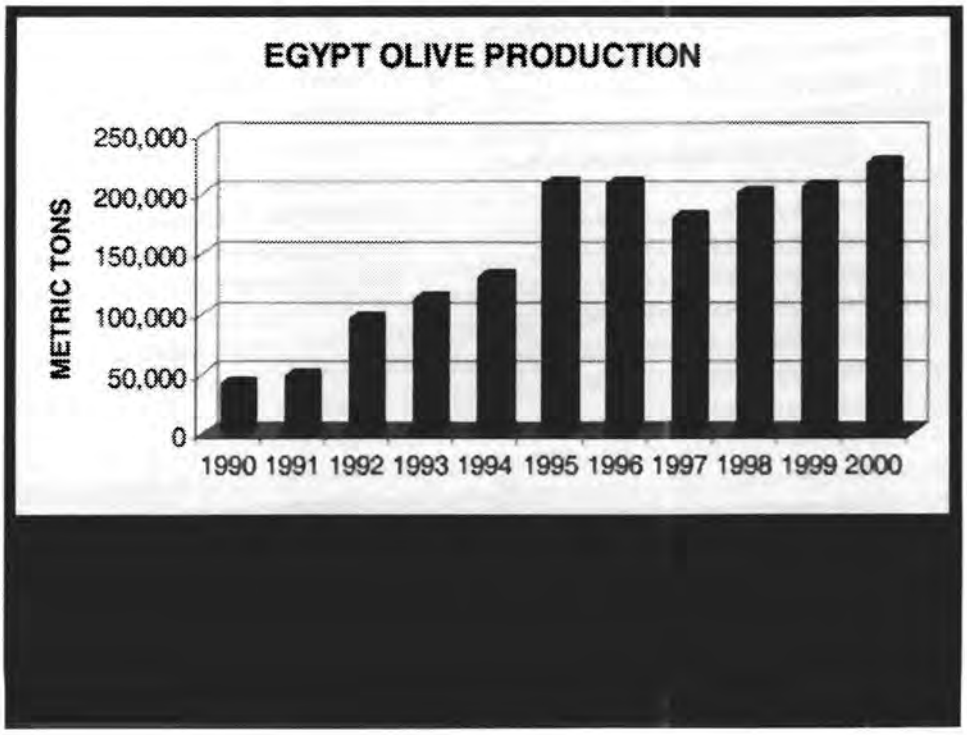

WORLD OLIVE PRODUCTION
METRIC TONS
16,000,000
14,000,000
12,000,000
10,000,000
8,000,000
6,000,000
4,000,000
2,000,000
0
1990
1992
1994
1996
1998
2000

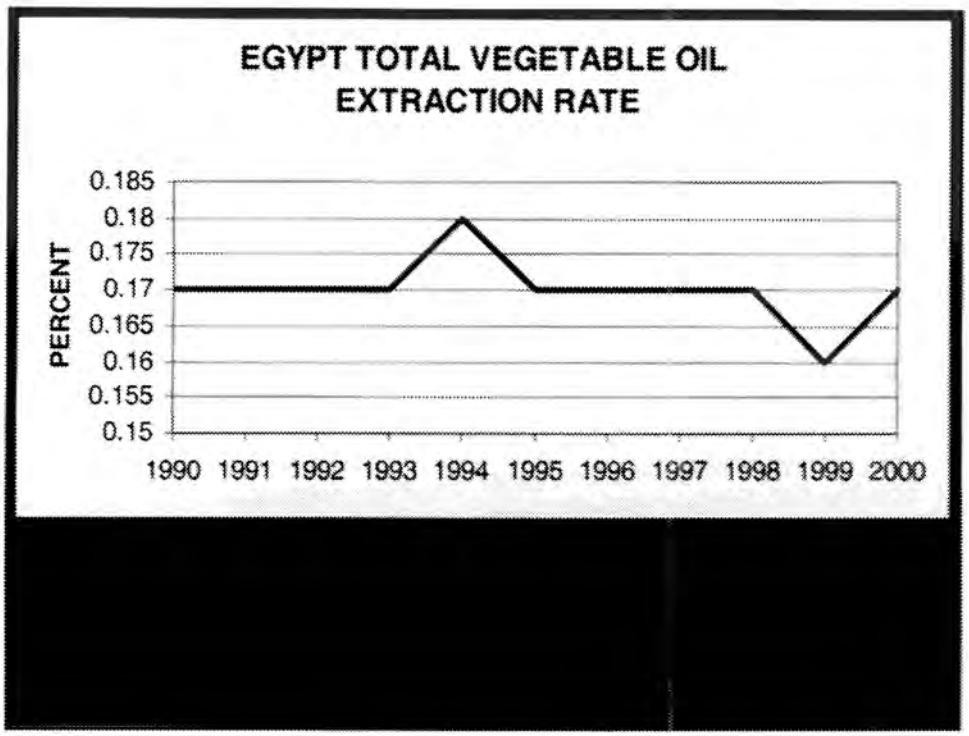

World Olive Oil
Oil Percent of Raw Olive Production
percent
18.5%
18.0%
17.5%
17.0%
16.5%
16.0%
15.5%
15.0%
14.5%
14.0%
1990
1991
1992
1993
1994
1995
1996
1997
1998
1999
2000

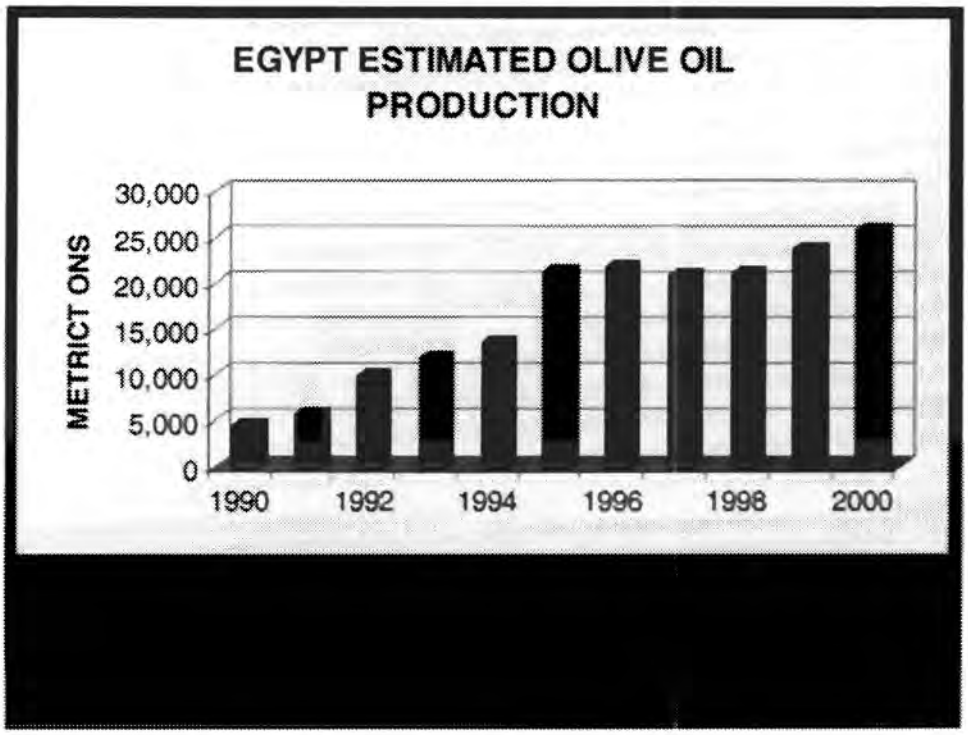

WORLD OLIVE OIL PRODUCTION
METRIC TONS
3,000,000
2,500,000
2,000,000
1,500,000
1,000,000
500,000
0
1990
1992
1994
1996
1998
2000

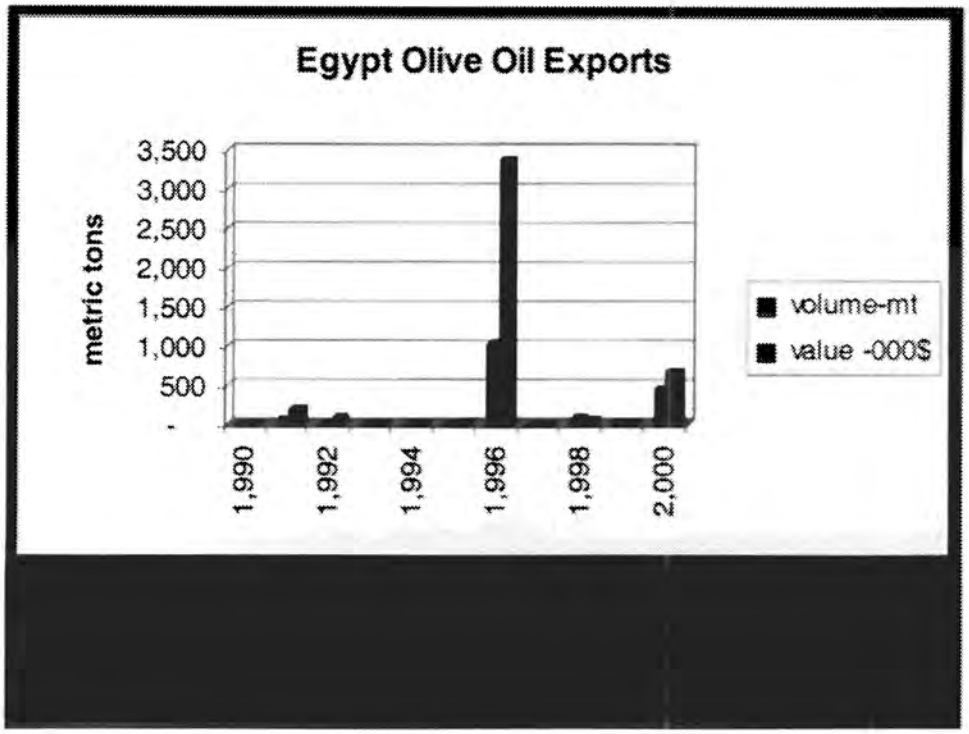
57

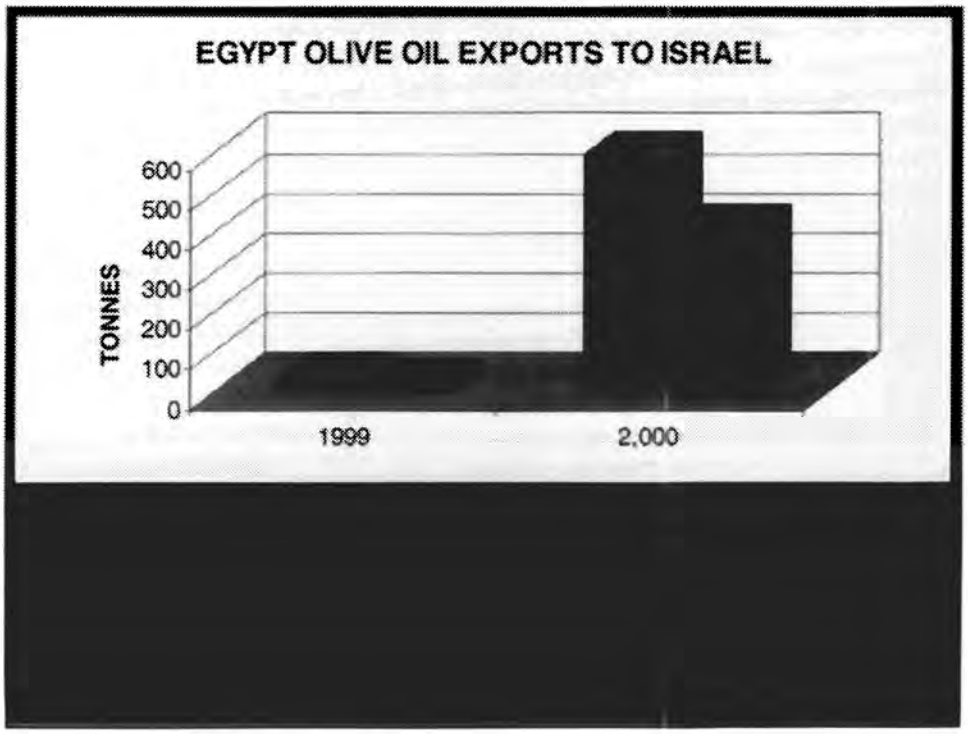

EGYPT OLIVE AREA
HECTARES
30,000
25,000
20,000
15,000
10,000
5,000
0
1990
1992
1994
1996
1998
2000

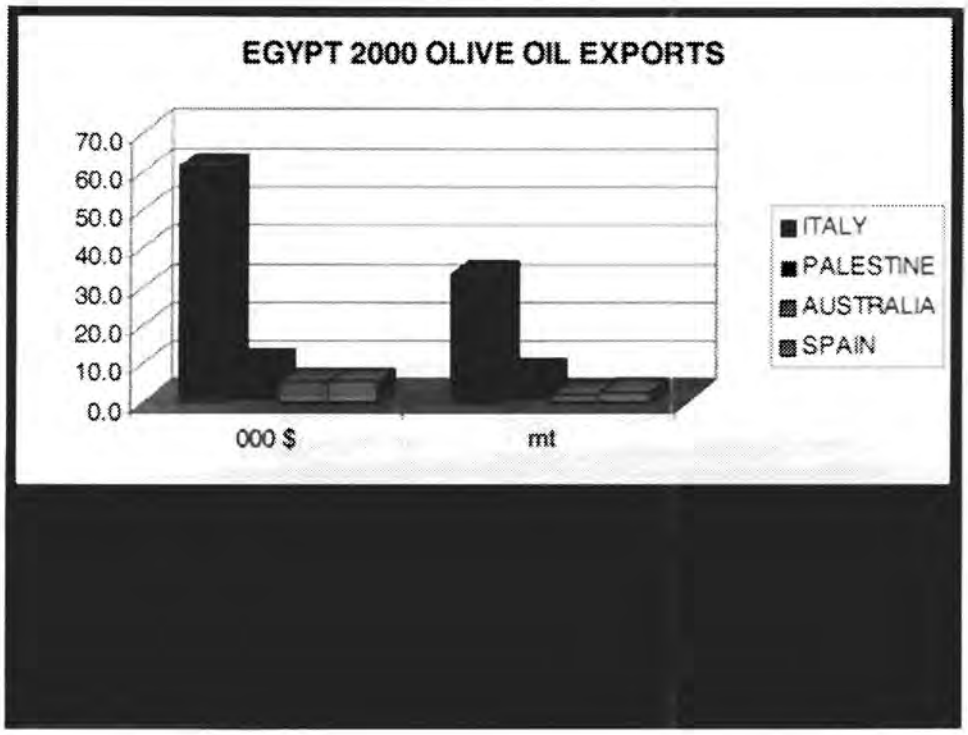

EGYPT OLIVE PRODUCTION
METRIC TONS
250,000
200,000
150,000
100,000
50,000
0
1990 1991 1992 1993 1994 1995 1996 1997 1998 1999 2000

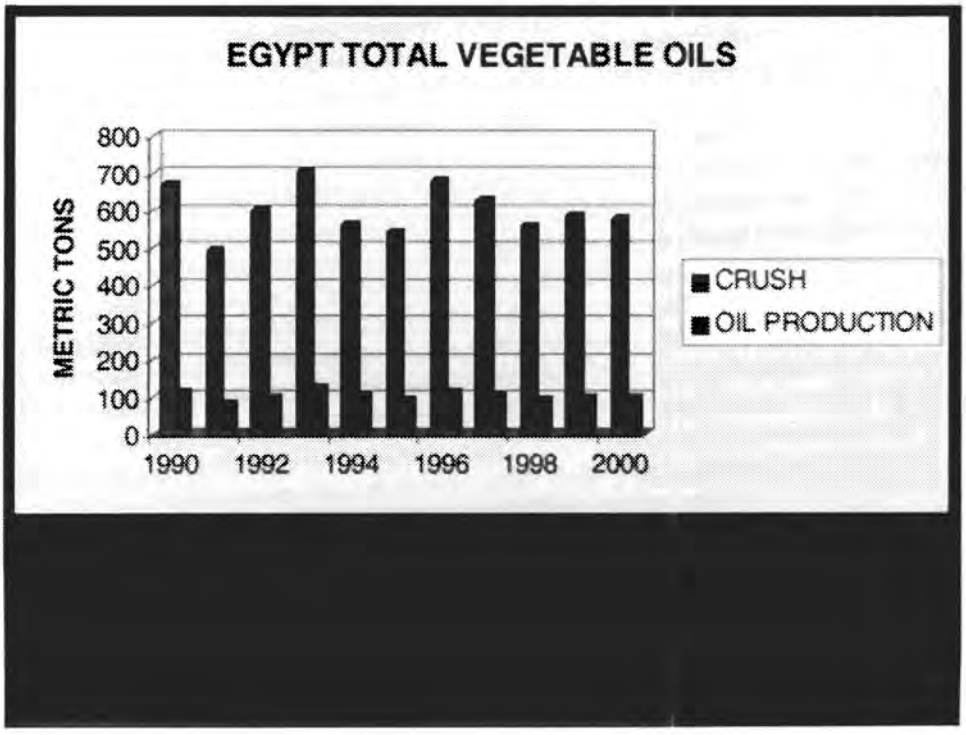

EGYPT TOTAL VEGETABLE OIL
EXTRACTION RATE
PERCENT
0.185
0.18
0.175
0.17
0.165
0.16
0.155
0.15
1990 1991 1992 1993 1994 1995 1996 1997 1998 1999 2000

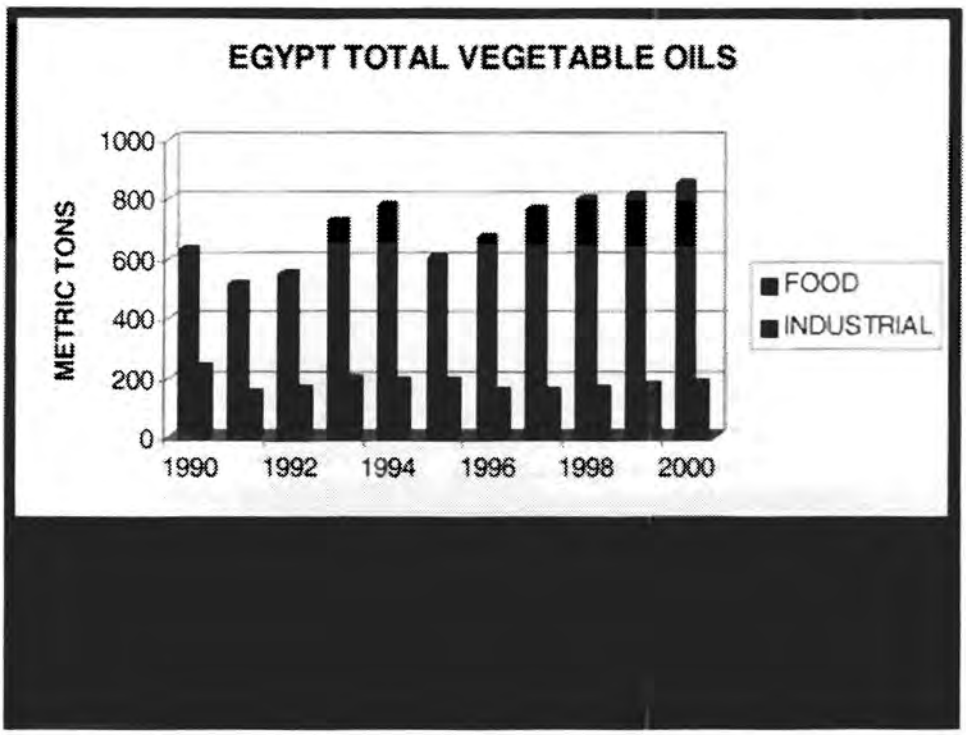

EGYPT ESTIMATED OLIVE OIL
PRODUCTION
METRICT ONS
30,000
25,000
20,000
15,000
10,000
5,000
0
1990 1992 1994 1996 1998 2000

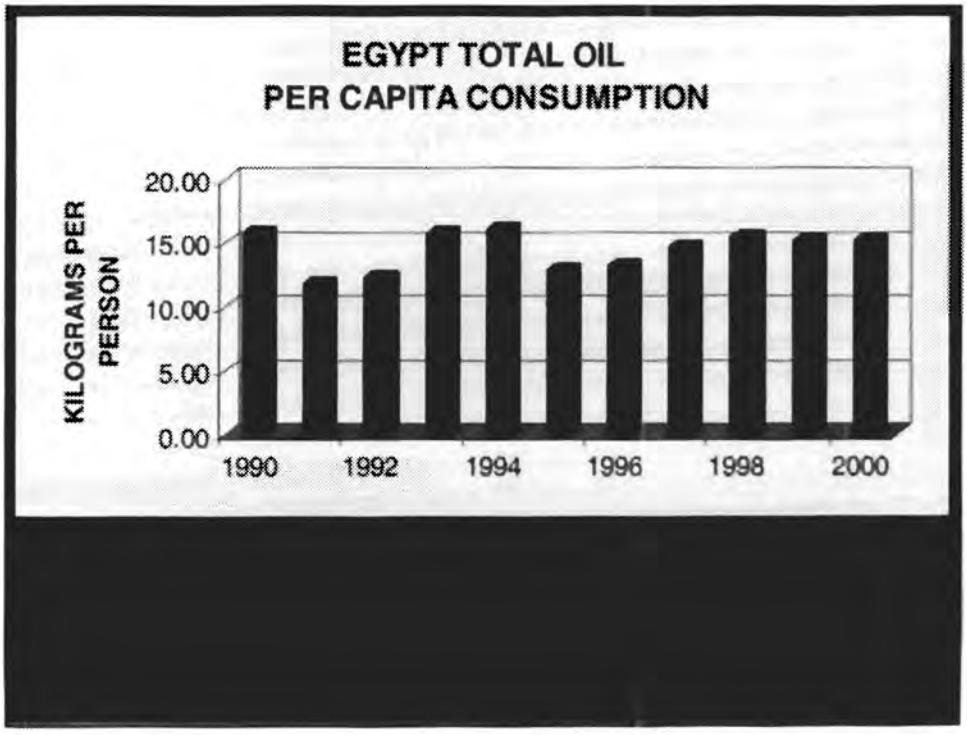

Egypt Olive Oil Exports
metric tons
3,500
3,000
2,500
2,000
1,500
1,000
500
-
1,990
1,992
1,994
1,996
1,998
2,000
volume-mt
value -000$

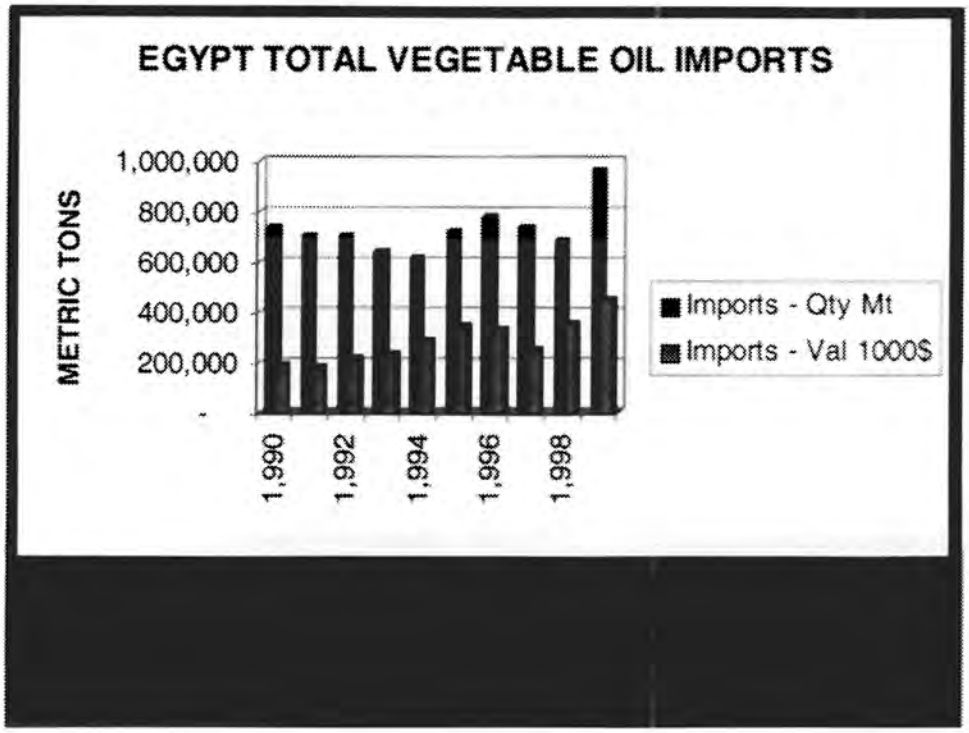

EGYPT OLIVE OIL EXPORTS TO ISRAEL
TONNES
600
500
400
300
200
100
0
1999
2,000

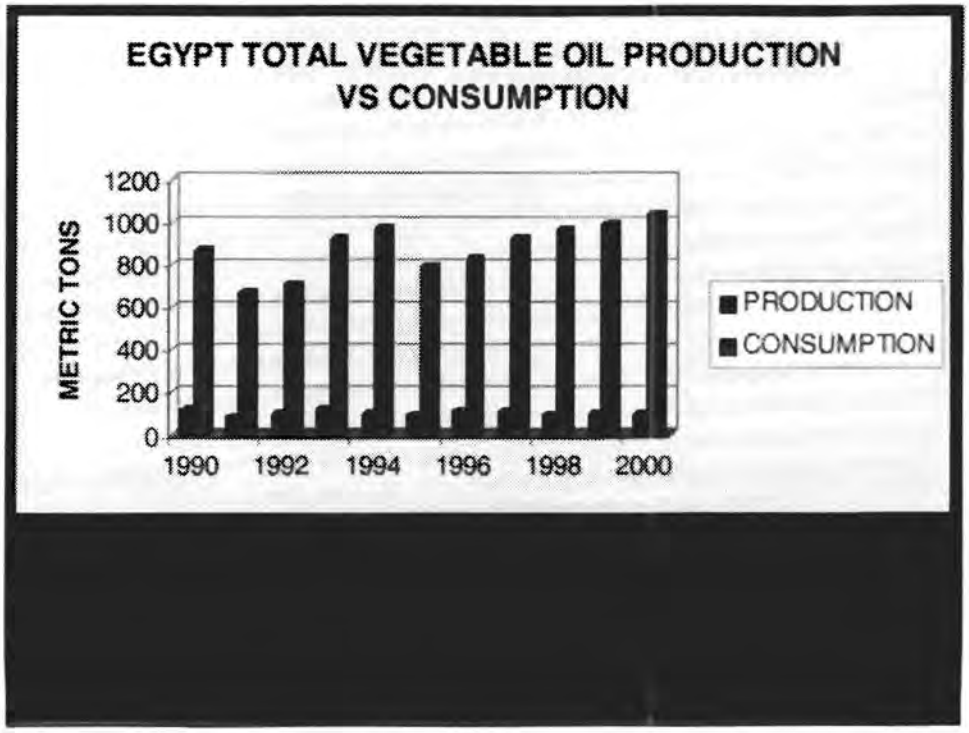

EGYPT 2000 OLIVE OIL EXPORTS
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
000 $
mt
ITALY
PALESTINE
AUSTRALIA
SPAIN

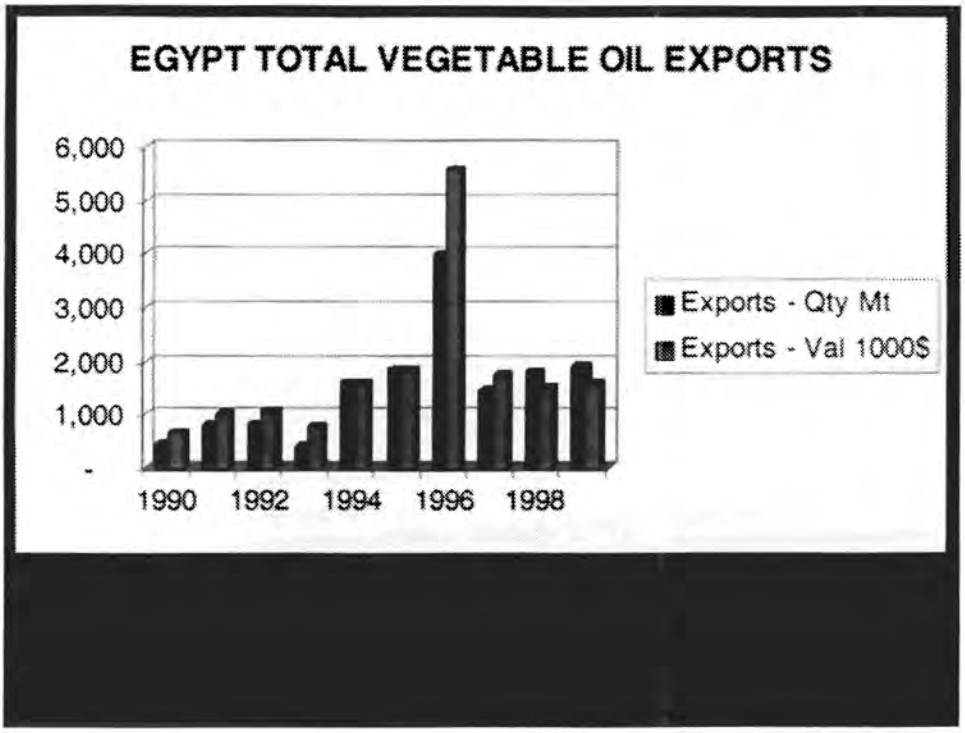

EGYPT TOTAL VEGETABLE OILS
METRIC TONS
800
700
600
500
400
300
200
100
0
1990
1992
1994
1996
1998
2000
CRUSH
OIL PRODUCTION

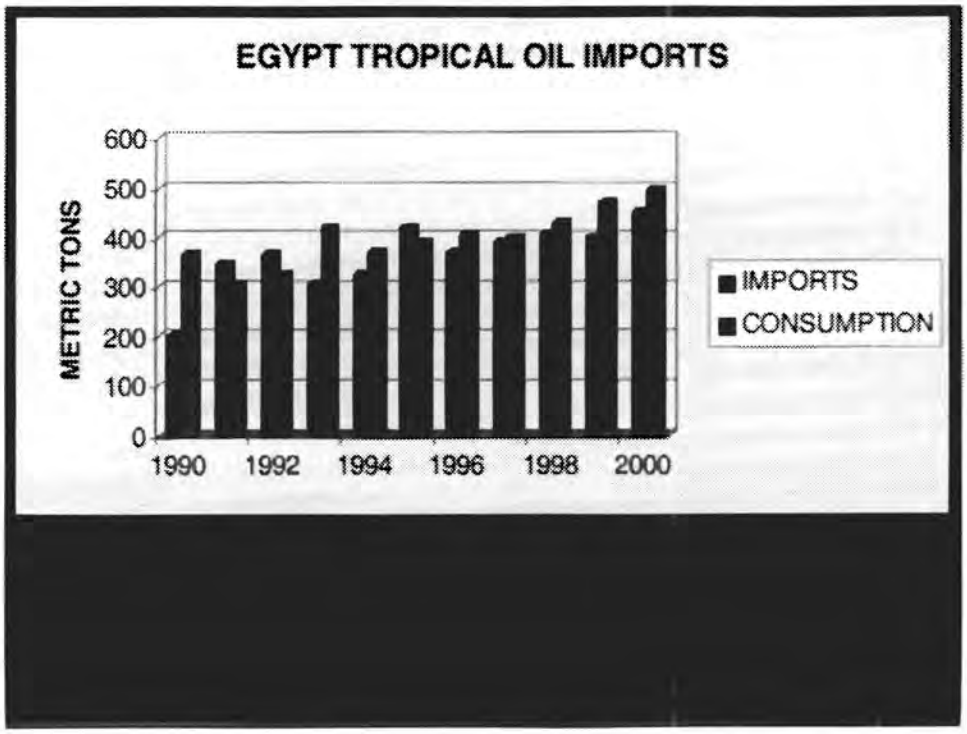

EGYPT TOTAL VEGETABLE OILS
METRIC TONS
1000
800
600
400
200
0
1990
1992
1994
1996
1998
2000
FOOD
INDUSTRIAL

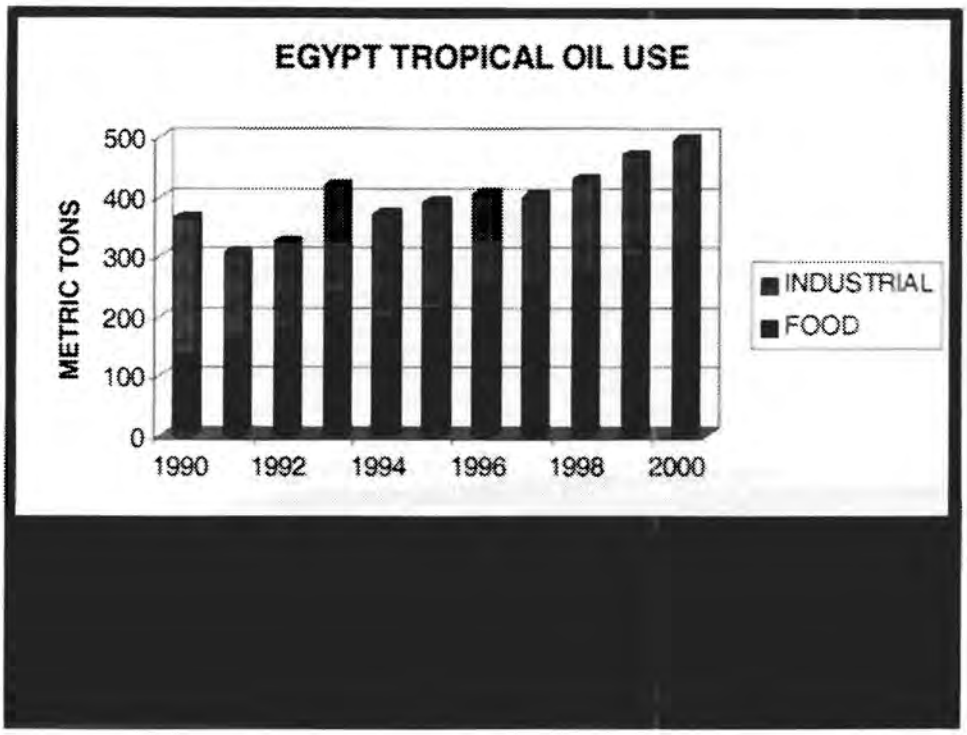

EGYPT TOTAL OIL
PER CAPITA CONSUMPTION
KILOGRAMS PER PERSON
20.00
15.00
10.00
5.00
0.00
1990
1992
1994
1996
1998
2000

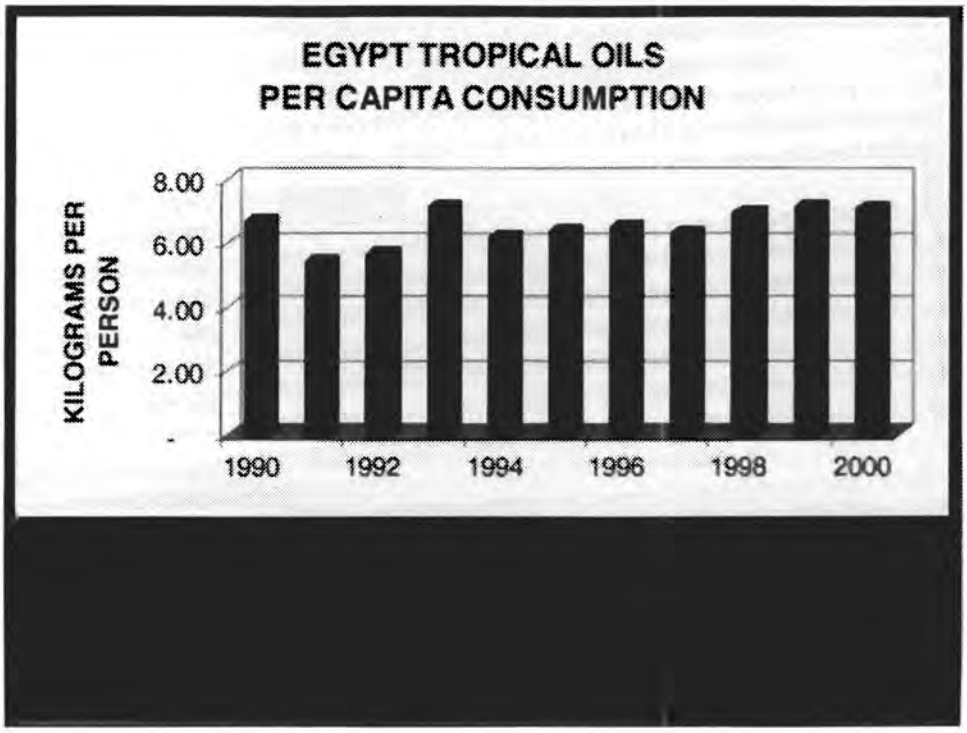

EGYPT TOTAL VEGETABLE OIL IMPORTS
METRIC TONS
1,000,000
800,000
600,000
400,000
200,000
-
1,990
1,992
1,994
1,996
1,998
Imports - Qty Mt
Imports - Val 1000$

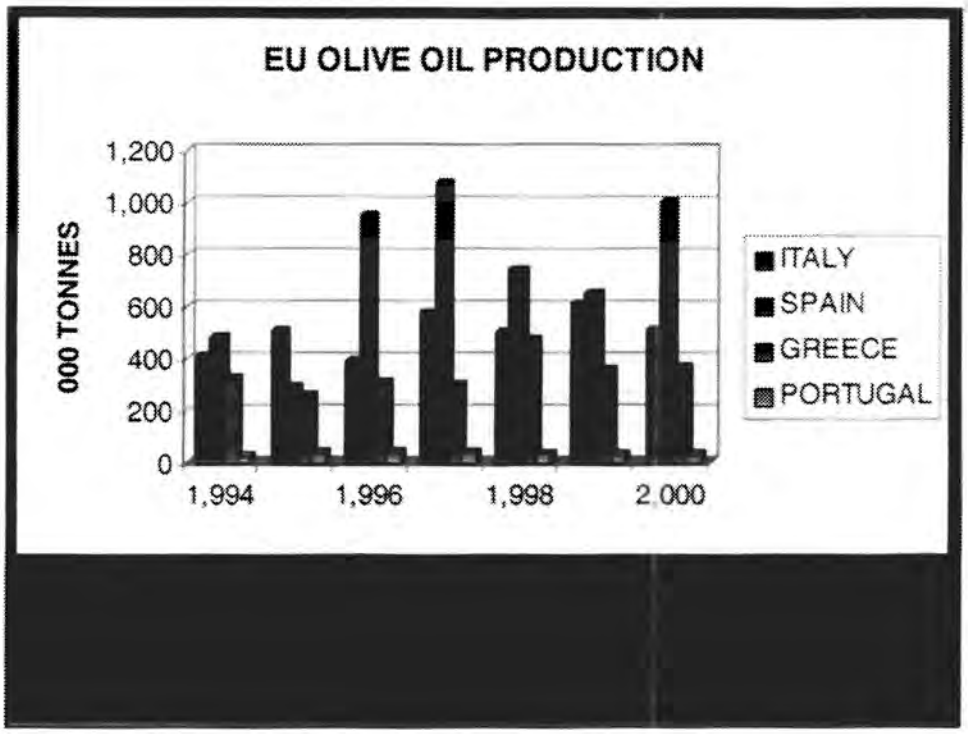

EGYPT TOTAL VEGETABLE OIL PRODUCTION VS CONSUMPTION
METRIC TONS
1200
1000
800
600
400
200
0
1990
1992
1994
1996
1998
2000
PRODUCTION
CONSUMPTION

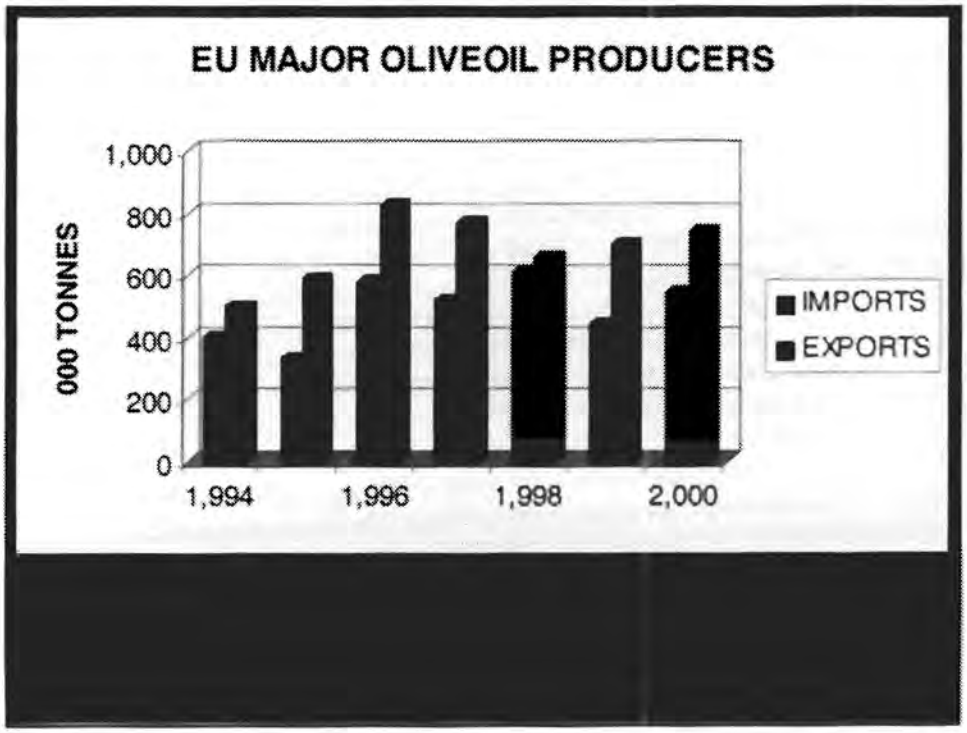

EGYPT TOTAL VEGETABLE OIL EXPORTS
6,000
5,000
4,000
3,000
2,000
1,000
-
1990
1992
1994
1996
1998
Exports - Qty Mt
Exports - Val 1000$

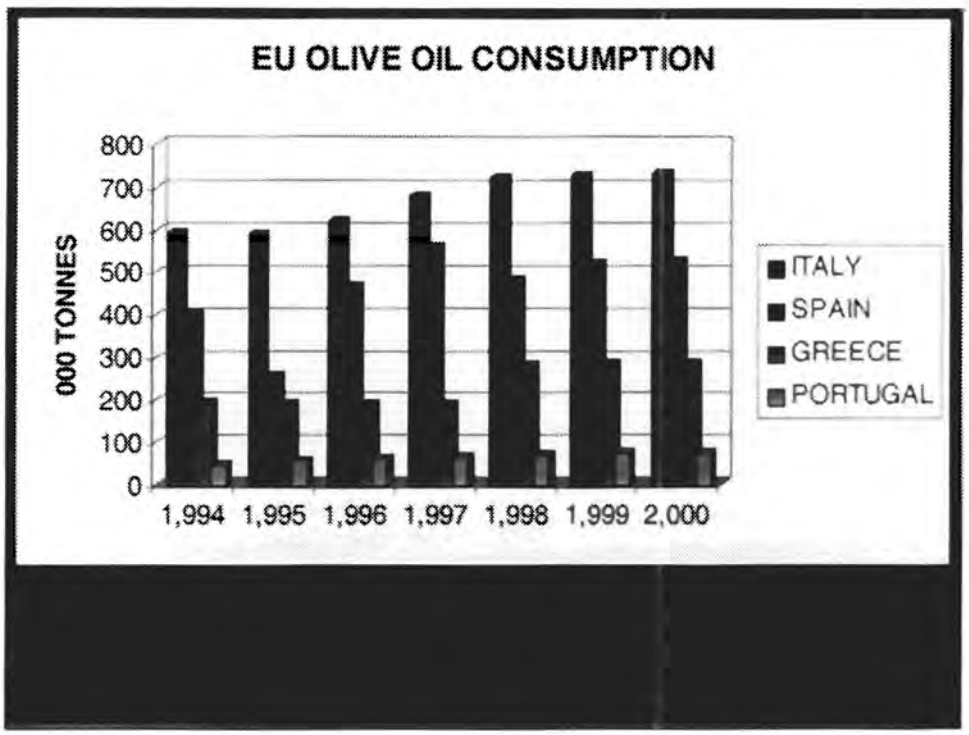

EGYPT TROPICAL OIL IMPORTS
METRIC TONS
600
500
400
300
200
100
0
1990
1992
1994
1996
1998
2000
IMPORTS
CONSUMPTION

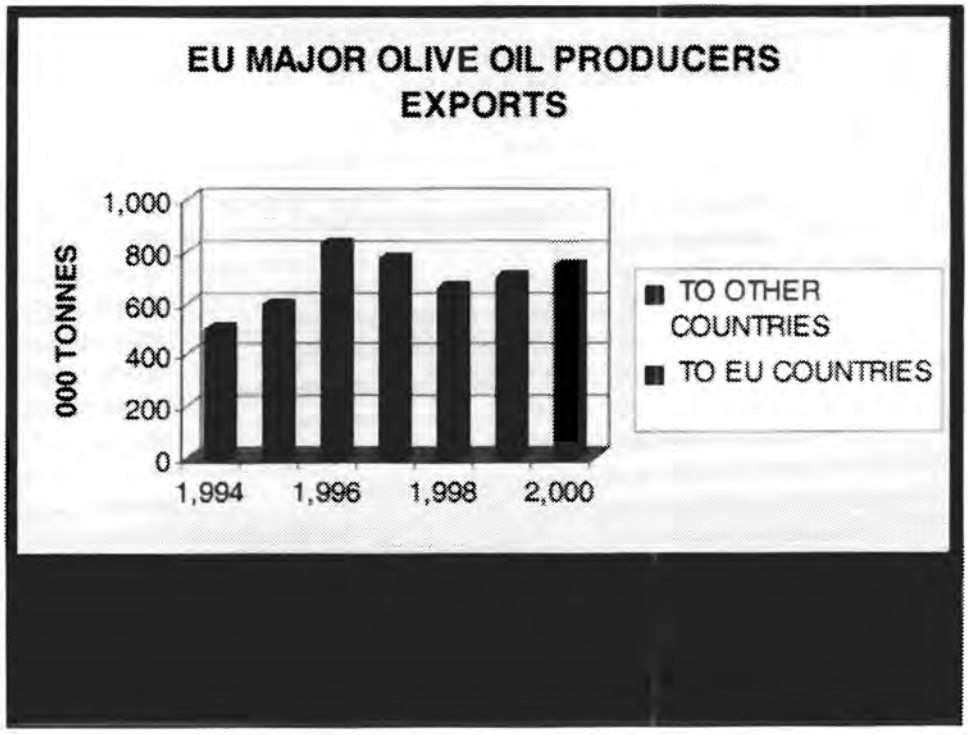

EGYPT TROPICAL OIL USE
METRIC TONS
500
400
300
200
100
0
1990
1992
1994
1996
1998
2000
INDUSTRIAL
FOOD

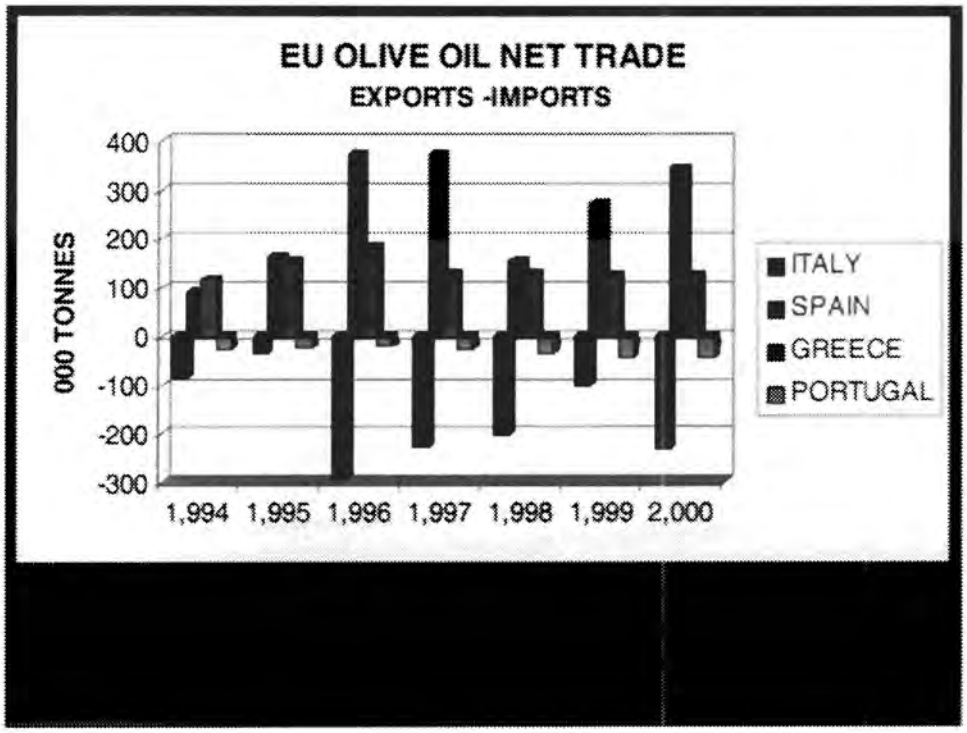

EGYPT TROPICAL OILS
PER CAPITA CONSUMPTION
KILOGRAMS PER PERSON
8.00
6.00
4.00
2.00
-
1990
1992
1994
1996
1998
2000

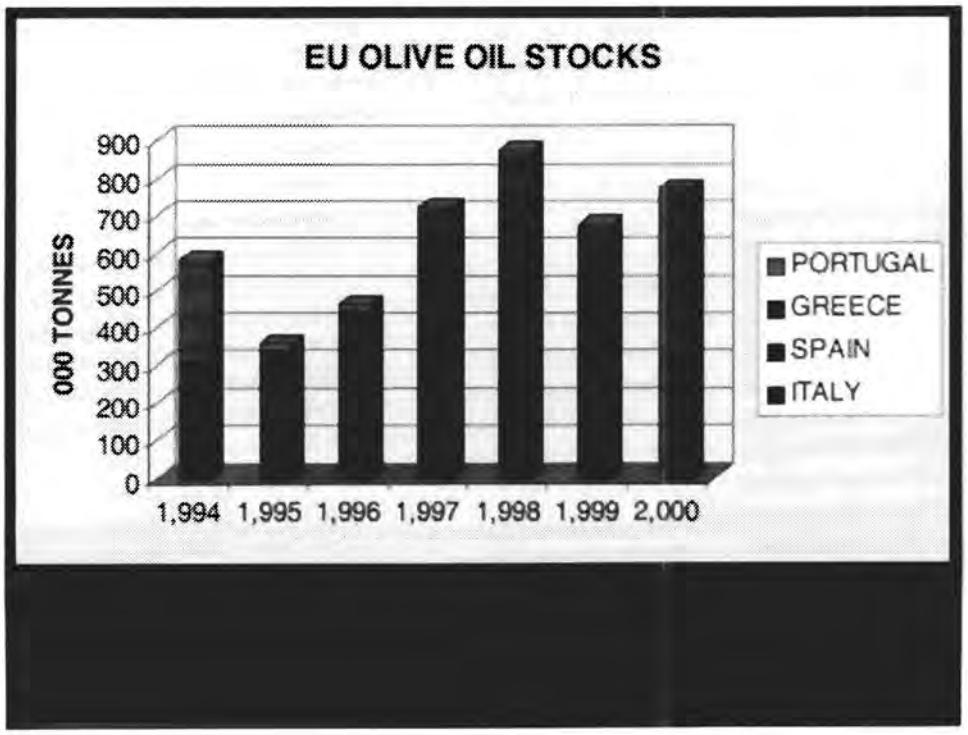

EU OLIVE OIL PRODUCTION
000 TONNES
1,200
1,000
800
600
400
200
0
1,994
1,996
1,998
2,000
ITALY
SPAIN
GREECE
PORTUGAL


EU MAJOR OLIVEOIL PRODUCERS
000 TONNES
1,000
800
600
400
200
0
1,994
1,996
1,998
2,000
IMPORTS
EXPORTS


EU OLIVE OIL CONSUMPTION
000 TONNES
800
700
600
500
400
300
200
100
0
1,994
1,995
1,996
1,997
1,998
1,999
2,000
ITALY
SPAIN
GREECE
PORTUGAL


EU MAJOR OLIVE OIL PRODUCERS EXPORTS
000 TONNES
1,000
800
600
400
200
0
1,994
1,996
1,998
2,000
TO OTHER COUNTRIES
TO EU COUNTRIES


EU OLIVE OIL NET TRADE
EXPORTS -IMPORTS
000 TONNES
400
300
200
100
0
-100
-200
-300
1,994
1,995
1,996
1,997
1,998
1,999
2,000
ITALY
SPAIN
GREECE
PORTUGAL


EU OLIVE OIL STOCKS
000 TONNES
900
800
700
600
500
400
300
200
100
0
1,994
1,995
1,996
1,997
1,998
1,999
2,000
PORTUGAL
GREECE
SPAIN
ITALY

Processing of Edible Oils
Processing, Quality & Safety

By
Monoj K Gupta
MG Edible Oil Consulting, Int'l
9 Lundy's Lane, Richardson, Texas 75080
Telephone; 972-234-1634, Fax: 972-234-5973
E-Mail: monoj.gupta@worldnet.att.net

تصنيع الزيوت القابلة للأكل
التصنيع وضمان الجودة والسلامة

Monoj K Gupta
MG Edible Oil Consulting, Int'l
9 Lundy's Lane, Richardson, Texas 75080
Telephone; 972-234-1634, Fax: 972-234-5973
E-Mail: monoj.gupta@worldnet.att.net

MAJOR SOURCES OF VEGETABLE OILS

- **SEEDS**
  - **SOYBEAN**
  - **EDIBLE RAPE SEED**
  - **SUNFLOWER**
  - **COTTONSEED**
  - **SAFFLOWER**
  - **GROUNDNUT**
  - **CORN**
- **FRUITS**
  - **PALM**
  - **OLIVE**

المصادر الرئيسية للزيوت النباتية

- **البذور**
  - **بذور فول الصويا**
  - **بذور اللفت القابلة للاكل**
  - **بذور عباد الشمس**
  - **بذور القطن**
  - **بذور العصفر**
  - **بذور فول السوداني**
  - **بذور الذرة**
- **الفاكهة**
  - **النخيل**
  - **الزيتون**

TYPICAL OIL APPLICATIONS

- WESTERN HEMISPHERE
  - BAKING
  - INDUSTRIAL FRYING
  - RESTAURANT & FOOD SERVICES
  - COOKING & SALAD DRESSING
  - MAYONNAISE
  - MARGARINE
  - SOUPS
  - INFANT FORMULA
  - COFFEE CREAMER
- ASIA, AFRICA, MIDDLE EAST
  - COOKING
  - INDUSTRIAL FRYING
  - BAKING
  - MAYONNAISE
  - MARGARINE

الاستخدامات الشائعة للزيوت

- العالم الغربي
  - الخبز
  - القلي الصناعي
  - المطاعم وخدمات تقديم الاغذية
  - الطهي وتوابل السلاطات
  - المايونيز
  - السمن الصناعي النباتي
  - الحساء
  - اغذية الاطفال
  - كريمة القهوة
- آسيا وافريقيا والشرق الاوسط
  - الطهي
  - القلي الصناعي
  - الخبز
  - المايونيز
  - السمن النباتي الصناعي

FUNCTIONS OF OILS IN FOOD APPLICATIONS

- **BAKING**
  - PROVIDES TEXTURE & MOISNESS TO THE PRODUCT
  - ACTS AS A VEHICLE TO INCORPORATE EMULSIFIERS
  - PROVIDES DESIRED MOUTH FEEL TO THE PRODUCT
- **TOPICAL APPLICATION AS SPRAY OIL**
  - ENHANCES FLAVOR OF THE PRODUCT
  - ADDS SMOOTHNESS TO PRODUCT TEXTURE
- **FRIED FOODS**
  - PROVIDES FRIED FOOD FLAVOR
  - PROVIDES THE DESIRED TEXTURE TO THE FRIED FOOD

وظيفة الزيوت في الاستخدامات الغذائية

- الخبز:
  - يعطي قواما وطراوة للمنتج
  - يعمل كاداة لادخال المستحلبات
  - يعطي مذاقا جيدا للمنتج
- الاستخدام الموضعي للزيت
  - يقوي من نكهة المنتج
  - يزيد من طراوة قوام المنتج
- الاغذية المقلية
  - يعطي نكهة جيدة للاغذية المقلية
  - يعطي القوام المطلوب للاغذية المقلية

FUNCTIONS OF OILS IN FOOD APPLICATIONS (CONT)

- **INSTANT OR PREPARED DRINKS**
  - ACTS AS A WETTING AGENT
  - ACTS AS A FLAVOR RELEASING AGENT
  - ACTS AS A MOUTH FEEL ENHANCER
- **SALAD DRESSING / SOUPS**
  - ADDS SMOOTHNESS TO THE DRESSING
  - ACTS AS A FLAVOR RELEASER
- **CANDY/ CHOCOLATE**
  - ACTS AS A FLAVOR RELEASING AGENT
  - PROVIDES SMOOTHNESS TO THE PRODUCT
- **INFANT FORMULA**
  - SUPPLIES ESSENTIAL FATTY ACIDS TO THE FORMULA

وظيفة الزيوت في الاستخدامات الغذائية (تابع)

المشروبات السريعة او الجاهزة

يعمل كعامل مرطب

- يعمل كعامل باعث للنكهة
- يساعد على تحسين المذاق

توابل السلاطات/الحساء

- يضيف طراوة للتوابل
- يعمل كباعث للنكهة

الحلوى/الشكولاته

- يعمل كعامل باعث للنكهة
- يعطي طراوة للمنتج

اغذية الاطفال الرضع

- مصدر للاحماض الدهنية الاساسية للاغذية

OTHER USES OF OILS

- **FOOD APPLICATIONS**
  - EMULSIFIERS
  - CANDY COATING
  - PREPARED AND INSTANT DRINKS
  - PREPARED MEALS
  - RELEASING AGENT IN CANDY AND BAKING PROCESS
  - SPRAY ON CEREAL PRODUCTS
- NON-FOOD APPLICATIONS
  - PAINT (DRYING AGENT)
  - LUBRICANT
  - INDUSTRIAL SOAPS
  - FATTY ACIDS
  - FATTY ALCOHOLS
  - TOILET SOAP
  - DETERGENTS
  - NEWSPRINT
  - CROP DUSTING

استخدامات أخرى للزيوت

الاستخدامات الغذائية

- المستحلبات
- تغطية الحلوى
- المشروبات السريعة والجاهزة
- الوجبات الجاهزة
- عامل مانع لالتصاق الحلوى والخبز
- يتم رشه على منتجات الحبوب

الاستخدامات غير الغذائية

- الطلاء (عامل مجفف)
- يستخدم كمادة تشحيم
- الصابون الصناعي
- الاحماض الدهنية
- الكحوليات الدهنية
- صابون التواليت
- المنظفات
- طباعة الصحف
- تعفير المحاصيل

BASIC OIL CHEMISTRY

الكيمياء الأساسية للزيت

WHAT IS AN OIL ?

- **IT CONSISTS OF:**
  - **96 TO 98 PERCENT TRIGLYCERIDES**
  - **2 TO 4 PER CENT NON-TRIGLYCERIDES**

ما هو الزيت؟

يتكون الزيت من:

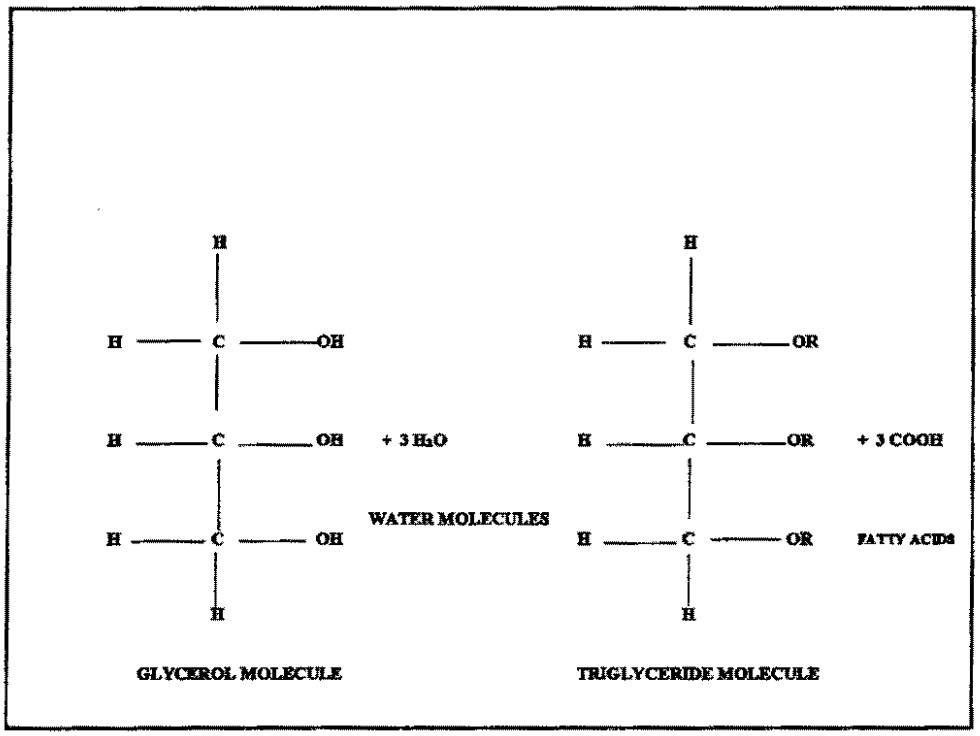
- من ٩٦ الى ٩٨% من المركبات العضوية الدهنية (الدهون الثلاثية)
- من ٢ إلى ٤% من الدهون غير الثلاثية

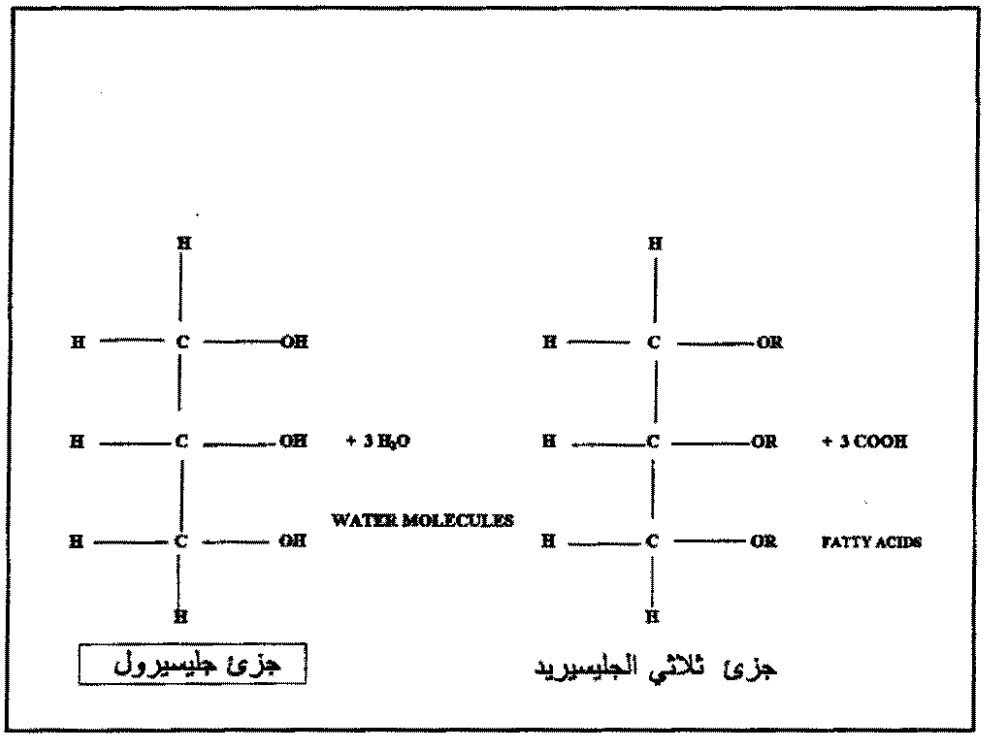
WHAT IS A TRIGLYCERIDE?

- IT IS A COMPOUND MADE OF :
  - GLYCERINE MOLECULE
  - THREE FATTY ACIDS
- GLYCERINE IS AN ALCOHOL
- REACTION PRODUCTS BETWEEN AN ALCOHOL AND AN ORGANIC ACID IS CALLED AN ESTER
- THEREFORE, TRIGLYCERIDES ARE ESTERS (TRIESTERS)

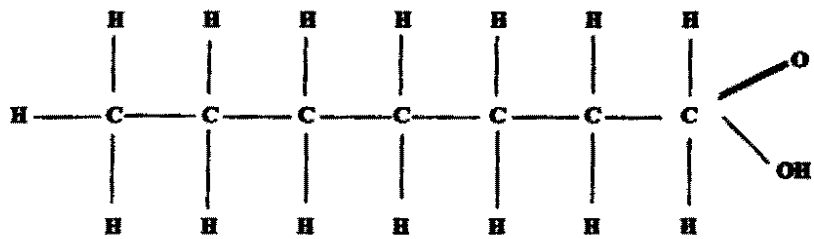
ما هو ثلاثي الجليسيريد؟

- هو مركب يتكون من:
  - جزىء جليسرين
  - أحماض ثلاثية الجليسيريد
- الجليسرين هو نوع من الكحول
- تسمى منتجات التفاعل بين الكحول والحامض العضوي بالاستر
- لذلك، تكون المركبات العضوية الدهنية عبارة عن استرات (استرات ثلاثية)


H
H — C — OH
H — C — OH
H — C — OH
H
+ 3 H₂O
WATER MOLECULES
GLYCEROL MOLECULE
H
H — C — OR
H — C — OR
H — C — OR
H
+ 3 COOH
FATTY ACIDS
TRIGLYCERIDE MOLECULE

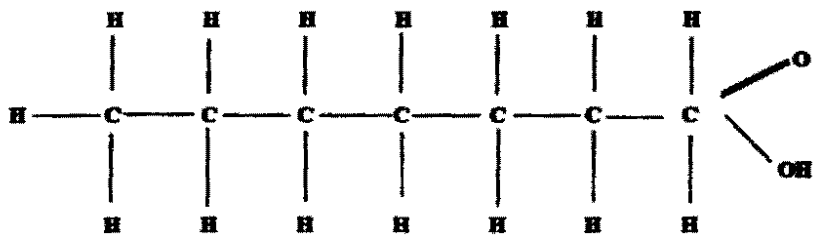

H
H — C — OH
H — C — OH
H — C — OH
H
+ 3 H₂O
WATER MOLECULES
جزئ جليسيرول
H
H — C — OR
H — C — OR
H — C — OR
H
+ 3 COOH
FATTY ACIDS
جزئ ثلاثي الجليسيريد

FATTY ACID MOLECULE



Fatty Acid Molecule (In Vegetable oils Typically Contain 12-22 Carbon Atoms)

جزئ الحامض الدهني



جزئ حامض دهني (يحتوي في الزيوت النباتية على ١٢-٢٢ ذرة كربون)

TYPES OF FATTY ACIDS

- SATURATED
- UNSATURATED

أنواع الاحماض الدهنية

مشبعة

غير مشبعة

SINGLE & DOUBLE BONDS

- -C-C-C-C- Saturated
- -C-C=C-C- Unsaturated

الروابط الفردية والمزدوجة

-C-C-C-C- مشبعة

-C-C=C-C- غير مشبعة

MOST COMMON FATTY ACIDS IN VEGETABLE OILS

- **PALMITIC ACID**
  - SATURATED FATTY ACID WITH 16 CARBON ATOMS
- **STEARIC ACID**
  - SATURATED FATTY ACID WITH 18 CARBON ATOMS
- **OLEIC ACID**
  - MONOUNSATURATED FATTY ACID WITH ONE DOUBLE BOND
- **LINOLEIC ACID**
  - POLYUNSATURATED FATTY ACID WITH TWO DOUBLE BONDS
- **LINOLENIC ACID**
  - POLYUNSATURATED FATTY ACID WITH THREE DOUBLE BONDS

الاحماض الدهنية الشائعة في الزيوت النباتية

حامض البلمتيك

– حامض دهني مشبع مع ١٦ ذرة كربون

حامض الاستايريك

– حامض دهني مشبع مع ١٨ ذرة كربون

حامض الاوليك

– حامض دهني أحادي غير مشبع مع رابطة واحدة مزدوجة

حامض اللينوليك

– حامض دهني متعدد غير مشبع مع رابطتين مزدوجتين

حامض اللينولينيك

– حامض دهني متعدد غير مشبع مع ثلاث روابط مزدوجة

NON-TRIGLYCERIDES

- IMPORTANT FROM THE STANDPOINT OF OIL QUALITY
- MOST OF THESE COMPONENTS ARE PRESENT IN MINUTE AMOUNTS(IN PARTS PER MILLION), BUT CAN GREATLY IMPACT OIL QUALITY & STABILITY
- SOME OF THESE NON-TRIGLYCERIDES ARE DESIRABLE TO MAINTAIN GOOD OIL STABILITY, WHILE THE OTHERS ARE DETREMENTAL TO OIL QUALITY, AND THEREFORE, MUST BE REMOVED FROM THE OIL THROUGH REFINING, BLEACHING AND DEODORIZATION

الدهون غير الثلاثية

- هامة بالنسبة لجودة الزيوت
- تتواجد معظم تلك المركبات بكميات صغيرة جدا (اجزاء في المليون) لكنها من الممكن ان تؤثر كثيرا على جودة الزيوت وثباتها
- يكون بعضها مطلوبا للحفاظ على الثبات الجيد للزيوت، في حين يكون البعض الاخر منها ضار بجودة الزيوت وعليه، ينبغي ان يتم ازالتها من الزيوت عن طريق التكرير والتبييض وازالة الروائح الكريهة

EXAMPLES OF NON-TRIGLYCERIDES

- MAJOR COMPONENTS
  - PHOSPHOLIPIDS (GUMS)
  - FREE FATTY ACIDS
  - DIGLYCERIDES
  - MONOGLYCERIDES
- MINOR COMPONENTS
  - TOCOPHEROLS
  - STEROLS & STEROL ESTERS
  - VOLATILE MATTER
  - COLOR COMPOUNDS
  - PHYTO CHEMICALS

أمثلة للدهون غير الثلاثية

المكونات الكبرى

- الفوسفوليبيدات (الصمغ)
- الاحماض الدهنية الحرة
- الجليسيريدات الثنائية
- الجليسيريدات الاحادية

المكونات الصغرى

- توكوفيرولات
- ستيرولات واسترات الستيرول
- مواد متطايرة
- المركبات الملونة
- كيماويات نباتية

MAJOR NON-TRIGLYCERIDES

- **PHOSPHOLIPIDS**
  - THESE ARE NATURALLY OCCURING COMPOUNDS, COMMONLY REFERRED TO AS "GUMS"
- **FREE FATTY ACIDS**
  - THESE ARE FATTY ACIDS FORMED IN THE OIL IN THE SEEDS, DUE TO ENZYMATIC HYDROLYSIS
- **MONO & DIGLYCERIDES**
  - THESE ARE FORMED WHEN THE FREE FATTY ACIDS ARE FORMED IN THE OIL IN THE SEEDS, DUE TO ENZYMATIC HYDROLYSIS

الدهون غير الثلاثية الكبرى

- **الفوسفوليبيدات**
  - هي عبارة عن مركبات حادثة بالطبيعة ويشار اليها عادة بالصمغ
- **الاحماض الدهنية الحرة**
  - هـي عبارة عن أحماض دهنية تتكون داخل الزيت في البذور بسبب التحلل المائي الانزيمي
- **الجليسيريدات الاحادية والثنائية**
  - تـتكون عندما تتكون الاحماض الدهنية الحرة في الزيت داخل البذور بسبب التحلل المائي الانزيمي

TYPICAL CONCENTRATION OF MAJOR NON-TRIGLYCERIDES

- PHOSPHOLIPIDS
  - TYPICALLY REFERRED TO AS PHOSPHORUS. IT'S CONCENTRATION VARIES WITH THE TYPE OF OIL
- FREE FATTY ACID
  - 0.5 - 1 %IN MOST SEED OILS & OLIVE OIL
  - 2 - 5 % IN CRUDE PALM OIL (CPO)
- MONOGLYCERIDES
  - < 0.2 - 0.3 % IN MOST SEED OILS & OLIVE OIL
  - 1-3 % IN CRUDE PALM OIL (CPO)
- DIGLYCERIDES
  - < 0.4 %IN MOST SEED OILS, OLIVE OIL (1-3 %)
  - 3-7 % IN CRUDE PALM OIL (CPO)

التركيز الامثل للدهون غير الثلاثية الكبرى

- الفوسفوليبيتيدات
  - يشار اليها بالفوسفور. ويتنوع تركيزها طبقا لنوع الزيت
- الحامض الدهني الحر
  - ٠,٥ – ١% في معظم زيوت البذور وزيت الزيتون
  - ٢ –٥ في زيت النخيل الخام
- الجليسيريدات الاحادية
  - <٠,٢ – ٠,٣% في معظم زيوت البذور وزيت الزيتون
  - ١ –٣ في زيت النخيل الخام
- الجليسيريدات الثنائية
  - <٠,٤ –في معظم زيوت البذور وزيت الزيتون (١ – ٣%)
  - ٣ – ٧ في زيت النخيل الخام

TYPICAL MINOR NON-TRIGLYCERIDES

- TOCOPHEROLS
  - THESE ARE NATURAL ANTIOXIDANTS PRESENT IN THE OIL
- STEROLS & STEROL ESTERS
  - THESE ARE ALSO NATURAL ANTIOXIDANTS PRESENT IN THE OIL BUT NOT COMMONLY DISCUSSED AS MUCH AS THE THE TOCOPHEROLS
- COLOR COMPOUNDS
  - CHLOROPHYLL'S, CAROTENES AND OTHER COLOR COMPOUNDS
- TRACE METALS
  - IRON, CALCIUM, MAGNESIUM, COPPER, SOME HEAVY METALS

الدهون غير الثلاثية الصغرى

- توكوفيرولات
  - عبارة عن مضادات اكسدة طبيعية موجودة في الزيت
- ستيرولات واسترات الستيرول
  - أيضـا عبارة عن مضادات اكسدة طبيعية موجودة في الزيت لكن لا يتم التعرض لها بنفس قدر التوكوفيرولات
- المركبات الملونة
  - الكلوروفيل والكاروتين ومركبات الالوان الاخرى
- نسب قليلة من المعادن
  - حديد، كالسيوم، ماجنيسيوم، نحاس، وبعض المعادن الثقيلة

DECOMPOSITION OF OILS

تحلل الزيوت

WHERE DOES THE OIL START DECOMPOSING ?

- OILS START TO DECOMPOSE SOON AFTER THE SEEDS ARE HARVESTED AND STORED.
- MORE DECOMPOSITION OCCURS AFTER THE SEEDS ARE CRACKED FOR OIL EXTRACTION
- FOR FRUIT PALM AND OLIVES, THE OIL STARTS TO DECOMPOSE IMMEDIATELY AFTER HARVEST

أين يبدأ الزيت في التحلل؟

- تبدأ الزيوت في التحلل مباشرة بعدما يتم حصاد البذور وتخزينها
- ويزيد التحلل بعد طحن البذور لاستخلاص الزيت
- بالنسبة لزيوت النخيل والزيتون، يبدأ الزيت في التحلل مباشرة بعد الحصاد

SEED QUALITY IS AFFECTED BY

- COOLER OR WARMER THAN NORMAL TEMPERATURE DURING GROWING SEASON
- INSUFFICIENT RAIN OR IRRIGATION
- DROUGHT
- EXCESSIVE RAIN BEFORE HARVEST
- INSECT OR MOLD DAMAGE IN THE FIELD
- STORAGE OF MOIST SEEDS IN SILO'S (MOISTURE CONTENT > 12 %)
- TEMPERATURE IN SILO'S IS NOT MAINTAINED AT < 45°C DURING STORAGE
- STORAGE AT ELEVATED TEMPERATURE AND MOISTURE LEVEL PROMOTES FORMATION OF NON-HYDRATABLE PHOSPHOLIPIDS. THIS INCREASES REFINING DIFFICULTY

تتأثر جودة البذور بما يلي:

- تكون ابرد او ادفأ من درجة الحرارة العادية اثناء فترات الانبات
- الامطار او الري الغير كافي
- الجفاف
- زيادة مطول الامطار قبل الحصاد
- التلف الذي تسببه الحشرات او الفطريات في الحقول
- تخزين البذور الرطبة في مخزن به محتوى رطوبة يبلغ >١٢%
- عدم الاحتفاظ بدرجة حرارة المخزن عند <٤٥°م اثناء التخزين
- التخزين عند درجات حرارة مرتفعة ومستوى معين من الرطوبة مما يساعد على تكون فوسفولبيتيدات غير قابلة للهدرجة. مما يزيد من صعوبة التكرير

DECOMPOSITION OF OIL NEVER STOPS !

- THE OIL CONTINUES TO DECOMPOSE DURING:
- THE REFINING PROCESS
- STORAGE, BEFORE, DURING AND AFTER REFINING
- LOADING & TRANSPORTATION
- UNLOADING & STORAGE AT THE DESTINATION
- DURING FOOD PROCESSING, PACKAGING, WAREHOUSING & DISTRIBUTION
- ON RETAIL STORE SHELF
- AT CONSUMERS' HOMES, UNTIL THE FOOD IS CONSUMED

لا تتوقف عملية تحلل الزيت مطلقا!

- يستمر الزيت في التحلل أثناء:
- عملية التصفية
- عملية التخزين قبل او اثناء او بعد التكرير
- التحميل والنقل
- التفريغ والتخزين في المكان المحدد
- أثناء تصنيع الاغذية وتعبئتها وتخزينها وتوزيعها
- اثناء التخزين في محال البيع بالتجزئة
- في منازل المستهلكين حتى يتم استهلاكه

MODES OF OIL DECOMPOSITION

- OXIDATION
  - AUTOXIDATION
  - ENZYMATIC OXIDATION
  - PHOTOOXIDATION
- HYDROLYSIS
  - ENZYMATIC
  - METAL CATALYZED
- THERMAL DECOMPOSITION

أنماط تحلل الزيت

- الاكسدة
  - الاكسدة التلقائية
  - الاكسدة الانزيمية
  - الاكسدة الضوئية
- التحلل المائي)
  - الانزيمي
  - محفز بالمعادن
- التحلل الحراري

ENIMIES OF OIL

- AIR (OXYGEN)
- WATER
- HEAT
- LIGHT
- ENZYMES
- CHEMICALS
- TIME

أعداء الزيت

- الهواء (الاكسجين)
- الماء
- الحرارة
- الضوء
- الانزيمات
- المواد الكيماوية
- الوقت

AUTOXIDATION

- THIS IS ONE OF THE PRIMARY, IF NOT "THE PRIMARY" SOURCE OF OIL DECOMPOSITION
- DEVELOPMENT OF AN "OFF-FLAVOR" OBSERVED IN THE OIL AS WELL AS IN THE FOOD PRODUCT CONTAINING THE OIL IS DUE TO OXIDATION OF THE TRIGLYCERIDES AND THE FREE FATTY ACIDS

الأكسدة التلقائية

- الاكسدة التلقائية هي احدى المصادر، ان لم تكن "المصدر الرئيسي"، لتحلل الزيت
- يكون سبب تغير الرائحة الملاحظ في الزيت وكذلك في المنتج الغذائي الذي يحتوى على زيت هو تأكسد الدهون الثلاثية والاحماض الدهنية الحرة

CONDITIONS LEADING TO AUTOXIDATION

- AUTOXIDATION REQUIRES:
- AN INITIATOR, TYPICALLY A METAL ION, SUCH AS IRON, NICKEL, COPPER, ETC.
- FORMATION OF FREE RADICALS FROM THE UNSATURATED FATTY ACIDS
- PRESENCE OF OXYGEN
- HIGHER TEMPERATURE ACCELERATES THE REACTION

الظروف المؤدية للأكسدة

- تتطلب عملية الأكسدة التلقائية:
- عـامل بدء، يفضا ان يكون أيون معدني مثل الحديد والنيكل والنحاس وما الى ذلك.
- تكون شقوق حرة من الاحماض الدهنية غير المشبعة
- وجود الاكسجين
- تعجل درجة الحرارة العالية من التفاعل

STEPS IN AUTOXIDATION

- UNSATURATED FATTY ACID (RH) + INITIATOR——————— -R* (FREE RADICAL)
- $R^* + O_2$ ————ROO* (FREE RADICAL)
- ROO* + RH ———ROOH (HYDROPEROXIDE) + R*
- R* + R* ————RR (DIMER OR POLYMER)

خطوات الاكسدة

حامض دهني غير مشبع (RH) + عامل بدء ——————— R* (شق حر)

- $R^* + O_2$ ————ROO* (شق حر)
- ROO* + RH ———ROOH (هيدروبيروكسيد) + R*
- R* + R* ————RR (دايمر او بوليمر)

ENZYMATIC OXIDATION

- LIPOXYGENASE, A TYPE OF ENZYME IS PRESENT IN MOST OIL SEEDS AND OIL BEARING FRUITS
- LIPOXYGENASE PROMOTES OXIDATION IN THE OIL JUST LIKE THE CHEMICAL OXIDATION PROCESS
- THESE ENZYMES ARE MORE ACTIVE WHEN THE TEMPERATURE IS 45°C TO 60°C
- THE REACTION REQUIRES THE PRESENCE OF OXYGEN

الاكسدة الانزيمية

- ليبوكسيجينيز، هو نوع من الانزيمات الموجدة في معظم البذور الزيتية او الفاكهة المحتوية على زيت
- ويحفز الليبوكسيجينيز الاكسدة في الزيت مثلما يحدث مع عملية الاكسدة الكيميائية
- تزيد فعالية تلك الانزيمات عندما تكون درجة الحرارة من ٤٥ °م الى ٦٠ °م
- يتطلب التفاعل وجود الاكسجين

PHOTOOXIDATION

- PHOTOOXIDATION TAKES PLACE ON UNSATURATED FATTY ACIDS, IN PRESENCE OF VISIBLE OR ULTRAVIOLET LIGHT
- PHOTOOXIDATION REACTIONS ARE OF TWO TYPES:
- PHOTOCHEMICAL
- PHOTOSENSITIZED

الاكسدة الضوئية

- تحدث الاكسدة الضوئية على الاحماض الدهنية الغير مشبعة في وجود ضوء مرئي او الاشعة فوق البنفسجية وتكون تفاعلات الاكسدة الضوئية تكون من نوعين:
- كيميائية ضوئية
- بالتمثيل الضوئي

TYPES OF PHOTOOXIDATION

- PHOTOCHEMICAL REACTION IS INITIATED BY THE FORMATION OF A FREE RADICAL IN PRESENCE OF ULTRAVIOLET LIGHT. THE REACTION PROCEDES AT THE SAME RATES AS AUTOXIDATION.

- PHOTOSENSITIZED REACTION TAKES PLACE IN PRESENCE OF THE VISIBLE LIGHT. A PHOTOSENSITIZER, LIKE CHLOROPHYLLS AND THEIR DECOMPOSITION PRODUCTS INITIATE THE REACTION. CHLOROPHYLLS ARE PRESENT IN CERTAIN TYPES OF OIL OR IN THE FOOD PRODUCT PREPARED WITH THE OIL. THIS REACTION PROCEDES 1500 TIMES FASTER THAN AUTOXIDATION OR PHOTOCHEMICAL REACTION

انواع الاكسدة الضوئية

- يبدأ التفاعل الكيميائي الضوئي بواسطة تكون شق حر في وجود الاشعة الفوق بنفسجية. ويستمر التفاعل بنفس معدلات الاكسدة التلقائية

- يحدث التفاعل بالتمثيل الضوئي في وجود ضوء مرئي. وتقوم الكلوروفيلات ومنتجات التحلل ببدء التفاعل. وتتواجد الكلوروفيلات في بعض انواع من الزيوت او في المنتجات الغذائية الذي تم تصنيعها بالزيت. ويسير هذا التفاعل بشكل اسرع بـ ١٥٠٠ مرة من عملية الاكسدة التلقائية او التفاعل الكيميائي الضوئي.

AUTOXIDATION VERSUS PHOTOOXIDATION

- AUTOXIDATION AND PHOTOCHEMICAL REACTIONS PROCEDE AT THE SAME RATE
- PHOTOSENSITIZED REACTION IS 1500 TIMES FASTER THAN AUTOXIDATION
- IN AUTOXIDATION, THE DOUBLE BONDS IN LINOLEIC ACID AND LINOLENIC ACID BECOME CONJUGATED BEFORE THE REACTION
- PHOTOOXIDATION IS NOT PRECEDED BY CONJUGATION
- THE TWO REACTION MECHANISMS PRODUCE DISTINCTLY DIFFRENT REACTION PRODUCTS. THEREFORE, THE MECHANISM OF OXIDATION CAN BE DETERMINED FROM THE COMPOSITIONS OF THE REACTION PRODUCTS.

الاكسدة التلقائية مقابل الاكسدة الضوئية

- تسير الاكسدة التلقائية والكيميائية الضوئية بنفس المعدل
- يكون التفاعل المعالج بالتمثيل الضوئي اسرع بـ ١٥٠٠ مرة من الاكسدة التلقائية
- في عملية الاكسدة التلقائية، تقترن الروابط المزدوجة في حامض اللينوليك وحامض اللينولينيك قبل التفاعل
- لا يسبق التقارن الاكسدة الضوئية
- ينتج عن اليتي التفاعل نواتج تفاعل مختلفة ل على حدة. ولذلك، يمكن تحديد الية الاكسدة من مكونات نواتج التفاعل.

PREVENTION OF AUTOXIDATION

– **AUTOXIDATION CAN BE RETARDED BY:**

- MINIMIZING EXPOSURE TO OXYGEN
- REFINING THE OILS TO VERY LOW TRACE METAL CONTENTS
- MAINTAINING LOW TEMPERATURE OF STORAGE
- USING NATURAL OR SYNTHETIC ANTIOXIDANTS
- BY REDUCING UNSATURATION VIA HYDROGENATION

– AS MENTIONED ABOVE, AUTOXIDATION CAN BE MINIMIZED BUT CANNOT BE STOPPED

منع التفاعل التلقائي

– **يمكن تأخير التفاعل التلقائي عن طريق:**

- الاقلال من التعرض للاكسجين
- تكرير الزيوت بحيث تحتوي على اقل قدر من المعادن
- الاحتفاظ بدرجة الحرارة منخفضة وكذلك درجة حرارة التخزين
- استخدام مضادات الاكسدة الطبيعية او الاصطناعية
- عن كريق اختزال عدم التشبع بواسطة الهدرجة

– وكما هو مذكور انفا، يمكن الإقلال من الاكسدة التلقائية لكن لا يمكن منعها على الاطلاق

PREVENTION OF PHOTOOXIDATION

- LIKE AUTOXIDATION, PHOTOOXIDATION CANNOT BE STOPPED COMPLETELY. IT CAN BE MINIMIZED BY:
- ELIMINATING THE SOURCE OF LIGHT BY PACKAGING THE PRODUCT IN METAL CONTAINERS OR METALLIZED FILMS
- MINIMIZING THE PHOTOSENSITIZERS, PRESENT IN THE OIL OR IN THE FOOD PRODUCT
- PHOTOOXIDATION CANNOT BE RETARDED BY USING ANTIOXIDANTS. IN SOME CASES, THE ANTIOXIDANTS BECOME PRO-OXIDANT
- CERTAIN COMPOUNDS, CALLED QUENCHERS CAN RETARD PHOTOOXIDATION. THIS HAS LIMITED VALUE BECAUSE MOST QUENCHERS IMPART UNDESIRABLE FLAVOR TO THE PRODUCT

منع الاكسدة الضوئية

- كما هو الحال مع الاكسدة التلقائية، فانه لا يمكن ايقاف الاكسدة الضوئية تماما، لكن يمكن الاقلال منها على النحو التالي:
- التخلص من مصدر الضوء بواسطة تعبئة المنتج في حاويات معدنية او رقائق معدنية
- الاقلال من عوامل التمثيل الضوئي الموجودة في الزيت او في المنتج الغذائي
- يصعب تأخير الاكسدة الضوئية عن طريق مضادات الاكسدة. في بعض الحالات تصبح مضادات الاكسدة عوامل مساعدة للاكسدة
- هناك بعض المركبات التي تعرف باسم عوامل الكبت التي تقوم بتأخير عملية الاكسدة الضوئية. ويكون لتلك المركبات قيمة محدودة نظرا لان معظمها يعطي نكهة غير مطلوبة للمنتج

WHERE DOES OXIDATION OCCUR ?

- IT STARTS FROM THE MOMENT THE SEEDS ARE HARVESTED AND STORED. DECOMPOSITION INCREASES AFTER THE SEEDS ARE CRACKED FOR OIL EXTRACTION
- FREE RADICALS ARE FORMED WHENEVER THE UNSATURATED FATTY ACIDS ARE EXPOSED TO TRACE METALS, OXYGEN, LIGHT, AND HEAT
- OXIDATION OCCURS DURING OIL PROCESSING, OIL STORAGE, TRANSPORTATION, UNLOADING & STORAGE AT THE POINT OF USE, FOOD PROCESSING, PACKAGED PRODUCT, IN STORAGE WHETHER FROZEN OR NOT
- THEREFORE, IT IS IMPORTANT TO MINIMIZE THE FORMATION OF THE FREE RADICALS AT EVERY STEP OF OIL HANDLING AND ITS USAGE

أين تحدث الاكسدة

- تبدأ عملية الاكسدة فورما يتم حصاد البذور وتخزينها، ويزيد التحلل بعد عصر البذور لاستخلاص الزيت
- تتكون الشقوق الحرة كلما تعرضت الاحماض الدهنية الغير مشبعة لقدر قليل جدا من المعادن والاكسجين والضوء والحرارة
- تحدث الاكسدة اثناء تصنيع الزيت وتخزينه ونقله وتفريغه وتخزينه وعند استخدامه وعند تصنيع الاغذية وفي المنتج المعبأ وعند التخزين سواء كان بالتجميد ام لا
- لذلك، يكون من المهم الاقلال من تكون الشقوق الحرة في كل مرحلة من مراحل مناولة الزيت واستخدامه

HYDROLYSIS

- HYDROLYSIS OCCURS WHEN A MOLECULE OF WATER REACTS WITH THE TRIGLYCERIDE MOLECULE AND RELEASES A MOLECULE OF FREE FATTY ACID

- FATTY ACID + HOH = DIGLYCERIDE + HCOOH

- DIGLYCERIDE + HOH = MONOGLYCERIDE + HCOOH

التحلل المائي

- يحدث التحلل المائي عندما يتفاعل جزئ من الماء مع جزئ ثلاثي الجليسيريد ويطلق جزئ من حامض دهني حر

- FATTY ACID + HOH = DIGLYCERIDE + HCOOH
- DIGLYCERIDE + HOH = MONOGLYCERIDE + HCOOH

MECHANISM OF HYDROLYSIS (CHEMICAL)

- OIL AND WATER MUST BE IN PERFECT SOLUTION FOR HYDROLYSIS TO OCCUR IN THE OIL
- THIS REQUIRES A SURFACTANT (EMULSIFIER) OR VERY HIGH TEMPERATURE
- EMULSIFIER CAN COME FROM:
- THE OIL – PHOSPHOLIPIDS, MONO & DIGLYCERIDES
- THE FOOD – BATTER COATINGS CONTAINING MONO & DICALCIUM PHOSPHATES
- SOME OF THE OIL BREAKDOWN PRODUCTS THAT ARE FORMED, ESPECIALLY DURING FRYING ARE SURFACE ACTIVE BY NATURE

آلية التحلل المائي (كيميائية)

يجب ان يكون الزيت والماء في محلول جيد بحيث تتم عملية التحلل المائي في الزيت

- يتطلب هذا الامر وجود عامل توتر سطحي (مستحلب) او درجة حرارة عالية جدا
- يمكن ان ياتي المستحلب مما يلي:
- الزيت: فوسفوليبيدات، ومركبات دهنية احادية وثنائية
- الاغذية: تغطيات العجائن تحتوي على فوسفاتات كالسيوم احادية وثنائية
- بعض منتجات الزيت التي قد تكونت وبخاصة أثناء القلي تكون نشطة على السطح بطبيعتها

SPECIAL REQUIREMENTS FOR FRYING OIL

- HYDROLYSIS OF OIL IS AN ISSUE IN THE FRYING PROCESS
- INCREASE OF FREE FATTY ACID IS NORMAL IN THE FRYER OIL DURING FRYING PROCESS, BATTER COATING MAY CAUSE A MORE RAPID RISE IN FREE FATTY ACIDS IN THE FRYER OIL
- THEREFORE, FRYING OIL NEEDS A TIGHT SPECIFICATION FOR A PHOSPHORUS CONTENT IN THE FRESH OIL (< 1.0 PPM) AND IRON,CALCIUM OR MAGNESIUM CONTENTOF < 0.5 PPM, EACH

متطلبات خاصة لزيت القلي

- إن تحلل الزيت امر هام بالنسبة لعملية القلي
- تكـون زيادة الاحماض الدهنية الحرة طبيعية في زيت القلي اثـناء عملية القلي، وقد تتسبب تغطية العجين بزيادة سريعة في الاحماض الدهنية الحرة في زيت القلي.
- وعـليه، فـان زيت القلي يتطلب مواصفات صارمة بالنسبة لمسـتوى الفوسـفور فـي الزيت الطازج (<١,٠ جزء في المـليون) ومحتوى الحديد و الكالسيوم او الماغنيسيوم <٠,٥ جزء في المليون، لكل منها

ENZYMATIC HYDROLYSIS

- LIPASE IS AN ENZYME PRESENT IN ALL OIL SEEDS AND OIL BEARING FRUITS
- THESE ENZYMES ARE ACTIVATED AT TEMPERATURE OF 45°C TO 60°C
- IN PRESENCE OF HIGH HUMIDITY, LIPASE CLEAVES THE -COOH GROUP AT THE END OF TRIGLYCERIDE MOLECULE, LIKE IN THE CHEMICAL HYDROLYSIS PROCESS

التحلل المائي الانزيمي

- الليباز هو انزيم موجود في كافة بذور الزيت وكافة الفاكهة المحتوية على زيت
- يتم تنشيط كافة تلك الانزيمات عند درجة حرارة تبلغ ٤٥°م الى ٦٠°م
- في وجود الرطوبة العالية، يقوم الليباز بتقسيم مجموعة - COOH عند نهاية جزئ ثلاثي الجليسيريد كما هو الحال مع عملية التحلل الكيميائي

CONDITIONS CONDUCIVE TO LIPASE ACTIVITY

- HIGH TEMPERATURE (> 45°C AND < 60°C)
- HIGH HUMIDITY

الظروف المساعدة على زيادة فعالية الليباز

- درجة الحرارة العالية (>٤٥°م و <٦٠ °م)
- الرطوبة المرتفعة

THERMAL DECOMPOSITION

- OIL STARTS FORMING FREE RADICALS AS SOON AS IT IS HEATED TO HIGH TEMPERATURE
- THESE FREE RADICALS LEAD TO THE FORMATION OF THERMAL POLYMERS

التحلل الحراري

- يبدأ الزيت في تكوين الشقوق الحرة مباشرة بعدما يتم تسخينة عند درجة حرارة عالية
- تؤدي تلك الشقوق الحرة الي تكون البوليمرات الحرارية

TIME FACTOR

- TIME OF STORAGE FOR SEEDS, OIL BEARING FRUITS AS WELL AS THE OILS, CAN BE A MAJOR FACTOR IN DETERMINING THE QUALITY OF THE OIL PRODUCED

العامل الزمني

- يمكـــن ان يكـــون زمن تخزين البذور والفاكهة المحتوية على زيوت وكذلك الزيوت عاملا هاما لتحديد جودة الزيت المنتج

IN SUMMARY

- QUALITY OF OIL STARTS WITH:
  - QUALITY OF SEEDS OR OIL BEARING FRUITS
  - PROPER EXTRACTION
  - PROPER HANDLING OF CRUDE OIL
  - PROPER REFINING, BLEACHING, HYDROGENATION, DEODORIZATION, STORAGE & DISTRIBUTION

وباختصار

تبدأ جودة الزيت بما يلي:

- جودة البذور او الفاكهة المحتوية على زيوت
- الاستخلاص الجيد للزيوت
- المناولة الجيدة للزيت الخام
- عمليات التكرير والتبييض والهدرجة والتخزين والتوزيع

PROTECTION OF OIL

حماية الزيت

WHY PROTECT OIL ?

- TO PRESERVE OIL (OR FAT) FLAVOR
- TO PRESERVE FLAVOR OF THE PRODUCT FORMULATED WITH THE OIL
- TO MAINTAIN SHELF-LIFE OF THE OIL
- TO IMPROVE PRODUCT SHELF-LIFE
- TO MAINTAIN PRODUCT CODE-DATE
- TO IMPROVE PRODUCT CODE DATE

لماذا يتم حماية الزيت

- للاحتفاظ بنكهة الزيت (او الدهون)
- للاحتفاظ بنكهة المنتج الذي تم ادخال الزيت في تصنيعه
- لزيادة فترة بقاء الزيت
- لتحسين فترة بقاء المنتج
- للاحتفاظ بتاريخ تكويد لوتات المنتج
- لتحسين تاريخ تكويد المنتج

HOW ARE THE OILS PROTECTED?

- **OILS ARE PROTECTED THROUGH:**
  - PREVENTION OF OXYGEN EXPOSURE
  - UNDUE EXPOSURE TO HEAT
  - FOLLOWING PROPER PROCEDURES DURING HANDLING OF CRUDE OIL, REFINING, BLEACHING, HYDROGENATION, DEODORIZATION, PRODUCT STORAGE AND DISTRIBUTIN

كيف يتم حماية الزيوت

- يتم حماية الزيوت عن طريق ما يلي:
  - منع التعرض للاكسجين
  - منع التعرض الغير ضروري للحرارة
  - اتباع الخطوات اللازمة أثناء مناولة الزيت الخام والتكرير والتبييض والهدرجة وازالة الروائح الكريهة وتخزين المنتج وتوزيعه

HANDLING CRUDE OILS

عمليات مناولة الزيت الخام

HANDLING CRUDE OIL AFTER CRUSHING

- CRUDE OIL MUST BE FILTERED IMMEDIATELY AFTER EXPELLING AND/OR EXTRACTION
- THE OIL MUST BE COOLED TO <40°C BEFORE STORAGE
- THE OIL MUST BE DISCHARGED AT THE BOTTOM OF THE STORAGE TANK, NEAR THE FLOOR TO AVOID AIR ENTRAINMENT
- TEMPERATURE OF OIL IN STORAGE MUST BE MAINTAINED AT <40°C
- DURING LOADING CRUDE OIL IN A TANK TRUCK, THE LOADING LINE (BOOM) MUST BE PLACED JUST A FEW CENTEMETERS FROM THE FLOOR OF THE TRUCK TO AVOID AIR ENTRAINMENT

مناولة الزيت الخام بعد الطحن

- يجب ترشيح الزيت الخام مباشرة بعد استخلاصه
- يجب ان يتم تبريد الزيت حتى درجة حرارة قدرها <٤٠°م قبل التخزين
- يجب ان يتم تصريف لزيت في الجزء السفلي من صهريج التخزين بالقرب من الارضية لتفادي احتباس الهواء في الزيت
- يجب ان تكون درجة حرارة الزيت في صهريج التخزين عند < ٤٠°م
- في اثناء تحميل الزيت الخام في الشاحنة، يجب وضع خط التحميل على بعد بضعة سنتيمترات من ارضية الشاحنة لتفادي احتباس الهواء في الزيت

UNLOADING & STORAGE OF CRUDE OILS

- IT IS RECOMMENDED TO DO THE FOLLOWING:
  - CRUDE OIL DISCHARGE MUST BE LOCATED AT THE BOTTOM OF THE TANK, PREFERABLY THROUGH A SUMP
  - THE TANK HAVE A MECHANICAL AGITATOR, THAT IS AUTOMATICALLY SHUT OFF WHEN THE OIL LEVEL IN THE TANK DROPS WITHIN ONE METER OF THE TIP OF THE AGITATOR BLADES
  - THE SEDIMENT AT THE BOTTOM OF THE TANK MUST BE REMOVED ONCE A YEAR, THOROUGHLY CLEANED BEFORE FILLING IT UP WITH FRESH CRUDE OIL
  - CRUDE OIL IN STORAGE MUST BE USED UP AS QUICKLY AS POSSIBLE

تفريغ الزيوت الخام وتخزينها

يوصى باجراء ما يلي:

- ينبغي ان تكون فتحة تصريف الزيت الخام عند الجزء السفلي من الصهريج ويفضل من خلال حوض الزيت او المجمع السفلي للزيت
- يحتوي الصهريج على اداة تحريك ميكانيكية تغلق اوتوماتيكيا عندما ينخفض مستوى الزيت في الصهريج بحوالي ١ متر عن الجزء العلوي من اتصال اداة التحريك
- يجب التخلص من الترسبات الموجودة في قاع الصهريج مرة في العام ويجب ان يتم تنظيفه جيدا قبل ملؤه بالزيت النقي الجيد
- يجب استهلاك الزيت الخام المخزن باسرع وقت ممكن

ANALYSIS OF CRUDE OILS DURING STORAGE

- CHECK PEROXIDE VALUE, ANISIDINE VALUE AND FREE FATTY ACID IN THE CRUDE OIL, AT LEAST ONCE A WEEK

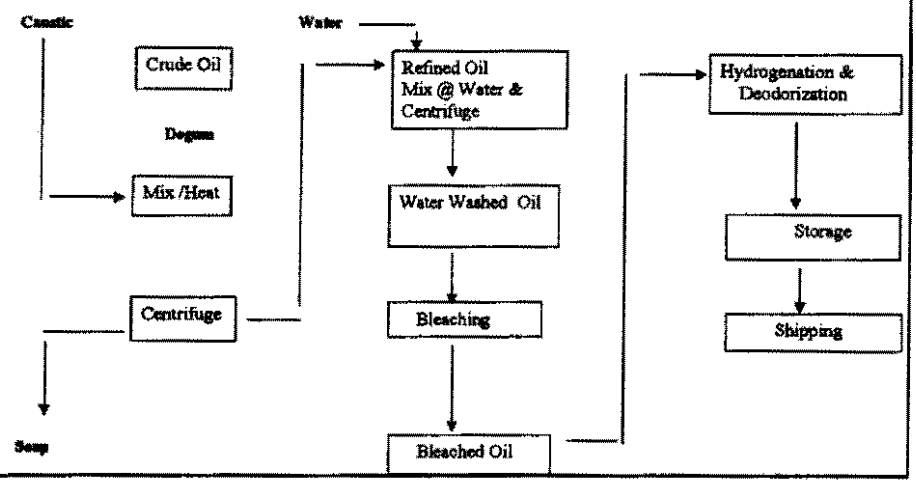
تحليل الزيوت الخام اثناء التخزين

- يجب فحص حجم البيروكسيد والانيسيدين والحامض الدهني الحر في الزيت الخام

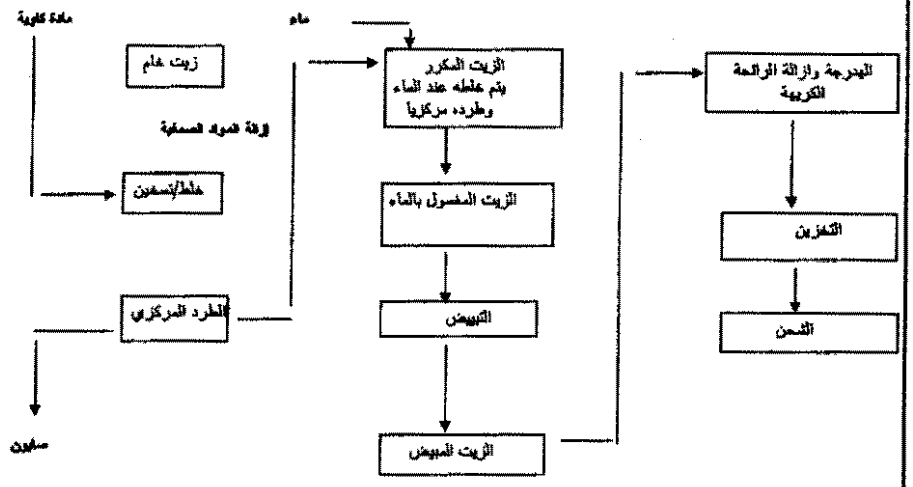
PROCESSING VEGETABLE OILS

تصنيع الزيوت النباتية

VEGETABLE OIL REFINING SCHEMATIC



رسم تخطيطي لعملية تكرير الزيت



FUNCTIONS OF THE PROCESSING STEPS

- CAUSTIC TREATMENT & CENTRIFUGING
- REDUCES FREE FATTY ACIDS, PHOSPHOLIPIDS, TRACE METALS, COLOR BODIES, OIL OXIDATION BYPRODUCTS
- WATER WASHING
  - REDUCES THE SOAP PRODUCED IN CAUSTIC REFINING
- BLEACHING
  - REDUCES PHOSPHOLIPIDS, TRACE METALS, COLOR BODIES AND OIL OXIDATION PRODUCTS TO VERY LOW VALUES
- HYDROGENATION
  - REDUCES UNSATURATED FATTY ACIDS, INCREASES OXIDATIVE STABILITY OF THE OIL, PRODUCES TRANS FATTY ACIDS
- DEODORIZATION
  - MAKES THE OIL ODORLESS, REDUCES ITS RED COLOR AND CAN PRODUCE TRANS FATTY ACIDS (TO A MUCH LESSER DEGREE THAN THE PROCESS OF HYDROGENATIONO)

وظائف الخطوات الخاصة بالعملية

المعالجة بالمواد الكاوية والطرد المركزي

- تقلل من الاحماض الدهنية الحرة والفوسفوليبيدات والنسب الصغيرة من المعادن والاجسام الملونة والمنتجات الثانوية للاكسدة
- الغسل بالماء
  - يقلل من الصابون الناتج عن عملية التكرير بالمواد الكيماوية
- التبييض
  - يقلل من الفوسفوليبيدات والنسب القليلة من المعادن والاجسام الملونة ومنتجات الاكسدة لمقادير بسيطة جدا
- الهدرجة
  - تقلل من الاحماض الدهنية الغير مشبعة وتزيد من ثبات تاكسد الزيت وينتج عنها احماض دهنية متحولة
- إزالة الروائح الكريهة
  - تجعل تلك العملية الزيت بدون رائحة وتقلل من اللون الاحمر (ويمكن ان تنتج احماض دهنية متحولة بدرجة اقل كثيرا من عملية الهدرجة)

DEGUMMING

OILS ARE DEGUMMED TO OBTAIN BETTER REFINING RESULTS AND OIL STABILITY

إزالة المواد الصمغية

يتم ازالة المواد الصمغية من الزيت للحصول على نتائج افضل للتكرير وثبات افضل للزيت

TYPES OF PHOSPHOLIPIDS

- **HYDRATABLE**
  - CAN BE REMOVED FROM THE CRUDE OIL BY WATER
- **NON-HYDRATABLE**
  - REQUIRES TREATMENT WITH PHOSPHORIC ACID, CITRIC ACID OR MALEIC ACID TO REDUCE THE LEVEL IN THE CRUDE OIL

أنواع الفوسفوليبتيدات

قابلة للتحلل المائي

- يمكن ازالتها من الزيت الخام بواسطة الماء

غير قابلة للتحلل المائي

- تتطلب المعالجة بحامض الفوسفوريك وحامض السيتريك او الماليك للاقلال من مستواها في الزيت الخام

WATER DEGUMMING PROCESS

- IN THIS PROCESS THE CRUDE OIL IS MIXED INTIMATELY WITH DEIONIZED WATER IN A TANK WITH A MECHANICAL AGITATOR, TEMPERATURE=60° - 65°C. THIS IS CALLED WATER DEGUMMING
- HYDRATABLE PHOSPHOLIPIDS PRECIPITATE AND ARE SEPARATED IN A CENTRIFUGE

عملية ازالة المواد الصمغية بالماء

- في تلك العملية، يتم مزج الزيت جيدا مع ماء منزوع الايون داخل صهريج يحتوى على محرك ميكانيكي. وينبغي ان تكون درجة الحرارة ٦٠°م – ٦٥ °م. وهذا ما يسمي بعملية ازالة المواد الصمغية بالماء.
- تترسب الفوسفوليبيدات القابلة للتحلل بالماء ثم يتم فصلها داخل جهاز طرد مركزي

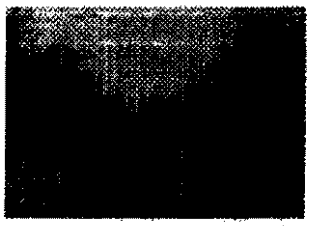
ACID DEGUMMING PROCESS

- SOMETIMES THE OIL IS FURTHER TREATED WITH PHOSPHORIC ACID TO REDUCE THE NON-HYDRATABLE PHOSPHOLIPIDS. THIS IS CALLED ACID DEGUMMING. THIS IS NEEDED IN SOME POOR QUALITY SEED OILS

عملية ازالة المواد الصمغية بالحامض

- في بعض الاحيان يتم معالجة الزيت بحامض الفوسفوريك لختزال الفوسفوليبيدات غير القابلة للتحلل بالماء. وهذا ما يسمي بعملية ازالة المكواد الصمغية بالحامض وتكون تلك العملية متطلبة في بعض انواع الزيوت منخفضة الجودة المستخلصة من البذور

Obtaining virgin oils

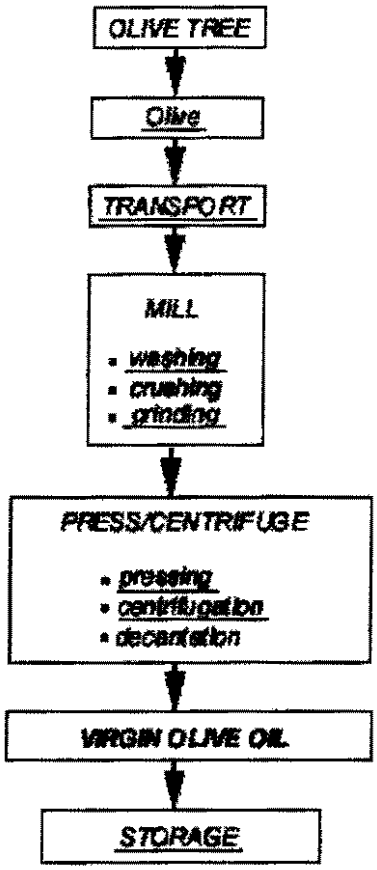


The olive tree is typical of the Mediterranean area as it needs a lot of sun and a climate characterised by mild winters, rainy autumns and springs, and warm, dry summers.

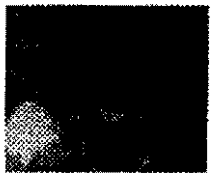
Olive trees grow slowly. They usually bear fruit 5-10 years after they are planted and are not fully developed until they are 20 years old. They are most mature and fully productive between the ages of 35 to 100-150. After that the trees get old and their performance is irregular.

Olive oil is obtained in the following steps:

Process flow chart



1. Harvesting the fruit



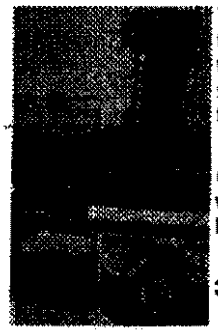
Olive trees flower in spring and the fruit starts to form and then to ripen, changing from green to black, between the summer and the end of the autumn / beginning of the winter when harvesting takes place.

Olives can be picked by hand, or beaten from the trees (with long,

126

flexible poles so that the olives fall onto the canvases placed at the foot of the trees), or harvested using mechanical tree shaking methods.

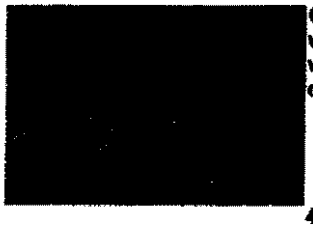
2. Transport to the oil mill



The harvested olives are transported to the oil mill for grinding. Those that are collected from the ground with nets (and which have to be transported separately) must be as free from stones, earth and impurities as possible to avoid breaking the skin during transport, as this would lead to the beginning of fermentation.

On arrival at the mill, the olives must not be piled up high or else they will heat up and start to ferment. Olives must be processed within 24 hours after they are harvested to obtain quality olive oil.

3. Washing



On the conveyor belt the olives cross a ventilation area where the leaves are separated by an air current. They are washed with normal water after they are selected for quality.

4. Grinding to prepare the paste

Grinding consists of crushing the fruit (including the pits) until a paste is formed which is then beaten. Water is added if necessary.

5. Separation of solid and liquid phases (oil extraction)

There are several methods, but in all of them it is essential that the temperature does not exceed 35° C

Traditional system (pressing)

- **Classic discontinuous plants**

  These are the classic plants maintained throughout the centuries. The ground paste is placed between round mats and is then pressed to squeeze out the oily liquid (mixture of oil and water). It is later left to decant, as the oil floats to the top due to density differences.

Continuous system (by centrifugation)

- **Continuous plants in 3 phases**

  The beaten olive paste is made more liquid by adding 1 litre of water per kilogram of paste and is taken to a horizontal centrifuge where solids are separated from the oily liquid. This liquid is then taken to a vertical centrifuge where the olive oil is separated from the fruit vegetable water.

- **Continuous plants in 2 phases**

  The process is practically the same as the one described above, with the difference that instead of adding water for the horizontal centrifugation, the vegetable water is recycled.

The continuous system is becoming more and more widespread and has the following advantages compared with the traditional system:

- high production capacity, which avoids having a stock of olives and therefore increases the oil's quality

- improves performance, cleanliness and hygiene

- in the 2 phase plants, recycling the vegetable water means the oil has a larger quantity of polyphenols, natural protectors against oxidation

6. Storage

The oil obtained is stored in cellars or warehouses until it is marketed.

The ideal storage room is a place where the walls and ceilings insulate against high temperatures and which does not add strange smells to the oil. The ideal temperature is between 15 and 18° C to allow the oils to mature without oxidation. There must not be much light.

The material of the storage containers must be inert (vitrified tile, stainless steel, glass polyester fibre, etc.). Iron or copper must never be used, as they encourage oxidation.

Obtaining non-virgin oils

Refining

Due to climate conditions, the type of soil and faults in the abovementioned process, virgin oils can have several defects, such as high acidity, a strange colour or aroma, etc. which makes them unsuitable for human consumption (they are called "lampante oils"). This means they have to undergo a refining process to eliminate defects. There are two types of processes:

1. Classic alkaline refining

This has 3 phases. Each one tends to eliminate a specific process:

-neutralisation
-bleaching
-deodorisation

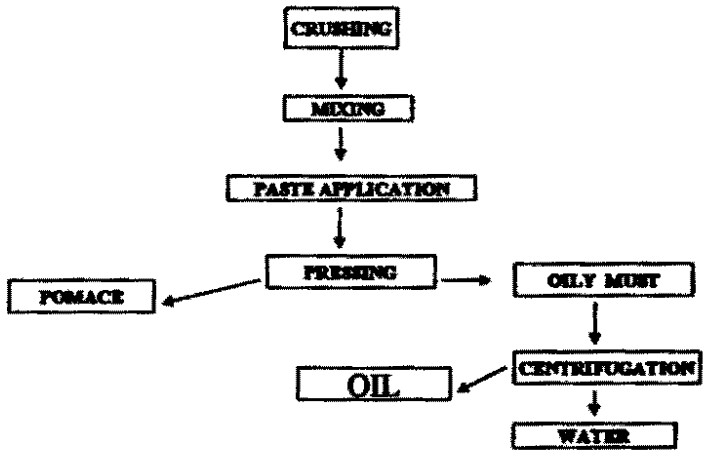
2. Physical refining

It is different from the previous method as acid water is used instead of soda to purify the oil and the temperature is higher.

128

OLIVE OIL

SINGLE PRESS METHOD



IMPORTANT VARIABLES

- TYPE OF OLIVE
- MATURITY OF THE OLIVES
- TRANSPORTATION & STORAGE OF OLIVES
- METHOD OR TYPE OF CRUSHING
  - STONE VS MECHANICAL GRINDING
  - EXTENT OF GRINDING
- MIXING TIME
  - LENGTH OF MIXING
  - TEMPERATURE
- PRESSURE IN THE PRESS
  - SINGL PRESS
  - DOUBLE PRESS
- RESIDENCE TIME IN THE PRESS
- POROSITY OF THE BED CREATED BY THE GROUND UP STONES FROM THE OLIVES

TYPE & MATURITY OF OLIVES

- TYPE OF OLIVES AS WELL AS THEIR MATURITY IMPACT THE QUALITY AND YIELD OF OLIVE OIL
- VARIOUS METHODS HAVE BEEN PROPOSED TO DETERMINE THE MATURITY OF OLIVES BY ANALYZING THEM IN THE OLIVE PASTE:
- RATIO OF ABSORPTION AT WAVE LENGTHS OF 665nm AND 525nm
- TOTAL VOLATILES
- MALEIC ACID/CITRIC ACID RATIO
- IOOC (INTERNATIONAL OLIVE OIL COUNCIL)METHOD TO ASSESS MATURITY

TRANSPORTATION & STORAGE OF OLIVES

- CRATES FOR TRANSPORTATION OF OLIVES CAN VARY FROM 25-KG TO 250 KG
- MATURE OLIVES ARE BEST TRANSPORTED IN SHALLOW CRATES, WITH 15 - 30 CM DEPTH AND STORED UNTIL CRUSHED IN COOL CONDITION
- FREE FATTY ACID INCREASES RAPIDLY IN THE OLIVES DURING STOTAGE
- SOME OF THE AROMATIC COMPOUNDS DIMINISH
- POLYPHENOLS IN THE OIL ARE REDUCED
- THIS IS WHY THE OLIVES MUST BE CRUSHED WITHIN 24 HOURS AFTER HARVESTING

IOOC METHOD FOR THE DETERMNATION OF MATURATY

- TAKE 100 OLIVES
- CALCULATE MATURITY USING THE FORMULA
  - $((0 \times n_0) + (1 \times n_1) + (2 \times n_2) + \text{——} + (7 \times n_7)) / 100$ WHERE,
  - 0 = OLIVES WITH DARK GREEN COLOR
  - 1 = OLIVES WITH YELLOWISH GREEN COLOR
  - 2 = OLIVES WITH YELLOWISH COLOR WITH REDDISH SPOTS
  - 3 = OLIVES WITH REDDISH OR LIGHT VIOLET SKIN COLOR
  - 4 = OLIVES WITH BLACK SKIN BUT GREEN INSIDE
  - 5 = OLIVES WITH BLACK SKIN AND THE FLESH IS VIOLET, HALF WAY THROUGH
  - 6 = OLIVES WITH BLACK SKIN COLOR AND THE FLESH WITH VIOLET COLOR ALMOST THROUGH
  - 7 = OLIVES WITH BLACK SKIN AND COMPLETELY BLACK FLESH
- THE DESIRED MATURITY IS: 5
- THIS IS NOT A PERFET APPROACH

EFFECT OF STORAGE

(OLIVES STORED AT ROOM TEMPERATURE, 10 - 15 CM DEEP)

OLIVE VARIETY	DATE OF EXTRACTION	FREE FATTY ACID AS OLEIC ACID %	PEROXIDE VALUE	ORGANOLEPTIC EVALUATION	TRANS-2 HEXENAL (PPM)	TOTAL VOLATILES (PPM)
	NOV. 20	0.56	5.6	7.1	43.0	138.6
	NOV. 26	0.62	7.1	.0	32.2	105.4
	DEC. 03	0.73	9.7	6.0	8.6	78.0
DRITTA	DEC. 11	1.24	10.3	5.2	3.6	66.6
	DEC. 17	1.86	10.0	5.0	1.6	17.6
	DEC. 30	3.83	9.8	4.6	TRACE	17.6
	JAN. 21	9.58	9.6	4.6	TRACE	1.4
	NOV. 20	0.28	4.5	7.8	55.0	75.5
	NOV. 26	0.30	5.5	7.9	37.2	70.0
	DEC. 04	0.34	6.0	7.1	15.4	34.8
LECCINO	DEC. 11	0.34	6.0	6.5	11.0	30.8
	DEC. 17	0.50	5.7	6.0	10.3	25.2
	DEC. 30	1.47	6.7	5.0	8.0	22.0
	JAN. 21	2.59	6.0	4.9	0.8	9.0

GRINDING & MIXING

- GRINDING OPENS UP THE CELLS WHERE THE OILS ARE
- FINER GRIND RELEASES MORE OIL
- FINER GRIND OF THE STONES ALLOWS BETTER PERCOLATION OF THE OIL
- FINER GRIND CAN ALSO CAUSE EMULSIFICATION. THIS IS WHY THE MIXING STEP IS NECESSARY
- TYPICAL MIXING TIME = 10 - 20 MINUTES FOR THE PRESSING PROCESS
- LONGER MIXING TIME CAN PRODUCE LARGER DROPLETS OF OIL WHICH CAN SEPARATE BETTER FROM THE VEGETATION WATER, IN THE CENTRIFUGAL SEPARATION PROCESS
- LONGER MIXING TIME CAN IMPROVE YIELD OF OIL
- HIGHER TEMPERATURE AT MIXING CAN INCREASE OIL YIELD

EFFECT OF MIXING

- **AFTER GRINDING**
- DIAMETER OF OIL DROPS BETWEEN:
  - 15-30 MICRONS 18 %
  - 30-45 MICRONS 18 %
  - 45-75 MICRONS 18 %
  - 75-150 MICRONS 19 %
  - > 150 MICRONS 25 %

- **AFTER MIXING**
- DIAMETER OF OIL DROPS BETWEEN:
  - 15-30 MICRONS 49 %
  - 30-45 MICRONS 21 %
  - 45-75 MICRONS 14 %
  - 75-150 MICRONS 4 %
  - > 150 MICRONS 6 %

EFFECTS OF MIXING TIME ON OLIVE OIL

- **OLIVES, EASY TO HANDLE**
- PER CENT YIELD VS MIXING TIME:
  - 82 % AFTER 15 MINUTES
  - 83 % AFTER 30 MINUTES
  - 84 % AFTER 45 MINUTES
  - 85 % AFTER 60 MINUTES
  - 88 % AFTER 90 MINUTES
- REDUCES THE PHENOLIC COMPOUNDS IN THE OIL

- **OLIVES HARD TO HANDLE**
- PER CENT YIELD VS MIXING TIME:
  - 73 % AFTER 15 MINUTES
  - 78% AFTER 30 MINUTES
  - 80 % AFTER 45 MINUTES
  - 81 % AFTER 60 MINUTES
  - 83 % AFTER 90 MINUTES
- REDUCES THE PHENOLIC COMPOUNDS IN THE OIL

EFFECTS OF MIXING TEMPERATURE ON OLIVE OIL

- **OLIVES, EASY TO HANDLE**
- PER CENT YIELD VS MIXING TEMPERATURE:
  - 81 % AT 15°C
  - 82 % AT 20°C
  - 84 % AT 25°C
  - 86 % AT 30°C
  - 88 % AT 35°C
- REDUCES PHENOLIC COMPOUNDS IN THE OIL

- **OLIVES HARD TO HANDLE**
- PER CENT YIELD VS MIXING TEMPERATURE:
  - 75 % AT 15°C
  - 76 % AT 20°C
  - 77 % AT 25°C
  - 79 % AT 30°C
  - 82 % AT 35°C
- REDUCES PHENOLIC COMPOUNDS IN THE OIL

EFFECT OF PRESSURE

VARIABLES	PRESSURE = 136 KG/SQ.CM	PRESSURE = 182 KG/SQ.CM
% YIELD	88.6	90.7
POMACE		
AMOUNT (KG/100 KG OF OLIVES)	31	29.4
MOISTURE, %	24.2	23.2
OIL, %	6.2	5.6
OIL, KG/100 KG OF OLIVES	1.9	1.7
VEGETATION WATER		
OIL (GRAMS/LITER	3.8	2.7
OIL (KG/100 KG OF OLIVES)	0.23	0.16

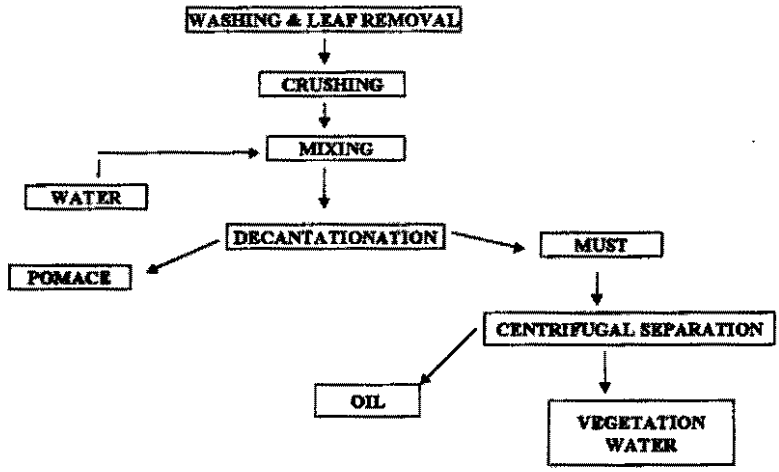
DOUBLE PRESSING

- THIS PROCESS IS STILL FOLLOWED IN SOME OPERATIONS
- OIL IS REMOVED FROM THE CRUSHED OLIVES AT 100 - 150 KG/SQ.CM PRESSURE
- THE POMACE FROM THE FIRST PRESS IS REGROUND IN STONE GRINDER
- THE REGROUND POMACE IS PRESSED UNDER PRESSURE 400 - 450 KG/SQ.CM
- THE OIL IS VERY GREEN IN COLOR
- THE OIL ALSO HAS DIFFERENT ODOR AND FLAVOR THAN THE OIL FROM THE FIRST PRESS

IMPORTANT VARIABLES (C0NT)

- TYPE AND THE DEGREE MATURITY OF OLIVES DETERMINE THE OIL YIELD, QUALITY AND FLAVOR
- IT IS BELIEVED THAT MECHANICAL GRINDING TENDS TO MAKE THE OIL BITTER, STRONGER AND SOMETIMES SPICIER (GRINDING TEMPERATURE : 18~19° C
- MIXING TIME INCREASES THE OIL YIELD. IT CAN ALSO AFFECT THE FLAVOR TO A DEGREE (MIXING TIME : 10 TO 20 MINUTES)
- PRESSURE AND RESIDENCE TIME IN THE PRES INCREASES THE YIELD. THIS CAN ALSO INCREASE THE VOLATILE MATTER AND REDUCE THE PHENOLIC COMPOUNDS
- FINER GRIND OF THE STONES IN THE OLIVES INCREASES POROSITY OF THE BED AND IMPROVES THE YIELD

CENTRIFUGE METHOD



CENTRIFUGE PROCESS

- THE PROCESS HAS CAPACITY RANGING FROM 0.5 TO 4 TONS OF OLIVE OIL PER HOUR
- USUALLY, A LARGER CAPACITY IS DISCOURAGED BY THE EQUIPMENT MANUFACTURERS
- THE PRODUCTION RATE CAN AND MUST BE VARIED DEPENDING ON THE EASINESS OF HANDLING PROPERTIES THE OF OLIVES
- IMPORTANT VARIABLES:
  - VARIETY & MATURITY OF OLIVES
  - CRUSHING, GRINDING AS DESCRIBED EARLIER
  - AMOUNT OF WATER ADDED IN THE OLIVE PASTE
  - TEMPERATURE OF THE WATER ADDED

IMPACT OF VARIOUS FACTORS ON OLIVE OIL QUALITY

- MATURITY OF THE OLIVES 30 %
- VARIETY 20 %
- HARVEST METHOD 05 %
- TRANSPORTATION & STORAGE 15 %
- METHOD OF EXTRACTION 30 %

GRADES OF OLIVE OIL

ANALYSIS	EXTRA VIRGIN OLIVE OIL	VIRGIN OLIVE OIL	ORDINARY VIRGIN OLIVE OIL	REFINED OLIVE OIL	OLIVE OIL	REFINED OLIVE-POMACE OIL	OLIVE-POMACE OIL
FREE FATTY ACID %, MAX	1	2	3.3	0.3	1.5	0.3	1.5
PEROXIDE VALUE MEQ/KG	20	20	20	5	15	5	15
ABSORBENCE AT 270nm	<0.25	< 0.25	< 0.30	< 1.10	< 0.90	< 2.0	< 1.70
% MOISTURE & VOLATILE MATTER	0.2 max	0.2 max	0.2 max	0.1 max	0.1 max	0.1 max	0.1 max
% INSOLUBLE IMPURITIES	0.1 max	0.1 max	0.1 max	0.05 max	0.05 max	0.05 max	0.05 max

ADDITIONAL PERINENT QUALITY INFORMATION

- ADITIVES:
  - NONE PERMITTED IN THE VIRGIN OILS
  - ALFA TOCOPHEROLS MAY BE ADDED TO REFINED OILS, ALLWING A MAXIMUM CONCENTRATION OF 200 PPM
- WAXES:
  - VIRGIN OLIVE OIL 250 PPM
  - REFINED OLIVE OIL 350 PPM
  - OLIVE OIL 350 PPM
- TOCOPHEROLS:
  - VIRGIN OLIVE OIL, UP TO 300 PPM
- STEROLS:
  - VIRGIN, REFINED, OLIVE OIL 1000 PPM, MINIMUM
  - REFINED-POMACE OIL 1800 PPM, MINIMUM
  - OLIVE-POMACE OIL 1600 PPM, MINIMUM

VOLATILE & AROMATIC COMPOUNDS

- VARIOUS TYPES OF COMPOUNDS HAVE BEEN REPORTED IN THE OLIVE OIL THAT ARE BELIEVED TO BE RESPONSIBLE FOR THE AROMA OF THE OIL. THESE INCLUDE COMPOUNDS, SUCH AS:
  - ALCOHOLS
  - ALDEHYDES
  - KETONES
  - ESTERS
  - ETHERS
  - PHENOLSACIDS
- TERPENE ALCOHOLS
- THIOLS
- SOME OF THESE COMPOUNDS IMPART MORE DESIRABLE AROMA THAN THEOTHERS
- THEIR THRESHOLD VALUES ARE NOT FULLY ESTABLISHED

OLIVE OIL PRODUCTION

PRODUCTION YEAR	1999-2000	2000-01(P)
WORLD PRODUCTION (MILLION TONS)	2,362	2,668
SPAIN	702	1,022
GREECE	432	435
ITALY	635	445
EU - 15	1,821	1,957
TUNISIA	225	163
SYRIA	88	183
TURKEY	61	210

- SOURCE: "OIL WORLD"
- P - PROJECTED

EGYPT'S OLIVE OIL PRODUCTION
(1999 - 2000)

TOTAL PRODUCTION	4,000 TONS
IMPORT	1,000 TONS*
EXPORT	1,000 TONS*
DOMESTIC EDIBLE CONSUMPTION	600 TONS
DOMESTIC NON-EDIBLE USE	3,400 TONS

- AVAILABLE STATISTICS IS DIFFICULT
- * SOURCE: OIL WORLD

STATE OF EGYPTIAN OLIVE OIL

- EGYPT IS NOT KNOWN AS A PRODUCER OF OLIVE OIL
- IT IS MORE OR LESS A COTTAGE INDUSTRY
- THE TRANSITION PROCESS FROM GROWER TO OLIVE PROCESSOR HAS NOT BEEN EFFECTIVE
- GROWERS WHO USE THEIR OWN OLIVES, DID NOT START THEIR ORCHARDS WITH OIL PRODUCTION IN MIND. PROCESSING OF OLIVES FOR OIL SIMPLY EVOLVED AS A GOOD IDEA
- THE ECONOMY OF SCALE IS WORKING AGAINST THE PROCESSORS
- UNFAVORABLE COMPETITION FROM OTHER OLIVE OIL PRODUCING COUNTRIES IS WORKING AGAINST THE GROWTH OF THIS INDUSTRY
- PROCESSORS CANNOT REACH FULL PRODUCTION DUE TO THE LACK OF SUPPLY OF OLIVES AND LOW YIEDING VARIETIES OF OLIVES

RECOMMENDATIONS

- THE OLIVE OIL INDUSTRY NEEDS AN EGYPTIAN OLIVE OIL SOCIETY OR AN ASSOCIATION, WHO CAN PROMOTE TECHNOLOGY, AGRONOMIC KNOW-HOW IN THE INDUSTY AND EDUCATE THE GROWERS WITH MORE MODERN TECHNIQUES ON SELECTION AND GROING OF HIGHER YIELDING AND BETTER VARIETIES OF OLIVES
- WITH THE HELP OF SOME CURRENT REPUTABLE AND UNBIASED RESEARCH WORKERS, EGYPTIAN OLIVE OIL SHOULD BE POSITIONED AGAINST THE WORLD'S BEST KNOWN BRANDS. THIS CAN BE ACCOMPLISHED THROUGH:
  - CONSUMER TESTS
  - ANALYTICAL CONFIRMATION OF GOOD TRAITS IN THE OIL
  - ENDORSEMENT FROM WELL KNOWN NUTRITIONISTS AND CHEFS AROUND THE WORLD
  - PUBLICITY ON OLIVE OIL OUTSIDE OF EGYPT
- CHANGE THE CURRENT MODE OF OPERATIONOF THE INDUSTRY TO APPLY AN ORGANIZED EFFORT TO PROMOTE THE INDUSTRY

BLEACHING

التبييض

PURPOSE OF BLEACHING

- REDUCE THE AMOUNT OF NON-HYDRATABLE AND HYDRATABLE PHOSPHOLIPIDS IN THE OIL
- REDUCE THE TRACE IMMETALS, SUCH AS IRON, COPPER, NICKEL, CALCIUM, MAGNESIUM
- REDUCE COLOR COMPONENTS, SUCH AS CHLOROPHYLLS, CAROTENES AND OTHER COLOR BODIES
- REMOVE BREAKDOWN PRODUCTS OF CHLOROPHYLLS, SUCH AS PHEOPHYTINES, PHEOPHORBIDES AND PYROPHEOPHORBIDES

الغرض من التبييض

- الاقلال من كمية الفوسفوليبيدات القابلة للتحلل بالماء وغير القابلة للتحلل بالماء في الزيت
- الاقلال من النسبة الضئيلة من المعادن مثل الحديد والنحاس والنيكل والكالسيوم والماجنيسيوم
- الاقلال من المكونات اللونية مثل الكلوروفيل والكاروتين والاجسام الملونة الاخرى
- ازالة المنتجات الناتجة عن الكلوروفيل مثل الفيوفيتينات والفيوفوربيدات والبيروفيوفوربيدات

CRITICAL VARIABLES

- CRUDE OIL AS WELL AS REFINED OIL QUALITY
- OIL TEMPERTURE
- OPERATING PRESSURE
- BLEACHING CLAY CONCENTRATION
- CITRIC ACID/PHOSPHORIC ACID
- TIME OF CONTACT
- DESIGN OF THE TANK AND AGITATOR
- USE OF HYDRATED SILICA

العوامل المتغيرة الهامة

- جودة الزيت الخام وكذلك جودة الزيت المكرر
- درجة حرارة الزيت
- ضغط التشغيل
- تركيز طين التبييض
- حامض السيتريك / حامض الفوسفوريك
- زمن التلامس
- تصميم الصهريج والمحرك
- استخدام السيليكا المتحللة بالماء

REFINING OF VEGETABLE OILS

تكرير الزيوت النباتية

WHY ARE VEGETABLE OILS REFINED?

- CRUDE OIL IS REFINED IN ORDER TO:
  - REDUCE THE AMOUNTS OF IMPURITIES PRESENT IN THE OIL
  - MAKE THE OIL SUITABLE FOR EDIBLE APPLICATIONS
  - IN MANY CASES THE OIL BECOMES SOMEWHAT LIGHTER IN COLOR

لماذا يتم تكرير الزيوت النباتية

يتم تكرير الزيت الخام نظرا لما يلي:

- الاقلال من كميات الشوائب الموجودة في الزيت
- لجعل الزيت ملائما للاستخدام في الاغذية
- في كثير من الاحوال، يصبح لون الزيت خفيفا بعض الوقت

METHODS FOR REFINING VEGETABLE OILS

- THERE ARE TWO MAIN METHODS FOR REFINING VEGETABLE OILS. THEY ARE:
  - CHEMICAL REFINING
  - PHYSICAL REFINING
- A COMBINATION METHOD IS ALSO USED. THEY ARE:
  - MODIFIED PHYSICAL REFINING
  - MODIFIED CHEMICAL REFINING

طرق تكرير الزيوت النباتية

- ثمة طريقتين اساسيتين لتكرير الزيوت النباتية، هما على الوجه التالي:
  - التكرير الكيميائي
  - التكرير الطبيعي
- وهناك طريقتين متحدتين يمكن استخدامهما:
  - التكرير المادي المعدل
  - التكرير الكيميائي المعدل

CHEMICAL REFINING

- IN THIS PROCESS THE CRUDE VEGETABLE OIL IS TREATED WITH SODIUM HYDROXIDE AND THEN CENTRIFUGED TO REMOVE THE EXCESS SOAP PRODUCED
- THE REFINED OIL IS WATER WASHED
- THE WATER WASHED OIL IS BLEACHED
- THE OIL IS EITHER HYDROGENATED, WINTERIZED AND/OR DEODORIZED
- DEODORIZED OIL IS STORED FOR:
  - BULK DISTRIBUTION FOR INDUSTRIAL USE
  - PACKAGED FOR THE RETAIL STORES

التكرير الكيميائي

- في تلك العملية، يتم معالجة الزيت النباتي الخام بهيدروكسيد الصوديوم ثم يتم وضعه في جهاز الطرد المركزي لازالة الصابون الزائد
- يتم غسل الزيت المكرر بالماء
- يتم تبييض الزيت المغسول بالماء
- يتم بعد ذلك اما هدرجة الزيت او تشتيته (تبريده للتخلص من الجزء المتبلور الذي يحتوي على احماض دهنية) و/او ازالة الروائح الكريهة منه
- يتم تخزين الزيت المزال منه الرائحة الكريهة ليتم:
  - توزيعه في عبوات كبيرة للاستخدام الصناعي
  - تعبئته لتوزيعه على محال البيع بالتجزئة

CRITICAL VARIABLES

- CRUDE OIL QUALITY
- CAUSTIC STRENGTH
- CAUSTIC DOSAGE
- MIXING TEMPERATURE
- CONTACT TIME
- CENTRIFUGING TEMPERATURE
- CENTRIFUGE BACK PRESSURE
- IMPORTANT ANALYSES:
  - SOAP INREFINED OIL (PARTS PER MILLION), 500 MAX. NOT TO EXCEED 1000PPM
  - PHOSPHORUS < 3 PARTS PER MILLION
  - FREE FATTY ACID < 0.2 %

العوامل المتغيرة الهامة

- جودة الزيت الخام
- قوة المادة الكيماوية
- جرعة المادة الكيماوية
- درجة حرارة الخلط
- زمن التلامس
- درجة حرارة الطرد المركزي
- الضغط المعاكس للطرد المركزي
- تحليل هام:
  - الصابون داخل الزيت المكرر (جزء في المليون)، يكون بحد القصى ٥٠٠ ويجب الا يزيد عن ١٠٠٠ جزء في المليون
  - الحامض الدهني الحر < ٠,٢ %

PHYSICAL REFINING

- CRUDE OIL IS NOT TREATED WITH CAUSTIC
- CRUDE OIL IS TREATED WITH CITRIC ACID OR PHOSPHORIC ACID. THEN IT IS BLEACHED WITH SILICA GEL AND BLEACHING CLAY AND FILTERED
- FILTERED OIL IS SENT TO WINTERIZATION, HYDROGENATION AND/OR DEODORIZATION
- DRAWBACKS:
  - THIS PROCESS IS USED ONLY IN REFINING PALM OIL, COCONUT OIL, PALM KERNEL OIL AND SUNFLOWER OIL
  - THE PROCESS MAY NOT REMOVE ALL IMPURITIES FROM THE CRUDE OIL
  - NOT SUITABLE FOR SEED OILS AND OLIVE OIL

التكرير المادى

- لا يتم معالجة الزيت الخام بالمواد الكاوية
- يتم معالجة الزيت الخام بحامض السيتريك او الفوسفوريك. ثم يتم تبييضه بجيل السيليكا والطين المبيض ثم يتم ترشيحه
- يتم ارسال الزيت المبيض لتتم تشتيته وهدرجته و/او ازالة الروائح الكريهة منه
- عيوب تلك الطريقة:
  - تستخدم تلك الطريقة فقط في تكرير زيت النخيل وزيت جوز الهند وزيت لب النخيل وزيت عباد الشمس
  - قد لا تزيل تلك العملية كافة الشوائب من الزيت الخام
  - تكون تلك الطريقة غير ملائمة للزيوت المستخلصة من الحبوب وزيت الزيتون

CRITICAL VARIABLES

- CONCENTRATION OF ACID
- CONCENTRATION OF HYDRATED SILICA
- CONCENTRATION OF BLEACHING CLAY
- TEMPERATURE OF TREATMENT
- TIME OF CONTACT
- DESIGN OF THE TANK AND AGITATOR
- FILTRATION DEVICE
- COOLING AFTER FILTRATION (NOT NEEDED IF GOES DIRECTLY TO THE DEODORIZER)

العوامل المتغيرة الهامة

- تركيز الحامض
- تركيز السيليكا المتحللة بالماء
- تحليل الطين المبيض
- درجة حرارة المعالجة
- زمن التلامس
- تصميم الصهريج والمحرك
- جهاز الترشيح
- التبريد بعد الترشيح (لا يكون متطلبا اذا تم نقل الزيت مباشرة الى جهاز ازالة الروائح الكريهة)

HYDROGENATION

الهدرجة

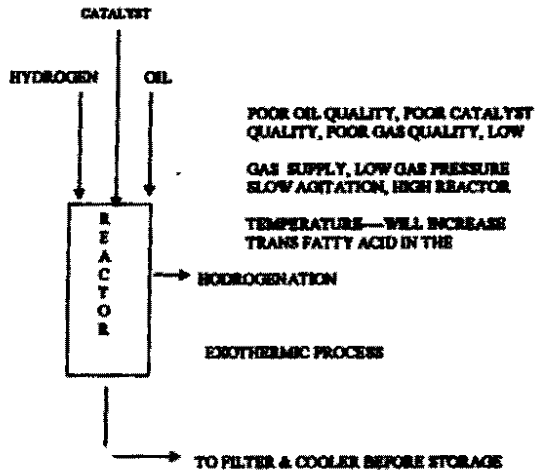
HYDROGENATION OF VEGETABLE OILS

- OIL IS REACTED WITH HYDROGEN GAS IN PRESENCE OF A CATALYST, UNDER PRESSURE
- THE PRESSURE CAN VARY FROM FRACTION OF A BAR TO 10 TO 15 BARS
- MOST COMMON COMMERCIAL CATALYST IS NICKEL HYDRIDE
- TEMPERATURE MAY RANGE FROM 160°C TO 220°C
- THE REACTION MIXTURE IS MECHANICALLY AGITATED
- HYDROGENATION PROCESS CAN BE EITHER CONTINUOUS OR BATCH
- MOST PRODUCTS ARE MADE IN BATCH REACTORS FOR SPECIFIC REASONS

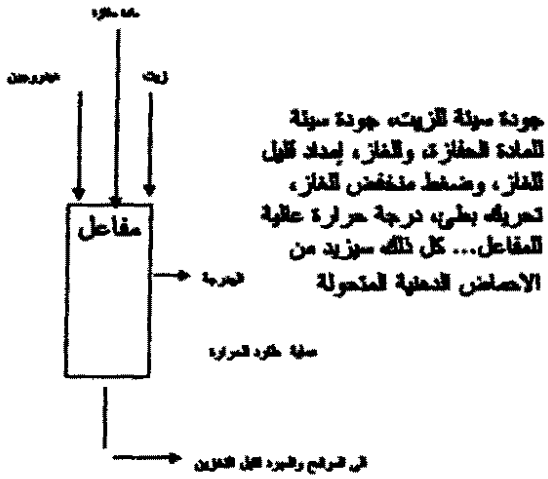
هدرجة الزيوت النباتية

- يتفاعل الزيت مع غاز الهيدروجين في وجود مادة حفازة
- يمكن ان يتنوع الضغط من جزء صغير من ١بار (وحدة قياس الضغط) الى ١٠ – ١٥ بار
- من اكثر المواد الحفازة التجارية الشائعة هو هيدريد النيكل
- قد تتراوح درجة الحرارة من ١٦٠°م الى ٢٢٠ °م
- يتم تحريك خليط التفاعل ميكانيكيا
- يمكن ان تكون عملية الهدرجة متواصلة او على دفعات
- يتم عمل معظم المنتجات في مفاعلات تعمل على دفعات لاسباب معينة

SCHEMATIC DIAGRAM FOR HYDROGENATION REACTOR



رسم تخطيطي لمفاعل الهدرجة



CRITICAL VARIABLES

- BLEACHED OIL QUALITY. CRITICAL IMPURITIES ARE:
  - PHOSPHORUS > 1 PARTS PER MILLION, SOAP AT ANY LEVEL, MOISTURE > 0.1 PER CENT, PEROXIDE VALUE . 4
- HYDROGEN GAS QUALITY. CEITICAL IMPURITIES ARE:
  - MOISTURE, SULFUR COMPOUNDS, HYDROCARBONS
- CONTINUOUS SUPPLY OF GAS
- OPERATING PRESSURE
- OPERATING TEMPERATURE
- DEGREE OF AGITATION
- CATALYST TYPE
- CATALYST QUALITY
- CATALYST CONCENTRATION

العوامل المتغيرة الهامة

- جودة الزيت المبيض، الشوائب الهامة هي:
  - الفوسفور < ١ جزء في المليون، الصابون عند أي مستوى ، الرطوبة < ٠,١ %، حجم البيروكسيد ٤,
- جودة غاز الهيدروجين: الشوائب الهامة هي:
  - الرطوبة ومركبات الكبريت والهيدروكربونات
- التغذية المتواصلة للغاز
- ضغط التشغيل
- درجة حرارة التشغيل
- درجة التحريك
- نوع المادة الحفازة
- جودة المادة الحفازة
- تركيز المادة الحفازة

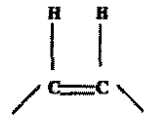
BENEFITS OF HYDROGENATION

- IMPROVES OXIDATIVE STABILITY OF THE OIL
- PROVIDES CERTAIN FUNCTIONAL PROPERTIES TO THE OIL FOR PROVIDING CONSISTENCY TO PRODUCTS, SUCH AS:
- MARGARINE
- ALL PURPOSE SHORTENING
- BAKING SHORTENING
- COOKIE FILLER FAT
- MANY OTHER PRODUCTS

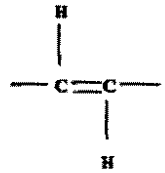
فوائد الهدرجة

- تحسن من ثبات الاكسدة الخاصة بالزيت
- توفر بعض الخواص الوظيفية للزيت لتزويد بعض المنتجات بقوام متماسك
- السمن النباتي الصناعي
- مقصر لكافة الاغراض
- مقصر للخبز
- الدهون التي يتم حشو الكعك
- منتجات كثيرة اخرى

DRAWBACK OF HYDROGENATION
(FORMATION OF TRANS ISOMERS)

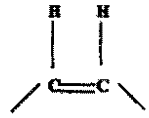


CIS ISOMER

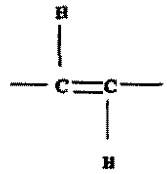


TRANS ISOMER

عيوب الهدرجة
(تكون الايسومرات المتحولة)



CIS ISOMER



TRANS ISOMER

COMMENTS ON TRANS FATTY ACIDS

- THESE ARE UNSATURATED FATTY ACIDS FORMED DURING HYDROGENATION AND TO SOME DEGREE DURING DEODORIZATION
- TRANS FATTY ACIDS HAVE HIGHER MELT POINTS THAN THEIR CORRESPONDING CIS ISOMERS
- TRANS FATTY ACIDS PROMOTE THE FORMATION OF LDL CHOLESTEROL AND TRIGLYCERIDES, JUST LIKE PALMITIC ACID OR MYRISTIC ACID
- EUROPE BECAME MORE CONCIOUS ABOUT THE HEALTH ISSUES CONNECTED WITH TRANS FATTY ACIDS
- UNITED STATES HAS FINALLY DECLARED SOME LABELING REQUIREMENTS FOR TRANS FAT IN FOODS

تعقيبات على الاحماض الدهنية المتحولة

- عبارة عن احماض دهنية غير مشبعة تتكون اثناء عملية الهدرجة واثناء عملية الرائحة بعض الشئ
- يكون لها نقاط انصهار اعلى من ايسومرات CIS المماثلة
- تعزز تلك الاحماض من تكون الكوليستيرول والدهون الثلاثية مثل حامض البلمانيك وحامض الميريستيك
- تهتم اوروبا كثيرا بقضايا الصحة المتعلقة بتلك الاحماض
- أعلنت الولايات المتحدة الامريكية مؤخرا عن بعض متطلبات البطاقات الخاصة بتلك الاحماض الدهنية في الاغذية

ALTERNATIVES TO HYDROGENATION

- FRACTIONATION
- INTERESTERIFICATION
- RANDOM REARRANGEMENT
- DIRECTED REARRANGEMENT
  - FRACTIONATION IS USED IN MAKING VARIOUS PRODUCTS FROM PALM AND PALM KERNEL OILS, THAT ARE FREE OF TRANS FATTY ACIDS
  - INTERESTERIFICATION IS ALSO USED TO MAKE COCOA BUTTER SUBSTITUTES
  - RANDOM AND DIRECTED REARRANGEMENTS ARE USED TO MAKE SPECIALTY PRODUCTS, SUCH AS CANDY COATINGS

بدائل الهدرجة

التجزئة (الفصل الى اجزاء)

- استرة تبادلية
- إعادة التوزيع العشوائي
- إعادة التوزيع المباشر
  - تستخدم التجزئة في عمل منتجات متعددة من زيوت النخيل وزيوت لب النخيل الخالية من الاحماض الدهنية المتحولة
  - تستخدم الاسترة التبادلية كذلكفي عمل بدائل زبدة الكاكاو
  - تستخدم عمليتي اعادة التوزيع العشوائي والمباشر في عمل بعض المنتجات مثل تغطية الحلوى

PRODUCT FORMULATION

- PRODUCTS ARE FORMULATED TO MEET THE REQUIRED PRODUCT PERFORMANCE
- THIS REQUIRES THE USE OF HYDROHENATED OILS WITH VARIOUS DEGREES OF HYDROGENATION AND SOME UNHYDROHENATED OILS

صياغة المنتج

- يتم صياغة تركيبة المنتجات للوفاء بمتطلبات اداء المنتج
- يتطلب ذلك الامر استخدام الزيوت المهدرجة مع درجات مختلفة من الهدرجة وبعض الزيوت الغير مهدرجة

BLENDING

- THIS IS A VERY IMPORTANT STEP IN MAKING SHORTENING, MAYONNAISE OR MARGARINE
- COMPONENTS WITH DIFFERENT DEGREES OF HYDROGENATION OR SOLID CONTENTS ARE BLENDED TOGETHER TO MAKE THE PRODUCTS SUCH AS BAKING SHORTENING, FRYING SHORTENING (DONUT FRYING), PUFF PASTRY, TABLE MARGARINE, SOFT MARGARINE, POURABLE SHORTENING, ETC.
- THE BLENDED PRODUCT IS THEN DEODORIZED

الخلط

- الخلط من الخطوات الهامة لعمل الدهون المقصرة والمايونيز او السمن النباتي الصناعي
- يتم خلط بعض المركبات بدرجات مختلفة من الهدرجة او التي يكون بها محتويات صلبة معا لعمل بعض المنتجات مثل مقصر الخبز ودهون قلي الفطائر والعجائن الهشة وسمن المائدة النباتي الصناعي واللين والدهون المقصرة القابلة للسكب، الخ
- يتم بعد ذلك ازالة الروائح الكريهة من المنتج المخلوط

DEODORIZATION

إزالة الروائح الكريهة

DEODORIZATION PROCESS

- DEODORIZATION IS THE FINAL STEP IN OIL REFINING PROCESS WHERE THE OIL IS STEAM DISTILLED UNDER HIGH VACUUM AND HIGH TEMPERATURE
- THERE ARE BOTH BATCH AND CONTINUOUS DEODORIZERS
- BATCH DEODORIZERS ARE USED WHEN THE PRODUCTION VOLUME IS LOW OR IT IS A VERY SPECIAL PRODUCT
- CONTINUOUS DEODORIZER IS SUITABLE FOR PRODUCING LARGE VOLUME OF OF THE SAME PRODUCT BECAUSE
  - IT IS DIFFICULT TO SHUT DOWN AND RESTART WITH THE NEW FEED
  - CROSS CONTAMINATION IS A MAJOR ISSUE IN THIS TYPE OF PROCESS
  - HOWEVER, THE PROCESS IS MUCH CHEAPER THAN THE BATCH PROCESS

عملية إزالة الروائح الكريهة

- تكـــون العمـــلية الاخيرة في عملية تكرير الزيت حيث يتم تنقية الزيت بالبخار تحت فراغ مرتفع ودرجة حرارة مرتفعة
- تكون العملية اما متواصلة او على دفعات
- تســتخدم عمــلية ازالـــة الروائح على دفعات عندما يكون حجم المنتج اقل او عندما يكون المنتج منتج خاصا
- تكـــون العمـــلية المســـتمرة مناسبة لانتاج حجم اكبر من نفس المنتج نظرا لانه:
  - يصعب غلق جهاز التغذية ثم اعادة تشغيله مرة اخرى
  - من الامور الهامة في هذا النوع من العمليات هي التلوث وانتقاله
  - مـــع ذلك، فان تلك العملية ارخص بكثير من العمليات التي تعمل على دفعات

GENERAL COMMENTS ON DEODORIZATION

- DEODORIZER TEMPERATURE VARIES FROM 240°C TO 260°C
- THE ABSOLUTE PRESSURE IN THE SYSTEM MUST BE < 3 OR < 6 MM OF MERCURY, DEPENDING ON THE DESIGN OF THE UNIT
- WITH POOR VACUUM THE OIL IS OXIDIZED AND PRODUCES OXIDATION POLYMERS, THERMAL POLYMERS, CYCLIC COMPOUNDS, TRANS FATTY ACIDS, ETC.
- THESE COMPOUNDS REDUCE THE SHELF LIFE OF THE OIL AS WELL AS THE PRODUCTS MADE WITH THE OIL
- DEODORIZED OILS ARE RECOMMENDED TO BE COOLED DOWN AND PROTECTED UNDER NITROGEN FOR MAXIMUM STORAGE LIFE

تعقيبات عامة على عملية ازالة الروائح الكريهة

- تتنوع درجة الحرارة في تلك العملية من ٢٤٠°م الى ٢٦٠ °م
- يجب ان يكون الضغط المطلق في النظام <٣ او < ٦مم استنادا على تصميم الوحدة
- مع وجود فراغ سيئ، يتاكسد الزيت وينتجبوليمرات اكسدة، وبوليمرات حرارية، ومركبات حلقية واحماض دهنية متحولة الخ
- تقلل تلك المركبات من فترة صلاحية الزيت وكذلك المنتجات المصنوعة منه
- يوصى بان يتم تبريد الزيوت المزال منها الروائح الكريهة وحمايتها عند النيتروجين للوصول الى اقصى درجة من التخزين

PROTECTING OILS AFTER PROCESSING

حماية الزيوت بعد تصنيعها

AREAS TO PAY ATTENTION

- STORAGE AFTER DEODORIZATION
- LOADING TRUCKS
- TRANSPORTATION
- RECEIVING / UNLOADING
- STORAGE AFTER UNLOADING
- UTILIZATION

بعض النقاط التي ينبغي مراعاتها

- التخزين بعد ازالة الروائح
- تحميل الشاحنات
- النقل
- الاستلام / التفريغ
- التخزين بعد التفريغ
- الاستخدام

STORAGE OF OILS AFTER DEODORIZATION

- SATURATE THE OIL WITH NITROGEN GAS AS THE OIL LEAVES THE DEODORIZER
- TEMPERATURE BELOW 30°C FOR LIQUID OILS
- TEMPERATURE NO HIGHER THAN COMPLETE ,ELTING POINT OG HYDROGENATED FAT
- FOR SHORTENINGS OR MARGARINE, NO HIGHER THAN 5°C ABOVE MELT POINT
- SATURATE WITH NITROGEN BEFORE STORAGE

تخزين الزيوت بعد ازالة الروائح الكريهة منها

- تشبيع الزيت بغاز النيتروجين عندما يترك الزيت جهاز ازالة الروائح
- ينبغي ان تكون درجة الحرارة اقل من ٣٠ °م بالنسبة للزيوت السائلة
- لا يجب ان تزيد درجة الحرارة عن درجة الانصهار أعلى من الدهون المهدرجة
- بالنسبة للسمن الصناعي النباتي، لا يجب ان تكون درجة الحرارة اعلى من درجة الانصهار بـــ ٥°م
- تشبيع الزيت بالنيتروجين قبل التخزين

STORAGE OF OILS AFTER DEODORIZATION (CONT)

- **IMPORTANT CONSIDERATIONS**
  - NITROGEN BLANKET
  - OXYGEN LESS THAN 0.5 % IN HEAD-SPACE
  - CHECK PV, FLAVOR DAILY
  - FOR HYDROGENATED FAT, USE MECHANICAL AGITATION IN THE STORAGE TANK

تخزين الزيوت بعد ازالة الروائح الكريهة منها (تابع)

اعتبارات هامة

- الغطاء النيتروجيني
- يكون الاكسجين اقل من ٠,٥% في الفراغ العلوي
- فحص حجم البيروكسيد والنكهة يوميا
- بالنسبة للدهون المهدرجة يتم استخدام التحريك الميكانيكي في صهريج التخزين

LOADING TRUCKS

- BOTTOM FILL TRUCKS TO AVOID AIR ENTRAINMENT
- SATURATE THE OIL WITH NITROGEN AS THE TRUCK IS BEING LOADED
- FOLLOW THE SAME TEMPERATURE GUIDELINES FOR TRUCK LOADING AS THAT FOR THE STORAGE OF DEODORIZED OILS

تحميل الشاحنات

- ملء الجزء السفلى من الشاحنات لتفادي انحباس الهواء
- تشبيع الزيت بالنيتروجين عندما يتم تحميل الشاحنة
- اتـباع نفس الخطوط الارشادية لدرجة الحرارة بالنسبة لتحميل الشاحنة بحيث تكون مثل درجات حرارة تخزين الزيوت المزال منها الروائح

TRANSPORT OF OIL

- TRUCK DELIVERY IS FOR SHORT DISTANCE. IT IS TYPICALLY LESS THAN 24 HOURS BETWEEN LOADING AND DELIVERY
- FILL THE TRUCK TO THE TOP, TO MINIMIZE AIR ENTRAINMENT DURING TRANSPORT
- USE INSULATED TRUCKS OR TRUCKS WITH EXTERNAL HEATING COILS

نقل الزيت

- يكون التسليم بواسطة الشاحنات لمسافات قصيرة ويستغرق الامر اقل من ٢٤ ساعة بين التحميل والتسليم.
- ينبغي ملء الشاحنة تماما للاقلال من احتباس الهواء اثناء النقل.
- ينـــبغي استخدام الشاحنات المعزولة او الشاحنات المزودة بملفات تسخين خارجية.

RECEIVING

- CHECK INCOMING OIL QUALITY FOR: PV, FFA, AV, FLAVOR
- CHECK OIL TEMPERATURE - MAXIMUM RECOMMENDED TEMPERATURE IS 65°C
- PV FOR INCOMING OIL - 1.0 MAX.
- THE OIL MUST NOT BE UNLOADED IF THE ANALYTICAL STANDARDS ARE NOT MET

الاستلام

- فحص جودة الزيت الوارد وعلى وجه الخصوص: حجم البيروكسيد، والحامض الدهني الحر، وحجم الانيسيدين والنكهة
- فحص درجة حرارة الزيت – واقصى درجة حرارة يوصى بها هي ٦٥°م.
- ينبغي ان يكون الحد الاقصى لحجم البيروكسيد في الزيت الوارد هو ١,٠
- لا يجب ان يتم تفريغ الزيت في حالة عدم الوفاء بالمعايير التحليلية

UNLOADING

- USE STEAM WITH LESS THAN 15 PSIG PRESSURE
- USE THERMODYNAMIC STEAM TRAP ON THE EXHAUST COIL
- SATURATE OIL WITH NITROGEN WHILE UNLOADING
- USE NITROGEN BLANKET IN THE OIL STORAGE TANKS

التفريغ

- استخدام البخار باقل من ١٥ باوند لكل بوصة مربعة
- استخدام مصيدة بخار ديناميكية حرارية على ملف العادم
- تشبيع الزيت بالنيتروجين اثناء التفريغ
- استخدام غطاء نيتروجيني في صهاريج تخزين الزيت

STORAGE OF OIL

- CHECK OIL TEMPERATURE, PV, FLAVOR DAILY
- DO NOT STORE OIL FOR MORE THAN 4 WEEKS
- CLEAN OIL STORAGE TANKS ONCE EVERY YEAR
- MAINTAIN OIL TEMPERATURE AS RECOMMENDED EARLIER
- ADJUST NITROGEN BLANKET IF OXYGEN IN THE TANK HEAD SPACE IS HIGH

تخزين الزيت

- فحص درجة حرارة الزيت وحجم البيروكسيد والنكهة يوميا
- لا يجب تخزين الزيت لاكثر من ٤ اسابيع
- ينبغي تنظيف صهاريج تخزين الزيت مرة كل عام
- الاحتفاظ بدرجة حرارة الزيت كما هو موصى به مسبقا
- ضبط الغطاء النيتروجيني اذا كان الاكسجين مرتفعا في الفراغ العلوي من الصهريج

SPECIAL COMMENTS
(TRUCK LOADING)

- BOTTOM FILL
- USE COMPARTMENTED TRUCKS IF RECEIVING LESS THAN TRUCK-LOAD OF OIL
- MIX ANTIOXIDANTS PROPERLY - NOT IN THE TRUCKS

تعقيبات خاصة

- ملء الجزء السفلي
- استخدام الشاحنات المحتوية على حجيرات صغيرة بداخلها اذا كان سيتم استلام شحنات من الزيت اقل من حمولة الشاحنة
- مزج مضادات الاكسدة جيدا – ويتم هذا الاجراء خارج الشاحنات وليس بداخلها

SPECIAL NOTES
(OIL STORAGE TANK)

- CLEAN OIL STORAGE TANKS AT LEAST ONCE EVERY YEAR
- FOLLOW TEMPERATURE GUIDELINES AS MENTIONED EARLIER

ملاحظات خاصة

- ينبغي تنظيف صهاريج تخزين الزيت مرة واحدة كل عام على الاقل
- اتباع الخطوط الارشادية لدرجات الحرارة كما ذكرنا آنفا

OIL STORAGE & USE AT DESTINATION

- CHECK TEMPERATURE, PV, FLAVOR, NITOGEN BLANKET EVERYDAY
- CORRECT ANY TEMPERATURE OR NITROGEN BLANKET ISSUE
- USE UP THE OIL QUICKLY IF THE PV BEGINS TO RISE
- DO NOT USE OIL IF THE FLAVOR IS BAD

تخزين الزيت واستخدامه في الجهة المقصودة

- ينبغي فحص درجة الحرارة وحجم البيروكسيد والنكهة والغطاء النيتروجيني يوميا
- تصحيح أي مشكلة تتعلق بدرجة الحرارة او الغطاء النيتروجيني
- ينبغي استهلاك الزيت سريعا على الفور اذا بدأ حجم البيروكسيد في الارتفاع
- لا يجب استخدام الزيت اذا كانت النكهة سيئة

QUALITY CONTROL

مراقبة الجودة

WHY IS QUALITY CONTROL NEEDED?

- QUALITY CONTROL IS NEEDED INORDER TO:
- MAINTAIN CONSISTANT PRODUCT STANDARD THIS INDIRECTLY HELPS MAINTAIN PROPER PROCESS STANDARDS
- IT REDUCES PRODUCT LOSSES DUE TO REJECTION BY QUALITY CONTROL OR BY THE CUSTOMER
- IT REDUCES THE COST OF REPROCESSING OR REPACKING FINISHED PRODUCTS
- IT REDUCES PROCESS LOSSES
- **FINALLY, IT REDUCES COST!**

لماذا تكون مراقبة الجودة متطلبة؟

- تكون مراقبة الجودة متطلبة للاسباب التالية:
- للاحـــتفاظ بالمعايير الثابتة للمنتج. وهذا الامر يساعد بشكل غير مباشر على الحفاظ على المعايير السليمة والدقيقة للعملية التشغيلية
- لا ن مراقـــبة الجودة تقلل من خسائر المنتج نظرا لرفض المنتج الغير سليم بسبب مراقبة الجودة او المستهلك نفسه
- لأنها تقلل من تكلفة اعادة التصنيع او اعادة تعبئة المنتجات النهائية
- لأنها تقلل من الفاقد في عملية التصنيع
- **وأخيراً، لأنها تقلل من التكاليف!**

FACTORS ESSENTIAL FOR QUALITY MANAGEMENT

- **MUST HAVE WELL DEFINED OBJECTIVES ON:**
  - QUALITY STANDARDS
  - PRODUCT STANDARDS
  - PROCESS OPERATING STANDARDS
- **DEVELOP & IMPLEMENT A QUALITY PROGRAM**
- **HAVE PROGRAM IN PLACE TO:**
  - TRAIN PROCESS PERSONNEL TO DEVELOP AN UNDERSTANDING OF GOOD OIL PROCESSING
  - TRAIN MAINTENANCE PERSONNEL ON ROUTINE AND PREVENTATIVE MAINTENANCE

العوامل الضرورية لإدارة الجودة

- يجب ان يكون هناك اهداف محددة فيما يتعلق بما يلي:
  - معايير الجودة
  - معايير المنتج
  - معايير تشغيل العملية
- وضع برنامج الجودة وتنفيذه
- إعداد برنامج جيد:
  - لـتدريب العاملين المشغلين للعملية لمساعدتهم على التفهم الجيد لعملية تصنيع الزيت السليمة
  - لتدريب عمال الصيانة على عمليات الصيانة الروتينية والوقائية

EXAMPLES OF QUALITY OBJECTIVES

- 100 PER CENT COMPLIANCE WITH THE FINISHED PRODUCT STANDARDS
- 100 PER CENT COMPLIANCE WITH THR PROCESS OPERATING STANDARDS
- ZERO DEFECTS ON ALL PRODUCTS SHIPPED
- ZERO CUSTOMER REFUSAL FOR NON-COMPLIANCE OF QUALITY
- PRODUCT REPROCESSING IS < 2 PER CENT OF PRODUCTION
- PRODUCT MEET THE REQUIRED SHELF LIFE

أمثلة لاهداف الجودة

- الالتزام بدرجة ١٠٠ % بمعايير المنتج النهائي
- الالتزام بدرجة ١٠٠ % بالمعايير التشغيلية للعملية
- عدم وجود عيوب على الاطلاق في المنتجات التي يتم شحنها
- عدم رفض العملاء على الاطلاق للمنتج بسبب عدم مطابقته للجودة
- تكون عملية اعادة تصنيع المنتج < ٢% من الانتاج
- وفاء المنتج بفترة بقائه المتطلبة

QUALITY STANDARDS

- **THIS IS ESTABLISHED ON THE BASIS OF:**
- SPECIFIC PRODUCT AND ITS APPLICATION
- QUALITY REQUIREMENTS TO MEET CUSTOMER'S NEEDS
- SHELF LIFE REQUIREMENT FOR MARKETING AND DISTRIBUTION OF THE PRODUCT
- COMPETITIVE EDGE OVER COMPETITOR'S PRODUCTS

معايير الجودة

يتم وضع تلك المعايير على اساس ما يلي:

- منتج معين ومواصفاته
- متطلبات الجودة للوفاء باحتياجات العملاء
- احتياجات فترة بقاء المنتج ليتم تسويقه وتوزيعه
- وجود ميزة تنافسية عن منتجات المنافسين

QUALITY GUIDELINES

- **CRUDE OIL**
- FILTER & COOL CRUDE OIL TO 40°C AFTER SOLVENT REMOVAL AND STORAGE
- KEEP THE OIL COOL AND AVOID EXPOSURE TO AIR
- MAINTAIN PEROXIDE VALUE < 4.0 AND AN ANISIDINE VALUE OF <2 DURING STORAGE

- **REFINED OIL**
- FFA = 0.01 - 0.02 %
- SOAP = < 500 PPM, & NEVER > 1000 PPM
- PHOSPHORUS <3 PPM

الخطوط الارشادية للجودة

الزيت الخام

- يتم ترشيح الزيت الخام وتبريده عند ٤٠°م بعد ازالة المادة المذيبة وتخزينه
- الاحتفاظ بالزيت باردا وتفادي تعرضه للهواء
- الاحتفاظ بحجم البيروكسيد عند <٤,٠ وبحجم الانسيدين عند <٢ طوال فترة التخزين

الزيت المكرر

- الحامض الدهني الحر: ٠,٠١ – ٠,٠٢ %
- الصابون: <٥٠٠ جزء في المليون ولا يجب ان يكون > ١٠٠٠ جزء في المليون على الاطلاق
- الفوسفور: <٣ جزء في المليون

QUALITY GUIDELINES (CONT)

- **WATER WASHED OIL**
- FFA = 0.02 - 0.05 %
- SOAP < 100 PPM

- **BLEACHED OIL**
- SOAP = 0 PPM
- PHOSPHORUS <1 PPM
- IRON <0.3 PPM
- CALCIUM < 0.2 PPM
- MAGNESIUM < 0.2 PPM
- CHLOROPHYLL(Soybean & Canola) < 30 PPB
- PEROXIDE VALUE = 0
- ANISIDINE VALUE <2

الخطوط الارشادية (تابع)

الزيت المغسول بالماء

- الحامض الدهني الحر: ٠,٠٢ – ٠,٠٥ %
- الصابون < ١٠٠ جزء في المليون

الزيت المبيض

- الصابون = صفر جزء في المليون
- الفوسفور <١ جزء في المليون
- الحديد < ٠,٣ جزء في المليون
- الكالسيوم < ٠,٢ جزء في المليون
- الماجنيسيوم < ٠,٢ جزء في المليون
- الكلوروفيل (فول الصويا و كانولا < ٣٠ جزء في البليون
- حجم البيروكسيد = صفر
- حجم الانيسيدين < ٢

QUALITY GUIDELINES (CONT)

- **HYDROGENATED OIL**
  - IODINE VALUE= MEET SPECIFICATION
  - SOLIDS (SFI or SFC) = MEET SPECIFICATIONS
  - NICKEL < 0.5 PPM
  - FFA NOT TO EXCEED 0.1 % OVER FFA IN THE BLEACHED OIL

- **DEODORIZED OIL**
- FFA < 0.05 %
- PV = 0
- ANISIDINE VALUE < 4
- FLAVOR = BLAND (EXCEPT FOR OLIVE OIL)
- FILTER GRADE = 8 MIN.
- PHOPHORUS < 1 PPM
- IRON < 0.3 PPM
- CALCIUM < 0.2 PPM
- MAGNESIUM < 0.2 PPM
- LOVIBOND COLOR = MEET STANDARD
- AOM or OSI = MEET STANDARD

الخطوط الارشادية (تابع)

الزيت المهدرج

- حجم اليود – المطابقة مع المواصفات
- المواد الصلبة (مؤشر المواد الصلبة أو محتوى الدهون الصلبة)– المطابقة مع المواصفات
- النيكل < ٠,٥جزء في المليون
- لا يجب ان تزيد الاحماض الدهنية الحرة عن ٠,١عن الاحماض الدهنية الحرة الموجودة في الزيت المبيض

الزيت المزال منه الروائح الكريهة

- الاحماض الدهنية الحرة < ٠,٠٥ %
- حجم البيروكسيد – صفر
- حجم الانيسيدين < ٤
- النكهة – غير مثيرة (فيما عدا زيت الزيتون)
- درجة الترشيح – ٨ دقائق
- الفوسفور < ١ جزء في المليون
- الحديد ٠,٣ جزء في المليون
- الكالسيوم < ٠,٢ جزء في المليون
- الماجنيسيوم < ٠,٢ جزء في المليون
- تركيز الالوان في المحاليل – تفي بالمعايير
- طريقة الاكسجين النشط أو مؤشر الثبات التأكسدي – يفي بالمعايير

TYPICAL QUALITY ANALYSES

- FREE FATTY ACID
- PEROXIDE VALUE (PV)
- ANISIDINE VALUE (AV)
- LOVIBOND COLOR
- OXIDATIVE STABILITY INDEX (OSI)
- ACTIVE OXYGEN METHOD (AOM)
- SOLID FAT INDEX (SFI)
- SOLID FAT CONTENT (SFC)
- IODINE VALUE
- MELT POINT
- SMOKE POINT
- POLAR MATERIAL
- POLYMERS
- IRON
- NICKEL
- PHOSPHORUS
- CALCIUM/MAGNESIUM
- OTHER METALS
- FLAVOR

التحليل الامثل للجودة

- حامض دهني حر
- حجم البيروكسيد (PV)
- حجم الانيسيدين (AV)
- تركيز الالوان في المحاليل
- مؤشر الثبات التأكسدي (OSI)
- طريقة الاكسجين النشط (AOM)
- مؤشر الدهون الصلبة (SFI)
- محتوى الدهون الصلبة (SFC)
- حجم اليود
- نقطة الانصهار
- نقطة التدخين
- مادة قطبية
- بوليمرات
- حديد
- نيكل
- فوسفور
- كالسيوم / ماجنيسيوم
- معادن اخرى
- النكهة

QUALITY METHODS

ANALYSIS	AOCS METHOD	IU PAC METHOD / ISO	AOAC METHOD
% FFA	C_a - 5a - 40	2.201 / 660.1996	940.28
PEROXIDE VALUE (PV)	C_d - 8b - 90		965.33
ANISIDINE VALUE (AV)	C_d – 18 - 40		
LOVIBOND COLOR	C_c – 13b – 45		
OXIDATIVE STABILITY INDEX (OSI) OR EQUIVALENT ACTIVE OXYGEN METHOD (AOM)	C_d – 12 - 57		

طرق الجودة

التحليل	طريقة جمعية كيميائي الزيوت الامريكية	طريقة IU PAC / ISO	طريقة جمعية الحلفين الكيميائيين الرسمية
الحامض الدهني الحر%	C_a – 5a- 40	2.201 / 660.1996	940.28
حجم البيروكسيد	C_d-8b-90		965.33
حجم الانيسيدين	C_d-18-40		
تركيز اللون في السائل	C_c-13b-45		
مؤشر الثبات التأكسدي او طريقة الاكسجين	C_d-12-57		

QUALITY METHODS

ANALYSIS	AOCS METHOD	IU PAC METHOD / ISO	AOAC METHOD
SOLID FAT INDEX (SFI)	C_d - 10 – 57		
SOLID FAT CONTENT (SFC)	C_d – 16b – 81		
IODINE VALUE	C_D – 1c – 85		993.20
SMOKE POINT			
MELT POINT	C_d – 20 – 90		
POLAR MATERIAL	C_d – 22 – 91		982.27

طرق الجودة

التحليل	طريقة جمعية كيميائي الزيوت الامريكية	طريقة IU PAC / ISO	طريقة جمعية المحللين الكيميائيين الرسمية
مؤشر الدهون الصلبة	C_d – 10- 57		
محتوى الدهون الصلبة	C_d-16b-81		
حجم اليود	C_d-1c-85		993.20
نقطة التدخين			
نقطة الانصهار	C_d-20-90		
المادة القطبية	C_d-22-91		982.27

QUALITY METHODS

ANALYSIS	AOCS METHOD	IU PAC METHOD/ISO	AOAC METHOD
FATTY ACID COMPOSITION		2.301,302,303 / 5508-1990,5509-2000	
TRANS FATTYACIDS		2.304 / 15304-2001	
STEROLS		2.403 / 12228-1999	
ERITHRODIOL		2.431	
ALA TOCOPHEROL		2.432 / 9936-1997	
TRIGLYCERIDE ANALYSIS		2.507 (PURIFICATION PRIOR TO ANALYSIS, 2.324)	986.19

طرق الجودة

التحليل	طريقة جمعية كيميائي الزيوت الامريكية	طريقة IU PAC / ISO	طريقة جمعية المحللين الكيميائيين الرسمية
تركيب الحامض الدهني		2.301,302,303 / 5508-1990,5509-2000	
الاحماض الدهنية المتحولة		2.304 / 15304-2001	
الستيرولات		2.403 / 12228-1999	
اريثروديول		2.431	
الاوكوفيرول		2.432 / 9936-1997	
ثلاثي الجليسريد	2.507(التنقية قبل التحليل، 2.324)		986.19

QUALITY METHODS

ANALYSIS	AOCS METHOD	IU PAC METHOD/ISO	AOAC METHOD
POLYMERS			993.25
SAMPLE PREPARATION		/ 661-1989, 5555-2001	981.11

طرق الجودة

التحليل	طريقة جمعية كيميائي الزيوت الامريكية	طريقة IU PAC / ISO	طريقة جمعية المحللين الكيميائيين الرسمية
بوليمرات			993.25
			981.11
اعداد العينات		/ 661-1989, 5555-2001	

OLIVE OIL QUALITY

Olive oil is a *natural product*; a real "*fruit juice*" compared with the vast majority of vegetable oils which are extracted from ground oily seeds and therefore need the help of solvents.

Quality olive oil is an oily juice obtained from olives in perfect ripe conditions from a *healthy olive tree*. The oil is obtained from *fresh fruit* and any handling or treatment that alters the *chemical properties* of its components must be avoided, both during *extraction* and *storage*.

It is important to differentiate between "oil varieties" and "oil quality". Two different olive tree families produce different olive oil varieties, each one with a different colour, aroma and flavour, but both can have the same quality, because quality depends on a combination of factors, such as climate, the type of soil, the care taken during the production process, storage, etc.

An oil's quality is defined by the following criteria, among others:

- Quality parameters
- Sensory analysis

1. Chemical parameters

- degree of acidity (quantity of fatty acids expressed as oleic acid). This is a result of, among others, the bad condition of the fruit, bad handling or bad storage. The degree of acidity of an oil IS NOT RELATED TO THE FLAVOUR
- peroxide index This determines the initial oxidation of the oil and the deterioration of the natural antioxidants, such as tocopherols and polyphenols, may have suffered.
- ultraviolet absorbency This is used to detect any oil's abnormal component.

2. Sensory analysis

- organoleptic characteristics (sensations sense organs can detect, mainly related to smell and taste) They are defined by experts by tasting.

Each type of tree, depending on the producing area, has a different variety of fruit and therefore produces an oil with different chemical and organoleptic properties. And within the same variety, different oils are produced depending on factors such as microclimate, the type of soil, altitude...

Tasting is therefore necessary to appreciate the character and personality of an olive oil. Tasting tells you the characteristic aroma and flavour of each oil. It is important to realise that THE COLOUR DOES NOT REFLECT THE QUALITY, which is why professional tasters use a dark blue glass so that they are not influenced by the colour.

An olive oil's aroma is appreciated by gently heating the glass and breathing in the fragrances several times to compare them with vegetable aromas (grass, fruit, etc.). As far as flavour is concerned, a few drops are enough to notice the oil's sweetness. An acid flavour is detected towards the centre of the tongue and when the oil is in contact with the palate. Bitterness or a peppery flavour is noticed at the back of the mouth. Tasters normally pour a little oil onto the tip of their tongues and then, by breathing in slowly and continuously, the oil covers the whole palate and then goes back towards the throat.

Among the attributes that can define an oil are, for example:

- **Almondy**: There can be two types: the typical fresh almond one or the dry healthy almond one. It is appreciated as an aftertaste when the oil stays in contact with the tongue or the palate and it is associated with sweet oils with a mild aroma.

- **Bitter**: This is a characteristic flavour of oil obtained from green olives: it can be more or less pleasant, depending on its intensity.

- **Mild**: This is neither a negative nor a positive attribute of an oil whose organoleptic characteristics are very weak, as its aromatic components have been lost.

- **Sweet**: This is a gentle and pleasant flavour of an oil which is not exactly sugary, but the bitter, astringent or peppery attributes do not predominate.

- **Fruity**: It reminds us of the aroma and flavour of healthy, fresh fruit picked when it is just ripe.

- **Ripe fruit**: This recalls olive oil obtained from ripe fruit and generally has a mild aroma and sweet taste.

- **Grassy**: Aroma and flavour characteristic of some oils that remind us of cut grass.

- **Green leaves**: Aroma and flavour of oil obtained from over-green olives or which has been ground mixed with leaves and stalks.

- **Apple**: Aroma and flavour of olive oil that reminds us of apples.

Did you know

Oil facts

- Air, light and heat affect olive oil, so you should make sure the bottle is properly closed and store it at room temperature out of direct sunlight.
- As olive oil is a natural product, it does not improve with age, unlike wine. It should therefore be used as soon as possible in the year it was produced.
- However, depending on the variety, a well preserved oil can last up to 18 months without losing its organoleptic qualities.
- An oil's colour does not indicate quality.

Cultivation facts

- Between 4 and 5 kilos of olives are needed to produce 1 litre of olive oil.
- The tree's age only affects the quantity produced, not the quality. An olive tree starts to produce between the ages of 5 and 10, and its production starts to decline after it is 100 years old.
- An olive's colour does not depend on the variety but on how ripe it is. Olives are green at the beginning and become black as they ripen.

Health and beauty facts

- Virgin olive oil is a pure fruit juice with no additives or preservatives.
- Olive oil contains between 60 and 80% of monounsaturated fats (in this case, oleic acid), which help to reduce "bad cholesterol" (LDL) and preserve "good cholesterol" (HDL). It also has just the right amount of linoleic acid, which is essential for the human diet, although in excess it can cause oxidation, which is harmful for our health.
- Olive oil has vitamins A, D, K and especially E.
- Researchers confirm that olive oil reduces the risk of heart diseases and some types of cancer. It also helps to maintain a low blood pressure and to alleviate arthritis.
- The majority of the medical community thinks that olive oil is antioxidant, helps the cardiovascular flow and delays the cell ageing process.
- Olive oil aids digestion and helps the body to absorb calcium.
- Among other properties, olive oil helps to improve the appearance and texture of our skin.

Food notes

- When dressing salads and vegetables, pour the olive oil first, then add salt and vinegar or lemon. Adding the oil first provides a protective layer which helps the vegetables to stay fresh and crisp.
- Olive oil is excellent to spread on meat in a barbecue, as it helps to preserve the natural juices.
- Olive oil adds flavour to charcoal-grilled meat whilst it is browning. The meat must be cooked on a low heat with only a little oil.
- Any meat, fish or vegetable fried in olive oil is tastier, as the oil prevents the food from sticking and becoming greasy.
- Meat marinated in olive oil before cooking will be much tastier. As olive oil is a very delicate product, it helps the aromatic herbs and spices to mix.
- To enjoy a delicious snack, spread a little garlic on toast and then add some olive oil.
- If you use a little olive oil in the water when cooking pasta, it will prevent it from sticking.
- Sauté meat and vegetables in olive oil for a few minutes until they are tender and season.

PACKAGING & DISTRIBUTION

التعبئة والتوزيع

PACKAGING

- **CRITICAL FACTORS**
  - THE PRODUCT MUST BE ANALYZED AND CERTIFIED
  - STORAGE TANKS SHOULD HAVE NITROGEN GAS PROTECTION
  - OXYGEN IN TANK HEAD SPACE < 0.5 PER CENT
  - THE OIL IS SATURATED WITH NITROGEN WITH A DIFFUSER BEFORE IT GOES TO THE FILLER SURGE TANK
  - THE BOTTLES OR CANS SHOULD BE PURGED WITH NITROGEN BEFORE FILLING WITH OIL
  - OIL IS FILLED FROM BOTTOM UP WITH LITTLE SPLASHING
  - THE OXYGEN CONTENT IN THE BOTTLE OR CAN IS RECOMMENDED TO BE < 2 PER CENT

التعبئة

العوامل الهامة

- يجب أن يتم تحليل المنتج واصدار شهادة لاجازته
- ينبغي ان تحتوي صهاريج التخزين على غاز النيتروجين كنوع من الوقاية
- تكون نسبة الاكسجين في الفراغ العلوي من الصهريج < ٠,٥ %
- يتم تشبيع الزيت بالنيتروجين بواسطة جهاز نشر النيتروجين قبل ذهابه الى صهريج الملء
- ينبغي ان يتم تطهير الزجاجات او العلب بالنيتروجين قبل ملئها بالزيت
- يتم ملء الزيت من اسفل لاعلى مع الرش الخفيف
- يوصى ان يكون محتوى الاكسجين في الزجاجة او العلبة حوالب < ٢ %

STORAGE

- FOR LIQUID OILS, STORAGE ROOM SHOULD BE MAINTAINED AT 21°C, OR LOWER, AND NO EXPOSURE TO LIGHT
- FOR MARGARINE, TEMPERATURE SHOULD BE 5°C, OR LOWER
- FOR BAKING FAT OR OTHER TYPES OF SOLID SHORTENING, THE RECOMMENDED STORAGE TEMPERATURE IS 24°C OR LOWER
- PV FFA AND FLAVOR SHOULD BE CHECKED ONCE EVERY WEEK IN THE TANKS, ONCE EVERY MONTH IN THE BOTTLES AND CANS

التخزين

- بالنسبة للزيوت السائلة، ينبغي ان تكون درجة حرارة غرفة التخزين ٢١ °م أو اقل شريطة عدم تعرض الزيت للضوء
- بالنسبة للسمن النباتي الصناعي، ينبغي ان تكون درجة الحرارة ٥ °م او اكثر
- بالنسبة لدهون الخبز او الانواع الاخرى من السمن المقصر، تكون درجة الحرارة الموصى بها ٢٤ °م أو أقل
- ينبغي فحص حجم البيروكسيد والاحماض الدهنية الحرة والنكهة مرة كل اسبوع في الصهاريج، ومرة كل شهر في الزجاجات والعلب

SHIPPING

- **BULK**
  - INSPECT THE TANK TRUCK FOR CLEANLINESS. IT SHOULD BE FREE OF ODOR AND USED FOR FOOD PRODUCTS IN THE PREVIOUS SHIPMENT
  - SATURATE THE OIL WITH NITROGEN AS IT IS LOADED
  - FILL THE TANK TRUCK TO THE TOP
  - USE COMPARTMRNTALIZED TRUCK FOR SMALLER SHIPMENT
- **PACKAGED PRODUCT**
  - INSPECT THE TRUCK. IT MUST BE CLEAN AND WITH NO ROOF OR WALL DAMAGED OR LEAKS
  - MUST HAVE BEEN USED IN SHIPPING FOOD PRODUCTS BEFORE

الشحن

شحن المنتج الغير معبأ:

- فحص صهاريج الشاحنة للتأكد من نظافتها. وينبغي ان تكون خالية من الـروائح وان يكـون قـد تـم شحن منتجات غذائية فيها مسبقا وليس منتجات اخرى.
- تشبيع الزيت بالنيتروجين عند تحميله.
- ملء صهاريج الشاحنة لقمتها.
- استخدام الشاحنات المقسمة الى حجيرات صغيرة للحمولة الصغيرة

المنتج المعبأ:

- فحص الشاحنة والتاكد من نظافتها وعدم احتوائها شقوق في سطحها او جدرانها لمنع التسرب
- يجـب ان يكـون قد تم شحن منتجات غذائية فيها مسبقا وليس منتجات اخرى.

FRYING

القلـــي

DESCRIPTION OF FRYING

- FRYING IS A PROCESS WHERE THE FOOD PRODUCT IS EITHER DIPPED INTO A BED OF HOT OIL OR THE FOOD IS PLACED ON A THIN LAYER OF OILAND HEATED FROM THE BOTTOM OF THE PAN
- IN EITHER CASE THE OIL IS MAINTAINED AT A HIGH TEMPERATURE, RANGING FROM 150° TO 215°C
- FRYING PROCESS CONTINUES UNTIL THE BUBBLING STOPS, MOISTURE IN THE FOOD DROPS TO A LOW VALUE AND THE FOOD TURNS GOLDEN BROWN IN COLOR

وصف عملية القلي

- القلي هو عملية يتم فيها غمس المنتج الغذائي إما في حوض من الزيت الساخن أو يتم وضع المنتج الغذائي على طبقة رقيقة من الزيت ويتم تسخينه في قلاية
- وفي كلتا الحالتين، ينبغي ان تكون درجة حرارة الزيت مرتفعة بحيث تتراوح ما بين ١٥٠°م الى ٢١٥°م
- تستمر عملية القلي حتى تنتهي الفقاقيع، وحتى تنخفض رطوبة المنتج الغذائي الى اقل درجة ويتحول لون المنتج الى اللون البني الذهبى

DESCRIPTION OF FRYING (CONT)

- WHAT HAPPENS DURING FRYING?
  - A TEMPERATURE GRADIENT IS ESTABLISHED BETWEEN THE SURFACE AND THE INTERIOR OF THE FOOD
  - MOISTURE ESCAPES FROM THE SURFACE OF THE FOOD
  - BROWNING REACTION OCCURS ON THE SURFACE OF THE FOOD
  - A GOLDEN BROWN CRUST IS FORMED ON THE FOOD SURFACE
  - THE FOOD DEVELOPS FRIED FLAVOR
  - THE OIL UNDERGOES DEGRADATION

وصف عملية القلي (تابع)

ما الذي يحدث أثناء عملية القلي؟

- يحدث تدرج في درجة الحرارة بين سطح المنتج الغذائي والجزء الداخلي منه.
- تهرب الرطوبة من سطح الغذاء
- يحدث تفاعل في لون الغذاء بحيث يتحول الى اللون البني
- تتكون طبقة هشة بنية وذهبية اللون على سطح الغذاء
- تكون هناك نكهة القلي في الغذاء
- يبدأ الزيت في التحلل

TYPES OF FRYERS

- FRYING OPERATION CAN BE:
  - BATCH
  - CONTINUOUS
  - VACUUM BATCH

أنواع اجهزة القلي

يمكن ان تكون عملية القلي على هيئة:

- قلي على دفعات
- قلي مستمر
- على دفعات تحت تفريغ

TYPES OF FOOD FRIED

- THE FOOD FRIED CAN BE:
  - MEAT, COATED OR BATTERED
  - CHIPS
  - VEGETABLES/FRUITS
  - DONUTS

أنواع الاغذية المقلية

يمكن ان تكون الاغذية المقلية عبارة عن:

- لحوم مغطاة بالبقسماط او بعجينه من البيض والبقسماط
- رقائق من البطاطس
- خضراوات / فاكهة
- فطائر (دوناتس)

REACTIONS IN THE OIL DURING FRYING

- **THE OIL DECOMPOSES THROUGH CHEMICAL REACTIONS:**
  - AUTOXIDATION
  - HYDROLYSIS
  - THERMAL POLYMERIZATION
  - OXIDATIVE POLYMERIZATION

التفاعلات التي تحدث في الزيت أثناء عملية القلي

يتحلل الزيت بفعل التفاعلات الكيميائية التالية:

- الاكسدة التلقائية
- التحلل بالماء
- البلمرة الحرارية
- البلمرة المؤكسدة

RESULTS OF OIL DECOMPOSITION

- **HDROLYSIS**
  - PRODUCES FREE FATTY ACIDS (FFA)
  - FREE FATTY ACIDS UNDERGO OXIDATION AND POLYMERIZATION FASTER THAN THE TRIGLYCERIDES
- **AUTOXIDATION**
  - PRODUCES ALDEHYDES, KETONES, ACIDS, ALCOHOLS, POLYMERS THAT DEVELOP POOR FLAVOR IN THE WHICH MAY OR MAY NOT BE DETECTABLE IN THE FRESH PRODUCT BUT BECOME STRONG IN THE AGED PRODUCT
- **POLYMERIZATION**
  - HIGH LEVEL OF THERMAL POLYMERS MAKE THE PRODUCT BITTER
  - HIGH LEVEL OF OXIDATIVE POLYMERS MAY NOT BE DETECTABLE IN FRESH PRODUCT BUT REDUCE PRODUCT SHELF LIFE

نتائج تحلل الزيت

التحلل بالماء

- ينتج عنها الاحماض الدهنية الحرة
- التعرض الاحماض الدهنية الحرة الى عملية الاكسدة والبلمرة بشكل اسرع من دهون ثلاثي الجليسريد

الاكسدة التلقائية

- ينتج عنها الالديهيدات والكيتونات والاحماض والكحوليات والبوليمرات التي تنتج نكهة سيئة التي قد يتم اكتشافها او عدم اكتشافها في المنتج الطازج لكن تشتد حدتها كلما قدم المنتج

البلمرة

- زيادة مستوى البوليمرات الحرارية يؤدي الى مرارة طعم المنتج
- قد لا يتم اكتشاف زيادة مستوى البوليمرات المؤكسدة في المنتج الطازج لكنها تقلل من فترة بقاء المنتج

TYPICAL BATCH FRYER OPERATION

- FRYER IS STARTED IN THE MORNING
- FOOD IS ADDED INTO THE FRYER IN DISCRETE AMOUNTS
- THE TEMPERATURE DROPS AND THEN INCREASES AS THE MOISTURE IN THE PRODUCT DECREASES
- THE PRODUCT IS STIRRED MANUALLY OR WITH MECHANICAL STIRRERS
- THE PRODUCT IS REMOVED
- EXCESS OIL IS SEPARATED FOR REUSE
- A SMALL AMOUNT OF OIL IS ADDED TO MAKE UP FOR THE OIL LOST IN FRYING THE FRYER IS CLEANED AT NIGHT AND REFILLED WITH THE FILTERED OIL
- THE OIL IS FILTERED ONCE OR TWICE PER DAY
- SOMETIMES, THE HOT OIL IS LEFT IN THE FRYER WITH HEAT OFF

عملية القلي على دفعات

- يتم تشغيل المقلاة في الصباح
- يضاف الغذاء الى المقلاة بكميات منفصلة
- تنخفض درجة الحرارة ثم تزيد كلما زادت رطوبة المنتج
- يتم تحريك المنتج يدويا او بمحراك ميكانيكي
- يتم ازالة المنتج
- يتم ازالة الزيت الزائد لاعادة استخدامه
- يتم اضافة كمية صغيرة من الزيت لتعويض الزيت الذي تم فقده في المقلاة. ثم تنظف المقلاة في الليل ويعاد ملؤها بالزيت المرشح
- يتم ترشيح الزيت مرة او مرتين في اليوم
- في بعض الاحيان، يتم ترك الزيت في المقلاة دون تسخينه

CONSEQUENCES OF THIS OPERATION

- DECOMPOSITION OF THE OIL
- DARKENING OF THE OIL
- DEVELOPMENT OF "OFF" FLAVORIN THE FOOD
- REDUCTION IN SPECIFIC HEAT OF THE OIL AND HEAT TRANSFER COEFFICIENT
- INCOMPLETE FRYING OF FOODS
- ACCUMULATION OF CUMBS IN THE FRYER

عواقب تلك العملية

- تحلل لزيت
- تحول لون الزيت الي اللون الاسود الداكن
- تكون رائحة غير مرغوب فيها في الغذاء
- اختزال في حرارة الزيت النوعية ومعامل النقل الحراري
- عدم اكتمال قلي الغذاء
- تراكم الترسبات في المقلاة

EXPECTED REACTIONS IN THE OIL

- THE BATCH REACTORS ARE EXPECTED TO EXHIBIT:
  - RAPID RISE OF FREE FATTY ACIDS
  - EXCESSIVE OXIDATION
  - HIGH AMOUNT OF THERMAL & OXIDATIVE POLYMERS
  - NO LONG SHELF LIFE FOR THE PRODUCT

التفاعلات المتوقع حدوثها في الزيت

من المتوقع ان ينتج عن عملية القلي على دفعات ما يلي:

- ارتفاع سريع في الاحماض الدهنية الحرة
- زيادة الاكسدة
- كميات مرتفعة من البوليمرات الحرارية والمؤكسدة
- لن تكون فترة بقاء او صلاحية المنتج طويلة

METHODS FOR IMPROVEMENT OF BATCH FRYIG OPERATION

- MAXIMIZE THE AMOUNT OF FOOD FRIED WITH AS LITTLE DOWNTIME AS POSSIBLE
- INSTALL ACONTINUOUS SIDE STREAM FILTER TO TAKE OUT 5 % OF THE OIL, FILTER AND RETURN IT TO THE FRYER
- DESIGN THE FRYER SYSTEM WITH THE MINIMUM AMOUNT OF OIL VOLUME TO MINIMIZE THE OIL TURNOVER TIME
- COOL DOWN THE OIL TO 60° - 65°C AND FILTER IT AT THE END OF THE OPERATION.
- STORE THE USED OIL IN A NARROW, TALL TANK, NOT IN THE FRYER
- SANITIZE THE FRYER SYSTEM
- SATURATE THE OIL WITH NITROGEN AS IT LEAVES THE COOLER
- HAVE NITROGEN BLANKET IN THE USED OIL TANK
- CHECK THE FREE FATTY ACID ANF PEROXIDE VALUE IN THE OILAFTER TRANSFER AND BEFORE REUSE
- USE A BLEND OF FRESH AND USED OIL AT RE-START
- BLEND BACK THE USED OIL IN A MIXED STREAM WITH FRESH OIL

طرق تحسين عملية القلي على دفعات

- زيادة كمية الغذاء المقلي مع الاقلال بقدر الامكان من زمن التوقف
- تركيب مرشح انسيابي جانبي ومتواصل حتى يأخذ ٥% من الزيت ويقوم بترشيحه واعادته الى المقلاه
- تصميم نظام القلي بحيث يستوعب اقل كمية من حجم الزيت للاقلال من زمن القلب
- ينبغي تبريد الزيت الى درجة ٦٠ °م – ٦٥ °م وترشيحه في نهاية عملية التشغيل
- تخزين الزيت المستخدم في صهريج ضيق وطويل وليس في المقلاه
- تعقيم نظام القلي
- تشبيع الزيت بالنيتروجين عندما يترك جهاز التبريد
- ينبغي ان يكون هناك حاجب نيتروجيني في صهريج الزيت المستخدم من قبل
- فحص الحامض الدهني الحر وحجم البيروكسيد في الزيت بعد نقله وقبل استخدامه
- ينبغي استخدام خليط من الزيت الطازج والمستخدم من قبل عند اعادة بداية
- اعادة خلط الزيت المستخدم مع الزيت الطازج

OBJECTIVES OF FRYING

- PRODUCE PALATABLE FOOD
- MAINTAIN GOOD PRODUCT FLAVOR
- MAINTAIN GOOD PRODUCT TEXTURE
- DO NOT PRODUCE OIL-SOAKED PRODUCT
- DO NOT HAVE THE COATING FALL OFF THE PRODUCT
- MAINTAIN LONG FRY-LIFE FOR THE OIL
- HAVE ACCEPTABLE PRODUCT SHELF LIFE

أهداف القلي

- انتاج منتج غذائي جيد المذاق
- إضفاء نكهة جيدة على المنتج
- إضفاء قواما جيدا على المنتج
- لا يجب انتاج منتج مغمور بالزيت
- يجب ألا تقع تغطية المنتج المقلي
- الاحتفاظ بفترة بقاء المنتج المقلي طويلا
- أن تكون صلاحية او فترة بقاء المنتج مقبولة

PERFORMANCE OF BATCH FRYERS

- BATCH FRYER ALWAYS DAMAGES THE OIL MORE RAPIDLY
- PRODUCT WILL HAVE A TENDENCY TO GO RANCID FASTER THAN THAT MADE IN A CONTINUOUS FRYER
- SIGNIFICANT IMPROVEMENTS IN THE OIL AND PRODUCT QUALITY CAN BE ACHIEVED USING THE GUIDELINES PROVIDED EARLIER

أداء أجهزة التي تعمل على دفعات

- دائما ما تتلف المقلاة الزيت سريعا
- يـتزنخ الزيت بشكل اسرع من استخدامه في مقلاة تعمل بشكل متواصل
- يمكن إجراء عمليات تحسين ملحوظة على جودة الزيت والمنتج باستخدام الخطوط الارشادية المذكورة آنفا

CONTINUOUS FRYERS

- THIS IS USED FOR LARGE SCALE PRODUCTION
- THE FRYERS HAVE CONTINUOUS PRODUCT FLOW AND, SOMETIMES, CONTINUOUS OIL ADDITION
- QUALITY OF OIL CAN BE MAINTAINED BETTER THAT THAN IN A BATCH FRYER
- PRODUCTS CAN HAVE LONGER SHELF LIFE

أجهزة القلي التي تعمل بشكل متواصل

- تستخدم في عمليات الانتاج واسعة النطاق
- يكـــون لأجهـــزة القلي تدفق متواصل للمنتج وفي بعض الاحيان تكون هناك اضافة متواصلة للزيت
- يمكـــن الاحـــتفاظ بجودة المنتج بشكل افضل من المقلاه التي تعمل على دفعات
- يمكن ان تطول فترة بقاء المنتجات أكثر

CONTINUOUS FRYER OPERATION

- THE FRYERS ARE GENERALLY OPERATED 24 HOURS A DAY FOR SEVERAL DAYS IN A ROW
- THE PRODUCT AND HOT OIL ENTER THE FRYER AT ONE END
- THE FRIED PRODUCT AND THE COLDER OIL LEAVE THE FRYER AT THE OTHER END
- THE PRODUCT IS DE-OILED BY DRAINING ON PERFORATED BELT
- THE PRODUCT IS SALTED, SEASONED AND PACKED
- FOR BEST SHELF LIFE THE PRODUCT SHOULD BE PACKED IN METALLIZED FILM WITH GOOD GAS AND LIGHT BARRIER PROPERTY AND NITROGEN FLUSH WITH < 2 % OXYGEN
- THE FRYER SYSTEM MUST BE DESIGNED TO HAVE THE LOWEST POSSIBLE OIL TURNOVER TIME
- USED OIL SHOULD BE HANDLED AS DESCRIBED EARLIER UNDER BATCH FRYER OPERATION

عملية القلي المتواصلة

- يتم تشغيل القلايات عموما لمدة ٢٤ ساعة في اليوم لعدة ايام دون توقف
- يدخل المنتج والزيت الساخن المقلاة من جهة واحدة
- يترك المنتج المقلي والزيت المبرد المقلاة من الجهة الاخرى
- يزال الزيت من المنتج عن طريق التصفية او السير الثقب
- يتم إضافة الملح والتوابل على المنتج وتعبئته
- للحصول على فترة بقاء اطول للمنتج، فانه ينبغي ان يتم تعبئته في رقائق معدنية مع وجود عائق يجد لمنع دخول الغازات والضوء ومع تدفق نيتروجيني مع< ٢% من الاكسجين
- يجب ان يتم تصميم المقلاة بحيث يكون هناك أدنى زمن لتغيير الزيت
- ينبغي التعامل مع الزيت المستخدم كما هو مذكور انفا طبقا لنظام القلي على دفعات

KEYS FOR BEST FRYER PERFORMANCE

- DESIGNED OIL TURNOVER TIME MUST BE MINIMUM
- OPERATE THE FRYER AT THE DESIGNED RATE AND WITHOUT FREQUENT SHUT DOWNS
- COOL THE OIL TO 60° - 65°C AND FILTER OIL AT THE END OF OPERATION
- SATURATE THE OIL WITH NITROGEN AS IT LEAVES THE FRYER
- STORE THE USED OIL UNDER NITROGEN BLANKET
- SANITIZE THE FRYER AT THE END OF OIL TRANSFER
- PACKAGE PRODUCT IN METALLIZED BAGS WITH GOOD GAS AND MOISTURE BARRIER PROPERTY
- USE NITROGEN FLUSH IN THE BAGS WITH < 2 % OXYGEN

القواعد الاساسية لتحقيق الاداء الجيد للمقلاة

- يجب ان يقل زمن القلب الى الحد الأدنى
- يجب ان يتم تشغيل المقلاة بالمعدل المحدد ودون ايقاف متكرر
- يتم تبريد المقلاة عند درجة ٦٠ °م – ٦٥ °م وترشيح الزيت في نهاية عملية التشغيل
- ينبغي تشبيع الزيت بالنيتروجين عند تركه المقلاة
- يتم تخزين الزيت المستخدم تحت غطاء نيتروجيني
- يتم تطهير المقلاة عند نهاية قلب الزيت
- يتم تعبئة المنتج في لفائف معدنية مع وجود حاجز جيد للغاز والرطوبة
- استخدام دفق نيتروجيني في الاكياس مع < ٢% من الأكسجين

1999

UNIVERSITY OF CALIFORNIA - COOPERATIVE EXTENSION

SAMPLE COSTS TO ESTABLISH AN OLIVE ORCHARD AND PRODUCE

~ *OLIVE OIL*~

NORTH COAST OF CALIFORNIA

Prepared by:

Paul Vossen	U.C. Cooperative Extension Farm Advisor, Sonoma County
G. Steven Sibbett	U.C. Cooperative Extension Farm Advisor, Tulare County
Ridgely Evers	Grower, Sonoma County
Karen Klonsky	U.C. Cooperative Extension Economist, Department of Agricultural and Resource Economics, U.C. Davis
Pete Livingston	Staff Research Associate, Department of Agricultural and Resource Economics, U.C. Davis

212

INTRODUCTION

This study includes detailed costs and underlying assumptions for establishing an olive orchard and producing olives in the North Coast of California for either pressing and marketing for oil or selling the fruit to an oil processor are presented in this study. The hypothetical farm used in this report is 20 acres, 15 of which are in olive production. Annual production costs are presented both for operations growing olives and producing olive oil (Tables 1-4) and operations for selling olives to processors (Tables 9-11). Tables 5 and 6 pertain to both types of operations.

This study is intended as a guide only. It can be used to make production decisions, determine potential returns, prepare budgets and evaluate production loans. Sample costs given for labor, materials, equipment and contract services are based on current figures. Costs and practices detailed in this study will not be applicable to every situation. A blank, *Your Cost,* column is provided to enter your actual costs on.

List of tables:

Section 1: Orchard Investment Costs
Table 1. Costs Per Acre to Establish An Olive Orchard
Table 2. Whole Farm Annual Equipment, Investment and Business Overhead
Table 3. Hourly Equipment Costs

Section 2: Costs to Produce Olives and Process and Market Olive Oil
Table 4. Costs Per Acre to Produce Olive Oil
Table 5. Costs and Returns Per Acre to Produce Olive Oil
Table 6. Monthly Cash Costs Per Acre to Produce Olive Oil
Table 7. Ranging Analysis – Olive Oil
Table 8. Cost and Returns/Breakeven Analysis – Olive Oil

Section 3: Costs to Produce Olives Sold for Processing
Table 9. Costs and Returns Per Acre to Produce Olives Sold For Processing
Table 10. Ranging Analysis – Olive Sold For Processing
Table 11. Cost and Returns/Breakeven Analysis – Olive Sold For Processing

This and other studies can be obtained through the Department of Agricultural Economics, U.C. Davis (530-752-1515), or from selected county Cooperative Extension offices. For an explanation of calculations or assumptions used in this study refer to the attached General Assumptions or call the Department of Agricultural Economics, University of California - Davis, (530-752-3589).

The University of California, in accordance with applicable Federal and State law and University policy, does not discriminate on the basis of race, color, national origin, religion, sex, disability, age, medical condition (cancer–related), ancestry, marital status, citizenship, sexual orientation, or status as a Vietnam-era veteran or special disabled veteran.

Inquiries regarding the University's nondiscrimination policies may be directed to the Affirmative Action Director, University of California, Agriculture and Natural Resources, 1111 Franklin, 6th Floor, Oakland, CA 94607-5200 (510) 987-0096.

213

ASSUMPTIONS

The following is a description of general assumptions pertaining to sample costs of establishing an olive orchard, production of olives for oil, and processing and marketing olive oil in the North Coast of California. Practices described are not recommendations by the University of California, but represent production procedures and materials considered typical of a well managed orchard. Some costs, practices, and materials may not be applicable to your situation nor used during every year. Additional ones not indicated may be needed. Establishment and cultural practices vary by grower and region; variations can be significant. These costs are on an annual, per acre basis. *The use of trade names in this report does not constitute an endorsement or recommendation by the University of California nor is any criticism implied by omission of other similar products.*

Land. The farm consists of 20 acres of land. Fifteen acres are planted to olives and the remaining five acres include roads, irrigation systems, unused land, and farmstead. Property costs $15,000 per acre. Land is not depreciated.

Labor. Hourly wages for workers are $9.80, and $7.35 per hour for skilled, and field workers respectively. Adding 34% for Workers Compensation, Social Security, Medicare insurance, and other possible benefits gives the labor rates shown of $13.13 per hour for skilled labor, and $9.85 per hour for field labor. Labor for operations involving machinery are 20% higher than the operation time given in Table 1 to account for the extra labor involved in equipment set up, moving, maintenance, work breaks, and repair.

Wages for management are not included as a cash overhead cost. Any return above total costs is considered a return to management.

Trees. Mission is the olive cultivar assumed in this study. Mission olives will produce between 40-45 gallons of oil per ton. Some of the cultivars representing the oil acreage historically planted in California include Mission, Sevillano, and Ascolano. Traditional European oil producing varieties that have been established in limited plantings in the region are Frantoio, Leccino, Pendolino, Maurino, Moraiolo, and Arbequina. The cost of European nursery stock can be twice that of Mission. A more inclusive list of oil producing varieties and their characteristics can be found in *Olive Production Manual, DANR Publication 3353.*

The trees are planted at 12' X 20' spacing, 180 trees per acre. Olive trees have a long production life if they are well maintained. The life of the orchard at the time of planting in this study is estimated to be 60 years.

Irrigation. The water cost for irrigation is the pumping charge. The cost per acre-foot for water will vary by grower in this region depending on various well characteristics and other irrigation factors. In this study, water is calculated to cost $100 per acre-foot. Irrigation rates increase each year as the orchard matures and is shown for establishment and production years in Table A. One acre-foot of water is assumed to be available from rainfall.

Table A. Olive orchard water use

Year	Acre-Feet/Year
1	0.3
2	0.7
3	1.5
4+	2.5

214

Because the orchard is planted on sloped land, water is delivered to the orchard by microsprinklers in the tree row. The irrigation system is installed and completed before the trees are planted. The pump, filter station, mainlines, laterals, and risers have an expected useful life of 40 years. The life of the microsprinklers are estimated at 10 years. The irrigation system is considered an improvement to the property and is shown in the capital recovery sections of Tables 1-3 and 9 and the Investments section of Table 2.

ORCHARD ESTABLISHMENT CULTURAL PRACTICES AND MATERIAL INPUTS

Site Preparation. Olives grown in north coast counties are mainly planted on hillsides. Various county zoning ordinances require owners to manage erosion on disturbed soils on hillsides. In this study, an erosion control plan is developed by a professional engineer. County fees are paid and the control plan is implemented by a contract company.

Land preparation begins with shallow subsoiling the soil profile to 12–18 inches in order to break up any surface compaction which would affect root and water penetration. Subsoiling is performed by contract operators. The ground is disced twice to break up large clods of soil and smooth the soil in advance of planting the trees. All operations that prepare the orchard for planting are done in the year prior to planting. However, for this study, these costs are included with those incurred in the first year as shown in Table 1.

Planting. Planting the orchard starts by marking tree sites in spring. Holes are dug, trees planted. In the second year, one tree per acre will have to be replanted. Nursery trees should have a single trunk and three to four main scaffold branches developing.

Regular pruning, other than sucker removal, begins in the fourth year and hours required to perform this task, as well as costs, increase annually. Newly planted trees should have the 3-4 main scaffold branches developing at a height of approximately 36 inches. No shoots are allowed to develop below 36 inches. Pruning is performed in spring months.

Weed Management. A pre-emergent herbicide is applied immediately after planting. In spring and summer three applications of a contact herbicide are made to control perrenial weeds. In the first fall a residual herbicide is sprayed along the tree rows to control weeds the following growing season. During the summer of the second year a combination of residual herbicides are used to control weeds in the tree rows. Mowing the row middles to manage the orchard floor also starts the first year. The orchard is mowed three times each year; each mowing requires two passes with the mower.

Insect and Disease Management. During the developmental years, pest and disease controls are minimal in this study, and not needed until the fourth year. Peacock spot and olive knot are major diseases infecting leaves and shoots, causing defoliation and shoot death. In this study, copper is used to prevent these diseases. The copper/peacock spray is applied in the fall after harvest. Olive oil processors are generally unwilling to accept fruit treated with copper. Therefore, copper is custom applied after harvest in the fall. Control of insect pests is not required during the period of orchard establishment. Occasional control may be needed for black scale, but is not shown for the immature orchard in this report.

215

Fertilization. Nitrogen is the major nutrient required for proper tree growth and optimum yields. Young trees receive approximately 50% of the mature tree rate applied 3-4 times during the growing season. Nitrogen fertilizer is applied in a granular form (46% nitrogen), at increasing rates during orchard establishment. Annual rates of actual N are shown in Table B.

Table B. Applied nitrogen for olives

Year	Pounds of N	Pounds of urea
	per acre	
1	6	13
2	11	24
3	23	50
4	45	98
5+	*90	196

* Rate is applied every other year.

Establishment Cost. The establishment cost is the sum of cash costs for land preparation, planting, trees, production expenses, and cash overhead for growing olive trees through the first year oil is produced minus any returns from production. The *Total Accumulated Net Cash Cost* in the fourth year shown in Table 1, represents the establishment cost per acre. For this study, the cost is $7,765 per acre or $116,475 for the 15 acres planted to olives. Establishment cost is amortized over the remaining 56 years that the orchard is assumed to be in production. Establishment cost is used to determine the non-cash overhead, orchard capital recovery expense for production years.

Production Cultural Practices And Material Inputs

Pruning. Pruning strategy is critical to production and is dependent on several factors such as olive cultivar and planting density. In this study, pruning is done in the spring by hand. Prunings are placed in the row middles and shredded.

Fertilization. Mature tree nutrition is determined by leaf analysis in July. Nitrogen is applied at a rate of one pound of nitrogen every two years. In this study the cost fertilizer is shown as half the rate or 0.5 pound of N per tree annually. Fertilizer is in a granular form (urea – 46% nitrogen) and applied by hand in April.

Weed Control. Weeds in mature orchards are controlled with chemicals and mowing. Weeds within the tree rows are controlled with residual, pre-emergent herbicides applied in the fall. Different herbicides are used alternately each year. Three spot sprays of a contact herbicide manage weeds missed by residual herbicides. Row centers are mowed three times annually during the spring and summer.

Insect and Disease Management. One insect and two disease pests are treated. Black scale, an insect pest, requires occasional insecticide treatment. Adequate pruning controls this pest well. Only following cool years or in those orchards that have become too dense would insecticide treatment be required to reduce the population to manageable levels. Prevention of the fungal disease, peacock spot, and the bacterial disease, olive knot requires an annual spray of copper following harvest and prior to fall rains. In years of heavy rains a second application is made in the early spring.

216

For specific pesticide choices and rates consult the *UC IPM Olive Pest Management Guidelines, DANR Publication 3339* and *Olive Production Manual, DANR Publication 3353.* Written recommendations are required for many pesticides and are made by licensed pest control advisors. For information and pesticide use permits, contact the local county Agricultural Commissioner's office. Contact your county farm advisor for additional production information.

Equipment Cash Costs. Equipment costs are composed of three parts; capital recovery, cash overhead, and operating costs. The operating costs consist of fuel, lubrication, and repairs.

Repair costs are based on purchase price, annual hours of use, total hours of life, and repair coefficients formulated by the American Society of Agricultural Engineers (ASAE). Fuel and lubrication costs are also determined by ASAE equations based on maximum PTO hp, and type of fuel used. The fuel and repair cost per acre for each operation in Table 2 is determined by multiplying the total hourly operating cost in Table 6 for each piece of equipment used for the cultural practice by the number of hours per acre for that operation. Tractor time is 10% higher than implement time (Operation Time) for a given operation to account for fueling, moving equipment, and setup time. Prices for on-farm delivery of diesel and gasoline are $0.62 and $1.02 per gallon, respectively.

Harvest. Harvest starts in the fourth year by a contracted harvesting company. Costs for contracted harvest operations are based on fresh tons. Olives for oil are hand picked at the color change stage of purple/black skin and green flesh in December and January. Care must be taken when harvesting olives because damaged or groundfall fruit can spoil and develop undesired odors and flavors which are imparted to the oil. Frost can also damage olive fruit and lower oil quality. Maximum yield is reached in the six year.

Processing and Marketing. Processing olives for oil requires special equipment and expertise. Some oil producers also process their olives in their own facilities, but many do not. The two major options for growers without production facilities are to sell the olives to a processor or pay to have it processed and market the olive oil themselves. Both options are examined in this study. Costs and returns for oil which is produced and sold by the grower are shown in Tables 2-8 and the production costs and returns for olives sold to oil processors are presented in Tables 9-11. A description of the different processing procedures are described in the *Olive Production Manual, DANR Publication 3353* and *Producing Olive Oil in California DANR Publication 21516.*

Olive oil produced in California is marketed as a high quality product and sold for a premium price. This is because of the locally high cost of producing olives and competition from lower price imported olive oil that dominate the low and medium quality olive oil markets. Marketing costs include distribution, possible slotting fees and promotional materials. Selling olive oil in the gourmet market requires careful consideration. Product packaging and developing a market channel are essential to succeed in the competitive oil marketplace. In this study, the cost of processing and packaging is included as a cost of producing olive oil in the Processing and Marketing cost section of Tables 2-8. Marketing costs are shown as a separate expense under Processing and Marketing costs.

Yields. As noted in the previous section, olives begin bearing an economic crop in the fourth year after planting. In this study, olives yield 19% oil per fresh weight and the oil weighs 7.61 pounds per gallon. With a 90% extraction rate about 45 gallons of oil per ton of olives is produced. A case of olive oil consists of 12 - 500

217

milliliter bottles. Typical annual yields for olives are measured in tons per acre. Tonnage, oil, and case yields are shown in Table C.

Table C. Annual yield for establishment and production years

Year	Tons (Fresh Weight)	Gallons Oil	Cases
	per acre		
4	0.5	22.5	14.2
5	1.0	44.9	28.4
6	2.0	89.9	56.7
7+	2.5	112.4	70.9

Returns. Growers can market their olives in different ways. This study looks at two approaches; the grower processes and markets their oil (Tables 4-8) versus the grower selling raw olives to an oil processor (Tables 9-11). Returns, shown in Tables 7 and 10, will vary and the yields and prices used in this cost study are an estimate taking into consideration current situations. For grower processed and marketed oil, an estimated price of $120 per case of olive oil is used (Table 5). A range from $80 to $140 per case is used in Table 7.

Growers selling their olives for pressing typically receive in the range of $350 to $500 per ton and, on rare occasions, upwards of $1,000 per ton for certain olive varieties. A price of $500 per ton is used in Tables 9 and 11 which is similar to the price paid for canning olives. Table 10 includes a range from $350 to $650 per ton.

Risk. The risks associated with producing and marketing olive oil are significant. While this study makes every effort to model a production system based on typical, real world practices, it cannot fully represent financial, agronomic and market risks which affect the profitability and economic viability of olive oil production. A market channel should be determined before olives are planted and brought into production. Though, not used in this study, crop insurance is a risk management tool available to growers.

OVERHEAD COSTS

Cash Overhead. Cash overhead consists of various cash expenses paid out during the year that are assigned to the whole farm and not to a particular operation. These costs include property taxes, interest on operating capital, office expense, liability and property insurance, management services, and equipment repairs. Cash overhead costs are found in Tables 1, 4-6, and 9.

Property Taxes Counties charge a base property tax rate of 1% on the assessed value of the property. In some counties special assessment districts exist and charge additional taxes on property including equipment, buildings, and improvements. For this study, county taxes are calculated as 1% of the average value of the property. Average value equals new cost plus salvage value divided by 2 on a per acre basis. Costs and salvage value for investments are shown in Table 5.

Interest On Operating Capital. Interest on operating capital is based on cash operating costs and is calculated monthly until harvest at a nominal rate of 9.69% per year. A nominal interest rate is the going market cost of borrowed funds.

218

Management Fees. Professional management services are contracted by the orchard owner. These services include horticultural and pest management advising. A fee of $75 per acre is charged.

Insurance. Insurance for farm investments vary depending on the assets included and the amount of coverage. Property insurance provides coverage for property loss and is charged at 0.713% of the average value of the assets over their useful life. Liability insurance covers accidents on the farm and costs $469 for the entire farm.

Office Expense. Office and business expenses are estimated at $2000 annually. These expenses include office supplies, telephones, bookkeeping, accounting, legal fees, etc.

Capital Recovery Costs. Although farm equipment on olive orchards in the region might be purchased new or used, this study shows the current purchase price for new equipment. The new purchase price is adjusted to 60% to indicate a mix of new and used equipment. Annual ownership costs for equipment and other investments are shown in Tables 1, 2 , 4-5, and 9. They represent the capital recovery cost for investments on an annual per acre basis.

Capital recovery cost is the amount of money required each year to recover the difference between the purchase price and salvage value (unrecovered capital). Put another way, it is equivalent to the annual payment on a loan for the investment with the downpayment equal to the discounted salvage value. This is a more complex method of calculating ownership costs than straight-line depreciation and opportunity costs, but more accurately represents the annual costs of ownership because it takes the time value of money into account (Boehlje and Eidman). The calculation for annual capital recovery costs is as follows.

$$\left[\left(\begin{matrix}\text{Purchase}\\\text{Price}\end{matrix} - \begin{matrix}\text{Salvage}\\\text{Value}\end{matrix}\right)\times\left(\begin{matrix}\text{Capital}\\\text{Recovery}\\\text{Factor}\end{matrix}\right)\right] + \left[\begin{matrix}\text{Salvage}\\\text{Value}\end{matrix} \times \begin{matrix}\text{Interest}\\\text{Rate}\end{matrix}\right]$$

Salvage Value. Salvage value is an estimate of the remaining value of an investment at the end of its life. For farm machinery (e.g., tractors and implements) the remaining value is a percentage of the new cost of the investment (Boehlje and Eidman). The life in years is estimated by dividing the wear-out life, as given by the ASAE by the annual use in hours. Salvage value is calculated as:

$$New\ Price \times \%\ Remaining\ Value$$

Salvage value for other investments including irrigation systems, buildings, and miscellaneous equipment is zero. The salvage value for land is equal to the purchase price because land does not depreciate from use. The purchase price and salvage value for certain equipment and investments are shown in Table 5.

Capital Recovery Factor. Capital recovery factor is the amortization factor or annual payment whose present value at compound interest is 1. It is the function of the interest rate and years of life of the investment. *Interest Rate.* The interest rate of 7.40% used to calculate capital recovery cost is the USDA-ERS's ten year average of California's agricultural sector long-run rate of return to production assets from current income. It is used to reflect the long-term realized rate of return to these specialized resources that can only be used effectively in the agricultural sector. In other words, the next best alternative use for these resources is in another agricultural enterprise.

Acknowledgment. Appreciation is expressed to those cooperators who provided support for this study.

REFERENCES

American Society of Agricultural Engineers. 1994. *American Society of Agricultural Engineers Standards Yearbook.* Russell H. Hahn and Evelyn E. Rosentreter (ed.) St. Joseph, Missouri. 41st edition.

Boelje, Michael D., and Vernon R. Eidman. 1984. *Farm Management.* John Wiley and Sons. New York, New York

Klonsky, Karen, G. Steven Sibbett, Mark Freeman, and Pete Livingston. 1997. *Sample Costs to Establish A Manzanillo Olive Orchard And Produce Olives In The Southern San Joaquin Valley - 1997.* University of California, Cooperative Extension. Department of Agricultural and Resource Economics. Davis, CA.

Sibbett, G. Steven and Joseph Connell. 1993. *Producing Olive Oil in California.* Pub. 21516. University of California, Division of Agriculture and Natural Resources. Oakland, CA.

Statewide IPM Project. 1990. *UC Pest Management Guidelines, Olive. In* M. L. Flint (ed.) *UC IPM pest management guidelines.* Pub. 3339. IPM Education and Publ. University of California, Division of Agriculture and Natural Resources. Oakland, CA.

University of California. 1995. *Olive Production Manual.* Pub. 3353. University of California, Division of Agriculture and Natural Resources. Oakland, CA.

220

Table 1.

U.C. COOPERATIVE EXTENSION
SAMPLE COSTS PER ACRE TO ESTABLISH AN OLIVE ORCHARD
NORTH COAST OF CALIFORNIA - 1999

Labor Rate: $13.13/hr. machine labor
$9.85/hr. non-machine labor

Trees Per Acre: 180
Long Term Interest Rate: 7.4%

	Cost Per Acre					
Year	1st	2nd	3rd	4th	5th	6th
Fresh Tons Per Acre				0.5	1.0	2.0
Gallons Per Acre (19% oil & 90% extraction 1st press)				22.5	44.9	89.9
Planting Costs:						
Erosion Control Plan & Fees	$1,270					
Land Preparation - Erosion Control	200					
Land Preparation - Subsoil	125					
Land Preparation - Disc	50					
Trees: 180 Per Acre (1% in 2nd year)	724	$8				
Survey, Mark, Dig Holes & Plant	720	8				
Wrap Trees	224					
Weed Control - Pre-emergent Strip Spray	47					
TOTAL PLANTING COSTS	3,360	16				
Cultural Costs:						
Pruning And Suckering	11	17	$17	$180	$270	$360
Brush Disposal				21	21	21
Irrigate	38	78	158	258	258	258
Fertilizer - Nitrogen	12	13	16	18	22	22
Weed Control - Summer Residual Herbicide		47				
Weed Control - Spot Spray 3X	19	19	19	19	19	19
Weed Control - Mow Centers 3X	25	25	25	25	25	25
Weed Control - Dormant Residual Herbicide	25	35	11	11	11	11
Disease Control - Peacock Spot & Olive Knot				100	100	100
Pickup Truck Use	160	160	160	160	160	160
TOTAL CULTURAL COSTS	290	394	406	792	886	976
Harvest Costs:						
Hand Pick Olives				175	350	700
TOTAL HARVEST COSTS				175	350	700
Interest On Operating Capital @ 9.69%	363	17	22	38	39	32
TOTAL OPERATING COSTS/ACRE	4,013	427	428	1,005	1,275	1,708
Cash Overhead Costs:						
Office Expense	133	133	133	133	133	133
Liability Insurance	19	19	19	19	19	19
Management Fees	25	25	25	25	25	25
Property Taxes	173	174	173	181	181	181
Property Insurance	124	124	124	129	129	129
Investment Repairs	58	58	58	58	58	58
TOTAL CASH OVERHEAD COSTS	532	533	532	545	545	545
TOTAL CASH COSTS/ACRE	4,545	960	960	1,550	1,820	2,253
INCOME/ACRE FROM PRODUCTION				250	500	1,000
NET CASH COSTS/ACRE FOR THE YEAR	4,545	960	960	1,300	1,320	1,253
ACCUMULATED NET CASH COSTS/ACRE	4,545	5,505	6,465	7,765	9,085	10,338

221

UC COOPERATIVE EXTENSION
Table 1. continued

Year	Cost Per Acre					
	1st	2nd	3rd	4th	5th	6th
Fresh Tons Per Acre				0.5	1.0	2.0
Gallons Per Acre (19% oil & 90% extraction 1st press)				22.5	44.9	89.9
Capital Recovery Cost:						
Land @ $15,000/Producing Acre	1,110	1,110	1,110	1,110	1,110	1,110
Shop Building	97	97	97	97	97	97
Irrigation System	80	80	80	80	80	80
Shop Tools	19	19	22	22	22	22
Pruning Tools	5	5	5	5	5	5
Equipment	247	247	233	388	388	388
TOTAL CAPITAL RECOVERY COST	1,558	1,558	1,547	1,702	1,702	1,702
TOTAL COST/ACRE FOR THE YEAR	6,103	2,518	2,507	3,252	3,522	3,955
TOTAL COST/CASE FOR THE YEAR				229	124	70
INCOME/ACRE FROM PRODUCTION				250	500	1,000
TOTAL NET COST/ACRE FOR THE YEAR	6,103	2,518	2,507	3,002	3,022	2,955
TOTAL ACCUMULATED NET COST/ACRE	6,103	8,621	11,128	14,130	17,152	20,107

ESTABLISHMENT YEAR PROCESSING AND MARKETING COSTS

Year	Cost Per Acre					
	1st	2nd	3rd	4th	5th	6th
Fresh Tons Per Acre				0.5	1.0	2.0
Gallons Per Acre (19% oil & 90% extraction 1st press)				22.5	44.9	89.9
Cases Per Acre (12 - 500 ml bottles per case)				14.2	28.4	56.7
Processing and Marketing Costs:						
Press, Process, Bottle, Label & Cork				675	1,358	2,713
Market Olive Oil				312	625	1,247
TOTAL PROCESSING AND MARKETING COSTS				987	1,983	3,960

222

Table 2.

U.C. COOPERATIVE EXTENSION
WHOLE FARM ANNUAL EQUIPMENT, INVESTMENT, AND BUSINESS OVERHEAD COSTS
FOR PRODUCING OLIVE OIL
NORTH COAST OF CALIFORNIA - 1999

ANNUAL EQUIPMENT COSTS

Yr	Description	Price	Yrs Life	Salvage Value	Capital Recovery	- Cash Overhead - Insur-ance	Taxes	Total
99	55 HP 4WD Tractor	31,102	12	7,776	3,575	139	194	3,908
99	Mower - Flail 9'	7,372	10	1,304	976	31	43	1051
99	Pickup Truck - 1/2 Ton	18,200	7	6,904	2,636	90	126	2851
99	Weed Sprayer - 50 Gal	1,500	15	144	163	6	8	177
	TOTAL	58,174		16,128	7,351	265	372	7,988
	60% of New Cost *	34,904		9,677	4,411	159	223	4,793

* Used to reflect a mix of new and used equipment.

ANNUAL INVESTMENT COSTS

Description	Price	Yrs Life	Salvage Value	Capital Recovery	--------- Cash Overhead --------- Insur-ance	Taxes	Repairs	Total
INVESTMENT								
Buildings	15,000	20		1,460	53	75	300	1,889
Irrigation System	15,350	40		1,205	55	77	450	1,787
Land	300,000	50	300,000	22,200	2,139	3,000		27,339
Olive Orchard Establishment	116,475	56		8,780	415	582		9,778
Pruning Tools	200	3	20	71	1	1	50	122
Shop Tools	3,000	15	300	326	12	16	70	424
TOTAL INVESTMENT	450,025		300,320	34,042	2,675	3,752	870	41,339

ANNUAL BUSINESS OVERHEAD COSTS

Description	Units/ Farm	Unit	Price/ Unit	Total Cost
Liability Insurance	20	Acre	19	378
Management Fee	15	Acre	75	1,125
Office Expense	15	Acre	400	6,000

Table 3.

HOURLY EQUIPMENT COSTS
FOR PRODUCING OLIVE OIL
NORTH COAST - 1999

Yr	Description	Actual Hours Used	Capital Recovery	----------COSTS PER HOUR---------- -- Cash Overhead -- Insur-ance	Taxes	---------- Operating ---------- Repairs	Fuel & Lube	Total Oper.	Total Costs/Hr.
99	55 HP 4WD Tractor	35.40	60.58	2.35	3.29	0.58	1.93	2.51	68.73
99	Mower - Flail 9'	17.20	34.08	1.08	1.51	3.00	0.00	3.00	39.68
99	Pickup Truck - 1/2 Ton	285.00	5.55	0.19	0.26	1.33	2.93	4.26	10.26
99	Weed Sprayer - 50 Gal	15.00	6.53	0.23	0.33	0.39		0.39	7.49

Table 4.

U.C. COOPERATIVE EXTENSION
COSTS PER ACRE TO PRODUCE OLIVE OIL
NORTH COAST - 1999

Labor Rate: $13.13/hr. machine labor
$9.85/hr. non-machine labor

Interest Rate: 9.69%
Yield per Acre: 71.0 Cases

Operation	Operation Time (Hrs/A)	Cash and Labor Costs per Acre: Labor Cost	Fuel,Lube & Repairs	Material Cost	Custom/ Rent	Total Cost	Your Cost
Cultural:							
Irrigate	0.80	8	0	250	0	258	
Weed Control - Spot Spray 3X	0.75	12	2	5	0	19	
Pruning & Sucker	0.00	0	0	0	360	360	
Weed Control - Mow Middles 3X	1.15	18	7	0	0	25	
Fertilizer - Nitrogen	1.00	10	0	13	0	22	
Brush Disposal	0.00	0	0	0	21	21	
Weed Control - Residual	0.25	4	1	18	0	23	
Disease Control - Peacock Spot	0.00	0	0	24	20	44	
Pickup Truck Use	8.00	126	34	0	0	160	
TOTAL CULTURAL COSTS	11.95	178	44	309	401	932	
Harvest:							
Hand Pick	0.00	0	0	0	875	875	
TOTAL HARVEST COSTS	0.00	0	0	0	875	875	
Process & Market:							
Press, Process, Bottle, Label & Cork	0.00	0	0	3,395	0	3,395	
Marketing	0.00	0	0	1,562	0	1,562	
TOTAL PROCESS & MARKET COSTS	0.00	0	0	4,957	0	4,957	
Interest on operating capital @ 9.69%						10	
TOTAL OPERATING COSTS/ACRE		178	44	5,267	1,276	6,774	
CASH OVERHEAD:							
Office Expense						133	
Liability Insurance						19	
Management Fee						25	
Property Taxes						189	
Property Insurance						134	
Investment Repairs						58	
TOTAL CASH OVERHEAD COSTS						558	
TOTAL CASH COSTS/ACRE						7,333	

CAPITAL RECOVERY COSTS (7.4% Interest Rate):

Investment:	Per producing Acre	-- Annual Cost -- Capital Recovery	
Land	15,000	1,110	1,110
Buildings	1,000	97	97
Irrigation System	1,023	80	80
Shop Tools	200	22	22
Pruning Tools	13	5	5
Olive Orchard Establisment	7,765	585	585
Equipment	1,905	233	233
TOTAL CAPITAL RECOVERY COSTS	26,907	2,132	2,132
TOTAL COSTS/ACRE			9,505

224

Table 5.

U.C. COOPERATIVE EXTENSION
COSTS AND RETURNS PER ACRE TO PRODUCE OLIVE OIL
NORTH COAST - 1999

Labor Rate: $13.13/hr. machine labor
$9.85/hr. non-machine labor

Interest Rate: 9.69%

	Quantity/Acre	Unit	Price or Cost/Unit	Value or Cost/Acre	Your Cost
GROSS RETURNS					
Olive Oil	71.00	Case	120.00	8,520	
TOTAL GROSS RETURNS FOR OLIVE OIL				8,520	
OPERATING COSTS					
Irrigation:					
Water	30.00	Acin	8.33	250.00	
Herbicide:					
Roundup Ultra	0.60	Pint	7.84	5.00	
Karmex DF	2.00	Lb	4.84	10.00	
Princep Caliber 90	2.00	Lb	4.22	8.00	
Custom:					
Prune Trees	180.00	Tree	2	360.00	
Shred Brush	1.00	Acre	21	21.00	
Hand Pick Fruit	2.50	Ton	350	875.00	
Fertilizer:					
46-0-0	45.00	Lb N	0.279	13.00	
Fungicide:					
Kocide	8.00	Lb	2.99	24.00	
Contract:					
Ground Application	1.00	Acre	20	20.00	
Processing & Marketing:					
Press & Process	2.50	Ton	350	875.00	
Bottling Charge	71.00	Case	3.5	249.00	
Bottle	71.00	Case	25	1,775.00	
Label & Cork	71.00	Case	7	497.00	
Marketing Charge	71.00	Case	22	1,562.00	
Labor (machine)	12.18	Hrs	13.13	160.00	
Labor (non-machine)	1.80	Hrs	9.85	18.00	
Fuel - Gas	20.00	Gal	1.02	20.00	
Fuel - Diesel	6.36	Gal	0.62	4.00	
Lube				4.00	
Machinery repair				16.00	
Interest on operatingcapital @ 9.69%				10.00	
TOTAL OPERATING COSTS/ACRE				6,774.00	
NET RETURNS ABOVE OPERATING COSTS				1,746.00	
CASH OVERHEAD COSTS:					
Office Expense				133.00	
Liability Insurance				19.00	
Management Fee				25.00	
Property Taxes				189.00	
Property Insurance				134.00	
Investment Repairs				58.00	
TOTAL CASH OVERHEAD COSTS/ACRE				558.00	
TOTAL CASH COSTS/ACRE				7,333.00	
CAPITAL RECOVERY COSTS (7.4% Interest Rate):					
Land				1,110	
Buildings				97	
Irrigation System				80	
Shop Tools				22	
Pruning Tools				5	
Olive Orchard Establishment				585	
Equipment				233	
TOTAL CAPITAL RECOVERY COSTS/ACRE				2,132	
TOTAL COSTS/ACRE				9,505	
NET RETURNS ABOVE TOTAL COSTS				-985	

225

Table 6.

U.C. COOPERATIVE EXTENSION
MONTHLY CASH COSTS PER ACRE TO PRODUCE OLIVE OIL
NORTH COAST OF CALIFORNIA - 1999

Beginning MAR 98 Ending FEB 99	MAR 98	APR 98	MAY 98	JUN 98	JUL 98	AUG 98	SEP 98	OCT 98	NOV 98	DEC 98	JAN 99	FEB 99	TOTAL
Cultural:													
Irrigate	18	23	33	38	51	46	28	18					258
Weed Control - Spot Spray	6	6		6									19
Pruning & Sucker		180	180										360
Weed Control - Mow Middle		8	8	8									25
Fertilizer - Nitrogen		22											22
Brush Disposal			21										21
Weed Control - Residual Herbicide								23					23
Disease Control - Peacock Spray										44			44
Pickup Truck Use	13	13	13	13	13	13	13	13	13	13	13	13	160
TOTAL CULTURAL COSTS	38	254	256	66	64	59	42	55	13	57	13	13	932
Harvest:													
Hand Pick										438	438		875
TOTAL HARVEST COSTS										438	438		875
Process & Market:													
Press, Process, Bottle, Label & Cork											3395		3395
Marketing											1562		1562
TOTAL PROCESS & MARKET COSTS											4,957		4,957
Interest on operating Capital 9.69% [1]	0	2	4	5	5	6	6	7	7	11	-44	0	10
TOTAL OPERATING COSTS/ACRE	38	256	260	71	70	65	48	61	20	508	5365	13	6774
CASH OVERHEAD:													
Office Expense	11	11	11	11	11	11	11	11	11	11	11	11	133
Liability Insurance	19												19
Management Fee	2	2	2	2	2	2	2	2	2	2	2	2	25
Property Taxes					94						94		189
Property Insurance					67						67		134
Investment Repairs	5	5	5	5	5	5	5	5	5	5	5	5	58
TOTAL CASH OVERHEAD COSTS	37	18	18	18	180	18	18	18	18	18	180	18	558
TOTAL CASH COSTS/ACRE	75	274	278	89	249	83	66	79	38	524	5544	31	7333

[1] Postharvest operation costs are discounted back to the time of the first harvest

226

Table 7.

U.C. COOPERATIVE EXTENSION
RANGING ANALYSIS
FOR PRODUCING OLIVE OIL
NORTH COAST OF CALIFORNIA - 1999

	YIELD(CASE/ACRE)						
	40	50	60	70	80	90	100
OPERATING COSTS/ACRE:							
Cultural Cost	932	932	932	932	932	932	932
Harvest, Process & Market Costs	3,286	4,107	4,929	5,750	6,572	7,393	8215
Interest on operating capital	28	22	17	11	5	0	-6
TOTAL OPERATING COSTS/ACRE	4,245	5,061	5,877	6,693	7,509	8,324	9140
TOTAL OPERATING COSTS/CASE	106	101	98	96	94	92	91
CASH OVERHEAD COSTS/ACRE	558	558	558	558	558	558	558
TOTAL CASH COSTS/ACRE	4,803	5,619	6,435	7,251	8,067	8,883	9699
TOTAL CASH COSTS/CASE	120	112	107	104	101	99	97
NON-CASH OVERHEAD COSTS/ACRE	2,132	2,132	2,132	2,132	2,132	2,132	2,132
TOTAL COSTS/ACRE	6,976	7,792	8,608	9,424	10,240	11,055	11,871
TOTAL COSTS/CASE	174	156	143	135	128	123	119

NET RETURNS PER ACRE ABOVE OPERATING COSTS FOR PRODUCING OLIVE OIL

PRICE (DOLLARS/CASE)	YIELD (CASES/ACRE)						
Olive Oil	40	50	60	70	80	90	100
80	-645	-561	-477	-393	-309	-224	-140
90	-245	-61	123	307	491	676	860
100	155	439	723	1,007	1,291	1,576	1,860
110	555	939	1,323	1,707	2,091	2,476	2,860
120	955	1,439	1,923	2,407	2,891	3,376	3,860
130	1,355	1,939	2,523	3,107	3,691	4,276	4,860
140	1,755	2,439	3,123	3,807	4,491	5,176	5,860

NET RETURNS PER ACRE ABOVE CASH COSTS FOR PRODUCING OLIVE OIL

PRICE (DOLLARS/CASE)	YIELD (CASES/ACRE)						
Olive Oil	40	50	60	70	80	90	100
80	-1,203	-1,119	-1,035	-951	-867	-783	-699
90	-803	-619	-435	-251	-67	117	301
100	-403	-119	165	449	733	1,017	1,301
110	-3	381	765	1,149	1,533	1,917	2,301
120	397	881	1,365	1,849	2,333	2,817	3,301
130	797	1,381	1,965	2,549	3,133	3,717	4,301
140	1,197	1,881	2,565	3,249	3,933	4,617	5,301

NET RETURNS PER ACRE ABOVE TOTAL COSTS FOR PRODUCING OLIVE OIL

PRICE (DOLLARS/CASE)	YIELD (CASES/ACRE)						
Olive Oil	40	50	60	70	80	90	100
80	-3,376	-3,292	-3,208	-3,124	-3,040	-2,955	-2,871
90	-2,976	-2,792	-2,608	-2,424	-2,240	-2,055	-1,871
100	-2,576	-2,292	-2,008	-1,724	-1,440	-1,155	-871
110	-2,176	-1,792	-1,408	-1,024	-640	-255	129
120	-1,776	-1,292	-808	-324	160	645	1,129
130	-1,376	-792	-208	376	960	1,545	2,129
140	-976	-292	392	1,076	1,760	2,445	3,129

227

Table 8.

U.C. COOPERATIVE EXTENSION
COSTS AND RETURNS / BREAKEVEN ANALYSIS
FOR PRODUCING OLIVE OIL
NORTH COAST OF CALIFORNIA - 1999

COSTS AND RETURNS - TOTAL ACREAGE

Crop	1. Gross Returns	2. Operating Costs	3. Net Returns Above Oper. Costs (1-2)	4. Cash Costs	5. Net Returns Above Cash Costs (1-4)	6. Total Costs	7. Net Returns Above Total Costs (1-6)
Olive Oil	8,520	6,774	1,746	7,373	1,147	9,505	-985

COSTS AND RETURNS - TOTAL ACREAGE

Crop	1. Gross Returns	2. Operating Costs	3. Net Returns Above Oper. Costs (1-2)	4. Cash Costs	5. Net Returns Above Cash Costs (1-4)	6. Total Costs	7. Net Returns Above Total Costs (1-6)
Olive Oil	127,800	101,616	26,184	110,594	17,206	142,581	-14,781

BREAKEVEN PRICES PER YIELD UNIT

CROP	Base Yield (Units/Acre)	Yield Units	Breakeven Price To Cover: Operating Costs	Cash Costs	Total Costs
			$ per Yield Unit		
Olive Oil	71	Case	95.41	103.84	133.88

BREAKEVEN YIELDS PER ACRE

CROP	Yield Units	Base Price ($/Unit)	Breakeven Yield To Cover: Operating Costs	Cash Costs	Total Costs
			Yield Units /Acre		
Olive Oil	Case	120	56.5	61.4	79.2

228

Table 9.

U.C. COOPERATIVE EXTENSION
COSTS AND RETURNS PER ACRE TO PRODUCE
OLIVES SOLD FOR OIL
NORTH COAST OF CALIFORNIA - 1999

Labor Rate: $13.13/hr. machine labor
$9.85/hr. non-machine labor

Interest Rate: 9.69%

	Quantity/Acre	Unit	Price or Cost/Unit	Value or Cost/Acre	Your Cost
GROSS RETURNS					
Olives Sold For Oil	2.50	Ton	500.00	1,250	
TOTAL GROSS RETURNS FOR OLIVE SOLD FOR OIL				1,250	
OPERATING COSTS					
Irrigation:					
Water	30.00	Acin	8.33	250	
Herbicide:					
Roundup Ultra	0.60	Pint	7.84	5	
Karmex DF	2.00	Lb	4.84	10	
Princep Caliber 90	2.00	Lb	4.22	8	
Custom:					
Prune Trees	80.00	Tree	2.00	360	
Shred Brush	1.00	Acre	21.00	21	
Hand Pick Fruit	2.50	Ton	350.00	875	
Fertilizer:					
46-0-0	45.00	Lb N	0.279	13	
Fungicide:					
Kocide	8.00	Lb	2.99	24	
Contract:					
Ground Application	1.00	Acre	20.00	20	
Labor (machine)	12.18	Hrs	13.13	160	
Labor (non-machine)	1.80	Hrs	9.85	18	
Fuel – Gas	20.00	Gal	1.02	20	
Fuel – Diesel	6.36	Gal	0.62	4	
Lube				4	
Machinery repair				16	
Interest on operatingcapital @ 9.69%				51	
TOTAL OPERATING COSTS/ACRE				1,857	
NET RETURNS ABOVE OPERATING COSTS				-607	
CASH OVERHEAD COSTS:					
Office Expense				133	
Liability Insurance				19	
Management Fee				25	
Property Taxes				189	
Property Insurance				134	
Investment Repairs				58	
TOTAL CASH OVERHEAD COSTS/ACRE				558	
TOTAL CASH COSTS/ACRE				2,415	
CAPITAL RECOVERY COSTS (7.4% Interest Rate):					
Land				1,110	
Buildings				97	
Irrigation System				80	
Shop Tools				22	
Pruning Tools				5	
Olive Orchard Establishment				585	
Equipment				233	
TOTAL CAPITAL RECOVERY COSTS/ACRE				2,132	
TOTAL COSTS/ACRE				4,588	
NET RETURNS ABOVE TOTAL COSTS				-3,338	

229

Table 10.

U.C. COOPERATIVE EXTENSION
RANGING ANALYSIS
OLIVES SOLD FOR OIL
NORTH COAST OF CALIFORNIA – 1999

COSTS PER ACRE AT VARYING YIELDS TO PRODUCE OLIVES SOLD FOR OIL

	YIELD (TON/ACRE)						
	1.75	2.00	2.25	2.50	2.75	3.00	3.25
OPERATING COSTS/ACRE:							
Cultural Cost	932	932	932	932	932	932	932
Harvest Cost	613	700	788	875	963	1,050	1,138
Interest on operating capital	51	51	51	51	51	51	51
TOTAL OPERATING COSTS/ACRE	1,595	1,682	1,770	1,857	1,945	2,032	2,120
TOTAL OPERATING COSTS/TON	911	841	787	743	707	677	652
CASH OVERHEAD COSTS/ACRE	558	558	558	558	558	558	558
TOTAL CASH COSTS/ACRE	2,153	2,240	2,328	2,415	2,503	2,590	2,678
TOTAL CASH COSTS/TON	1,230	1,120	1,035	966	910	863	824
NON-CASH OVERHEAD COSTS/ACRE	2,132	2,132	2,132	2,132	2,132	2,132	2,132
TOTAL COSTS/ACRE	4,326	4,413	4,501	4,588	4,676	4,763	4,851
TOTAL COSTS/TON	2,472	2,207	2,000	1,835	1,700	1,588	1,493

NET RETURNS PER ACRE ABOVE OPERATING COSTS FOR OLIVES SOLD FOR OIL

PRICE (DOLLARS/TON)	YIELD (TON/ACRE)						
Olives Sold For Oil	1.75	2.00	2.25	2.50	2.75	3.00	3.25
350	-982	-982	-982	-982	-982	-982	-982
400	-895	-882	-870	-857	-845	-832	-820
450	-807	-782	-757	-732	-707	-682	-657
500	-720	-682	-645	-607	-570	-532	-495
550	-632	-582	-532	-482	-432	-382	-332
600	-545	-482	-420	-357	-295	-232	-170
650	-457	-382	-307	-232	-157	-82	-7

NET RETURNS PER ACRE ABOVE CASH COSTS FOR OLIVES SOLD FOR OIL

PRICE (DOLLARS/TON)	YIELD (TON/ACRE)						
Olives Sold For Oil	1.75	2.00	2.25	2.50	2.75	3.00	3.25
350	-1,540	-1,540	-1,540	-1,540	-1,540	-1,540	-1,540
400	-1,453	-1,440	-1,428	-1,415	-1,403	-1,390	-1,378
450	-1,365	-1,340	-1,315	-1,290	-1,265	-1,240	-1,215
500	-1,278	-1,240	-1,203	-1,165	-1,128	-1,090	-1,053
550	-1,190	-1,140	-1,090	-1,040	-990	-940	-890
600	-1,103	-1,040	-978	-915	-853	-790	-728
650	-1,015	-940	-865	-790	-715	-640	-565

NET RETURNS PER ACRE ABOVE TOTAL COSTS FOR OLIVES SOLD FOR OIL

PRICE (DOLLARS/TON)	YIELD (TON/ACRE)						
Olives Sold For Oil	1.75	2.00	2.25	2.50	2.75	3.00	3.25
350	-3,713	-3,713	-3,713	-3,713	-3,713	-3,713	-3,713
400	-3,626	-3,613	-3,601	-3,588	-3,576	-3,563	-3,551
450	-3,538	-3,513	-3,488	-3,463	-3,438	-3,413	-3,388
500	-3,451	-3,413	-3,376	-3,338	-3,301	-3,263	-3,226
550	-3,363	-3,313	-3,263	-3,213	-3,163	-3,113	-3,063
600	-3,276	-3,213	-3,151	-3,088	-3,026	-2,963	-2,901
650	-3,188	-3,113	-3,038	-2,963	-2,888	-2,813	-2,738

230

Table 11.

U.C. COOPERATIVE EXTENSION
COSTS AND RETURNS / BREAKEVEN ANALYSIS
OLIVES SOLD FOR OIL
NORTH COAST OF CALIFORNIA - 1999

COSTS AND RETURNS - PER ACRE BASIS

Crop	1. Gross Returns	2. Operating Costs	3. Net Returns Above Oper. Costs (1-2)	4. Cash Costs	5. Net Returns Above Cash Costs (1-4)	6. Total Costs	7. Net Returns Above Total Costs (1-6)
Olives Sold For Oil	1,250	1,857	-607	2,456	-1,206	4,588	-3,338

COSTS AND RETURNS - TOTAL ACREAGE

Crop	1. Gross Returns	2. Operating Costs	3. Net Returns Above Oper. Costs (1-2)	4. Cash Costs	5. Net Returns Above Cash Costs (1-4)	6. Total Costs	7. Net Returns Above Total Costs (1-6)
Olives Sold For Oil	18,750	27,859	-9,109	36,837	-18,087	68,824	-50,074

BREAKEVEN PRICES PER YIELD UNIT

CROP	Base (Units/Acr	Yield Units	Operating Costs	Cash Costs	Total Costs
			---------- $ per Yield Unit ----------		
Olives Sold For Oil	2.5	Ton	742.91	982.32	1,835.31

BREAKEVEN YIELDS PER ACRE

CROP	Yield Units	Base Price ($/Unit)	Operating Costs	Cash Costs	Total Costs
			---------- Yield Units/Acre ----------		
Olives Sold For Oil	Ton	500	3.7	4.9	9.2

231

1999

UNIVERSITY OF CALIFORNIA - COOPERATIVE EXTENSION

SAMPLE COSTS TO ESTABLISH AN OLIVE ORCHARD AND PRODUCE

~ *OLIVE OIL*~

NORTH COAST OF CALIFORNIA

Prepared by:

Paul Vossen	U.C. Cooperative Extension Farm Advisor, Sonoma County
G. Steven Sibbett	U.C. Cooperative Extension Farm Advisor, Tulare County
Ridgely Evers	Grower, Sonoma County
Karen Klonsky	U.C. Cooperative Extension Economist, Department of Agricultural and Resource Economics, U.C. Davis
Pete Livingston	Staff Research Associate, Department of Agricultural and Resource Economics, U.C. Davis

232

INTRODUCTION

This study includes detailed costs and underlying assumptions for establishing an olive orchard and producing olives in the North Coast of California for either pressing and marketing for oil or selling the fruit to an oil processor are presented in this study. The hypothetical farm used in this report is 20 acres, 15 of which are in olive production. Annual production costs are presented both for operations growing olives and producing olive oil (Tables 1-4) and operations for selling olives to processors (Tables 9-11). Tables 5 and 6 pertain to both types of operations.

This study is intended as a guide only. It can be used to make production decisions, determine potential returns, prepare budgets and evaluate production loans. Sample costs given for labor, materials, equipment and contract services are based on current figures. Costs and practices detailed in this study will not be applicable to every situation. A blank, *Your Cost,* column is provided to enter your actual costs on.

List of tables:

Section 1: Orchard Investment Costs
Table 1. Costs Per Acre to Establish An Olive Orchard
Table 2. Whole Farm Annual Equipment, Investment and Business Overhead
Table 3. Hourly Equipment Costs

Section 2: Costs to Produce Olives and Process and Market Olive Oil
Table 4. Costs Per Acre to Produce Olive Oil
Table 5. Costs and Returns Per Acre to Produce Olive Oil
Table 6. Monthly Cash Costs Per Acre to Produce Olive Oil
Table 7. Ranging Analysis – Olive Oil
Table 8. Cost and Returns/Breakeven Analysis – Olive Oil

Section 3: Costs to Produce Olives Sold for Processing
Table 9. Costs and Returns Per Acre to Produce Olives Sold For Processing
Table 10. Ranging Analysis – Olive Sold For Processing
Table 11. Cost and Returns/Breakeven Analysis – Olive Sold For Processing

This and other studies can be obtained through the Department of Agricultural Economics, U.C. Davis (530-752-1515), or from selected county Cooperative Extension offices. For an explanation of calculations or assumptions used in this study refer to the attached General Assumptions or call the Department of Agricultural Economics, University of California - Davis, (530-752-3589).

The University of California, in accordance with applicable Federal and State law and University policy, does not discriminate on the basis of race, color, national origin, religion, sex, disability, age, medical condition (cancer-related), ancestry, marital status, citizenship, sexual orientation, or status as a Vietnam-era veteran or special disabled veteran.

Inquiries regarding the University's nondiscrimination policies may be directed to the Affirmative Action Director, University of California, Agriculture and Natural Resources, 1111 Franklin, 6th Floor, Oakland, CA 94607-5200 (510) 987-0096.

ASSUMPTIONS

The following is a description of general assumptions pertaining to sample costs of establishing an olive orchard, production of olives for oil, and processing and marketing olive oil in the North Coast of California. Practices described are not recommendations by the University of California, but represent production procedures and materials considered typical of a well managed orchard. Some costs, practices, and materials may not be applicable to your situation nor used during every year. Additional ones not indicated may be needed. Establishment and cultural practices vary by grower and region; variations can be significant. These costs are on an annual, per acre basis. *The use of trade names in this report does not constitute an endorsement or recommendation by the University of California nor is any criticism implied by omission of other similar products.*

Land. The farm consists of 20 acres of land. Fifteen acres are planted to olives and the remaining five acres include roads, irrigation systems, unused land, and farmstead. Property costs $15,000 per acre. Land is not depreciated.

Labor. Hourly wages for workers are $9.80, and $7.35 per hour for skilled, and field workers respectively. Adding 34% for Workers Compensation, Social Security, Medicare insurance, and other possible benefits gives the labor rates shown of $13.13 per hour for skilled labor, and $9.85 per hour for field labor. Labor for operations involving machinery are 20% higher than the operation time given in Table 1 to account for the extra labor involved in equipment set up, moving, maintenance, work breaks, and repair.

Wages for management are not included as a cash overhead cost. Any return above total costs is considered a return to management.

Trees. Mission is the olive cultivar assumed in this study. Mission olives will produce between 40-45 gallons of oil per ton. Some of the cultivars representing the oil acreage historically planted in California include Mission, Sevillano, and Ascolano. Traditional European oil producing varieties that have been established in limited plantings in the region are Frantoio, Leccino, Pendolino, Maurino, Moraiolo, and Arbequina. The cost of European nursery stock can be twice that of Mission. A more inclusive list of oil producing varieties and their characteristics can be found in *Olive Production Manual, DANR Publication 3353.*

The trees are planted at 12' X 20' spacing, 180 trees per acre. Olive trees have a long production life if they are well maintained. The life of the orchard at the time of planting in this study is estimated to be 60 years.

Irrigation. The water cost for irrigation is the pumping charge. The cost per acre-foot for water will vary by grower in this region depending on various well characteristics and other irrigation factors. In this study, water is calculated to cost $100 per acre-foot. Irrigation rates increase each year as the orchard matures and is shown for establishment and production years in Table A. One acre-foot of water is assumed to be available from rainfall.

Table A. Olive orchard water use

Year	Acre-Feet/Year
1	0.3
2	0.7
3	1.5
4+	2.5

234

Because the orchard is planted on sloped land, water is delivered to the orchard by microsprinklers in the tree row. The irrigation system is installed and completed before the trees are planted. The pump, filter station, mainlines, laterals, and risers have an expected useful life of 40 years. The life of the microsprinklers are estimated at 10 years. The irrigation system is considered an improvement to the property and is shown in the capital recovery sections of Tables 1-3 and 9 and the Investments section of Table 2.

ORCHARD ESTABLISHMENT CULTURAL PRACTICES AND MATERIAL INPUTS

Site Preparation. Olives grown in north coast counties are mainly planted on hillsides. Various county zoning ordinances require owners to manage erosion on disturbed soils on hillsides. In this study, an erosion control plan is developed by a professional engineer. County fees are paid and the control plan is implemented by a contract company.

Land preparation begins with shallow subsoiling the soil profile to 12–18 inches in order to break up any surface compaction which would affect root and water penetration. Subsoiling is performed by contract operators. The ground is disced twice to break up large clods of soil and smooth the soil in advance of planting the trees. All operations that prepare the orchard for planting are done in the year prior to planting. However, for this study, these costs are included with those incurred in the first year as shown in Table 1.

Planting. Planting the orchard starts by marking tree sites in spring. Holes are dug, trees planted. In the second year, one tree per acre will have to be replanted. Nursery trees should have a single trunk and three to four main scaffold branches developing.

Regular pruning, other than sucker removal, begins in the fourth year and hours required to perform this task, as well as costs, increase annually. Newly planted trees should have the 3-4 main scaffold branches developing at a height of approximately 36 inches. No shoots are allowed to develop below 36 inches. Pruning is performed in spring months.

Weed Management. A pre-emergent herbicide is applied immediately after planting. In spring and summer three applications of a contact herbicide are made to control perrenial weeds. In the first fall a residual herbicide is sprayed along the tree rows to control weeds the following growing season. During the summer of the second year a combination of residual herbicides are used to control weeds in the tree rows. Mowing the row middles to manage the orchard floor also starts the first year. The orchard is mowed three times each year; each mowing requires two passes with the mower.

Insect and Disease Management. During the developmental years, pest and disease controls are minimal in this study, and not needed until the fourth year. Peacock spot and olive knot are major diseases infecting leaves and shoots, causing defoliation and shoot death. In this study, copper is used to prevent these diseases. The copper/peacock spray is applied in the fall after harvest. Olive oil processors are generally unwilling to accept fruit treated with copper. Therefore, copper is custom applied after harvest in the fall. Control of insect pests is not required during the period of orchard establishment. Occasional control may be needed for black scale, but is not shown for the immature orchard in this report.

235

Fertilization. Nitrogen is the major nutrient required for proper tree growth and optimum yields. Young trees receive approximately 50% of the mature tree rate applied 3-4 times during the growing season. Nitrogen fertilizer is applied in a granular form (46% nitrogen), at increasing rates during orchard establishment. Annual rates of actual N are shown in Table B.

Table B. Applied nitrogen for olives

Year	Pounds of N	Pounds of urea
	per acre	
1	6	13
2	11	24
3	23	50
4	45	98
5+	*90	196

* Rate is applied every other year.

Establishment Cost. The establishment cost is the sum of cash costs for land preparation, planting, trees, production expenses, and cash overhead for growing olive trees through the first year oil is produced minus any returns from production. The *Total Accumulated Net Cash Cost* in the fourth year shown in Table 1, represents the establishment cost per acre. For this study, the cost is $7,765 per acre or $116,475 for the 15 acres planted to olives. Establishment cost is amortized over the remaining 56 years that the orchard is assumed to be in production. Establishment cost is used to determine the non-cash overhead, orchard capital recovery expense for production years.

PRODUCTION CULTURAL PRACTICES AND MATERIAL INPUTS

Pruning. Pruning strategy is critical to production and is dependent on several factors such as olive cultivar and planting density. In this study, pruning is done in the spring by hand. Prunings are placed in the row middles and shredded.

Fertilization. Mature tree nutrition is determined by leaf analysis in July. Nitrogen is applied at a rate of one pound of nitrogen every two years. In this study the cost fertilizer is shown as half the rate or 0.5 pound of N per tree annually. Fertilizer is in a granular form (urea – 46% nitrogen) and applied by hand in April.

Weed Control. Weeds in mature orchards are controlled with chemicals and mowing. Weeds within the tree rows are controlled with residual, pre-emergent herbicides applied in the fall. Different herbicides are used alternately each year. Three spot sprays of a contact herbicide manage weeds missed by residual herbicides. Row centers are mowed three times annually during the spring and summer.

Insect and Disease Management. One insect and two disease pests are treated. Black scale, an insect pest, requires occasional insecticide treatment. Adequate pruning controls this pest well. Only following cool years or in those orchards that have become too dense would insecticide treatment be required to reduce the population to manageable levels. Prevention of the fungal disease, peacock spot, and the bacterial disease, olive knot requires an annual spray of copper following harvest and prior to fall rains. In years of heavy rains a second application is made in the early spring.

236

For specific pesticide choices and rates consult the *UC IPM Olive Pest Management Guidelines, DANR Publication 3339* and *Olive Production Manual, DANR Publication 3353.* Written recommendations are required for many pesticides and are made by licensed pest control advisors. For information and pesticide use permits, contact the local county Agricultural Commissioner's office. Contact your county farm advisor for additional production information.

Equipment Cash Costs. Equipment costs are composed of three parts; capital recovery, cash overhead, and operating costs. The operating costs consist of fuel, lubrication, and repairs.

Repair costs are based on purchase price, annual hours of use, total hours of life, and repair coefficients formulated by the American Society of Agricultural Engineers (ASAE). Fuel and lubrication costs are also determined by ASAE equations based on maximum PTO hp, and type of fuel used. The fuel and repair cost per acre for each operation in Table 2 is determined by multiplying the total hourly operating cost in Table 6 for each piece of equipment used for the cultural practice by the number of hours per acre for that operation. Tractor time is 10% higher than implement time (Operation Time) for a given operation to account for fueling, moving equipment, and setup time. Prices for on-farm delivery of diesel and gasoline are $0.62 and $1.02 per gallon, respectively.

Harvest. Harvest starts in the fourth year by a contracted harvesting company. Costs for contracted harvest operations are based on fresh tons. Olives for oil are hand picked at the color change stage of purple/black skin and green flesh in December and January. Care must be taken when harvesting olives because damaged or groundfall fruit can spoil and develop undesired odors and flavors which are imparted to the oil. Frost can also damage olive fruit and lower oil quality. Maximum yield is reached in the six year.

Processing and Marketing. Processing olives for oil requires special equipment and expertise. Some oil producers also process their olives in their own facilities, but many do not. The two major options for growers without production facilities are to sell the olives to a processor or pay to have it processed and market the olive oil themselves. Both options are examined in this study. Costs and returns for oil which is produced and sold by the grower are shown in Tables 2-8 and the production costs and returns for olives sold to oil processors are presented in Tables 9-11. A description of the different processing procedures are described in the *Olive Production Manual, DANR Publication 3353* and *Producing Olive Oil in California DANR Publication 21516.*

Olive oil produced in California is marketed as a high quality product and sold for a premium price. This is because of the locally high cost of producing olives and competition from lower price imported olive oil that dominate the low and medium quality olive oil markets. Marketing costs include distribution, possible slotting fees and promotional materials. Selling olive oil in the gourmet market requires careful consideration. Product packaging and developing a market channel are essential to succeed in the competitive oil marketplace. In this study, the cost of processing and packaging is included as a cost of producing olive oil in the Processing and Marketing cost section of Tables 2-8. Marketing costs are shown as a separate expense under Processing and Marketing costs.

Yields. As noted in the previous section, olives begin bearing an economic crop in the fourth year after planting. In this study, olives yield 19% oil per fresh weight and the oil weighs 7.61 pounds per gallon. With a 90% extraction rate about 45 gallons of oil per ton of olives is produced. A case of olive oil consists of 12 - 500

milliliter bottles. Typical annual yields for olives are measured in tons per acre. Tonnage, oil, and case yields are shown in Table C.

Table C. Annual yield for establishment and production years

Year	Tons (Fresh Weight)	Gallons Oil	Cases
	per acre		
4	0.5	22.5	14.2
5	1.0	44.9	28.4
6	2.0	89.9	56.7
7+	2.5	112.4	70.9

Returns. Growers can market their olives in different ways. This study looks at two approaches; the grower processes and markets their oil (Tables 4-8) versus the grower selling raw olives to an oil processor (Tables 9-11). Returns, shown in Tables 7 and 10, will vary and the yields and prices used in this cost study are an estimate taking into consideration current situations. For grower processed and marketed oil, an estimated price of $120 per case of olive oil is used (Table 5). A range from $80 to $140 per case is used in Table 7.

Growers selling their olives for pressing typically receive in the range of $350 to $500 per ton and, on rare occasions, upwards of $1,000 per ton for certain olive varieties. A price of $500 per ton is used in Tables 9 and 11 which is similar to the price paid for canning olives. Table 10 includes a range from $350 to $650 per ton.

Risk. The risks associated with producing and marketing olive oil are significant. While this study makes every effort to model a production system based on typical, real world practices, it cannot fully represent financial, agronomic and market risks which affect the profitability and economic viability of olive oil production. A market channel should be determined before olives are planted and brought into production. Though, not used in this study, crop insurance is a risk management tool available to growers.

OVERHEAD COSTS

Cash Overhead. Cash overhead consists of various cash expenses paid out during the year that are assigned to the whole farm and not to a particular operation. These costs include property taxes, interest on operating capital, office expense, liability and property insurance, management services, and equipment repairs. Cash overhead costs are found in Tables 1, 4-6, and 9.

Property Taxes Counties charge a base property tax rate of 1% on the assessed value of the property. In some counties special assessment districts exist and charge additional taxes on property including equipment, buildings, and improvements. For this study, county taxes are calculated as 1% of the average value of the property. Average value equals new cost plus salvage value divided by 2 on a per acre basis. Costs and salvage value for investments are shown in Table 5.

Interest On Operating Capital. Interest on operating capital is based on cash operating costs and is calculated monthly until harvest at a nominal rate of 9.69% per year. A nominal interest rate is the going market cost of borrowed funds.

Management Fees. Professional management services are contracted by the orchard owner. These services include horticultural and pest management advising. A fee of $75 per acre is charged.

Insurance. Insurance for farm investments vary depending on the assets included and the amount of coverage. Property insurance provides coverage for property loss and is charged at 0.713% of the average value of the assets over their useful life. Liability insurance covers accidents on the farm and costs $469 for the entire farm.

Office Expense. Office and business expenses are estimated at $2000 annually. These expenses include office supplies, telephones, bookkeeping, accounting, legal fees, etc.

Capital Recovery Costs. Although farm equipment on olive orchards in the region might be purchased new or used, this study shows the current purchase price for new equipment. The new purchase price is adjusted to 60% to indicate a mix of new and used equipment. Annual ownership costs for equipment and other investments are shown in Tables 1, 2 , 4-5, and 9. They represent the capital recovery cost for investments on an annual per acre basis.

Capital recovery cost is the amount of money required each year to recover the difference between the purchase price and salvage value (unrecovered capital). Put another way, it is equivalent to the annual payment on a loan for the investment with the downpayment equal to the discounted salvage value. This is a more complex method of calculating ownership costs than straight-line depreciation and opportunity costs, but more accurately represents the annual costs of ownership because it takes the time value of money into account (Boehlje and Eidman). The calculation for annual capital recovery costs is as follows.

$$\left[\left(\begin{matrix}\text{Purchase}\\ \text{Price}\end{matrix} - \begin{matrix}\text{Salvage}\\ \text{Value}\end{matrix}\right)\times\left(\begin{matrix}\text{Capital}\\ \text{Recovery}\\ \text{Factor}\end{matrix}\right)\right] + \left[\begin{matrix}\text{Salvage}\\ \text{Value}\end{matrix} \times \begin{matrix}\text{Interest}\\ \text{Rate}\end{matrix}\right]$$

Salvage Value. Salvage value is an estimate of the remaining value of an investment at the end of its life. For farm machinery (e.g., tractors and implements) the remaining value is a percentage of the new cost of the investment (Boehlje and Eidman). The life in years is estimated by dividing the wear-out life, as given by the ASAE by the annual use in hours. Salvage value is calculated as:

$$New\ Price \times \%\ Remaining\ Value$$

Salvage value for other investments including irrigation systems, buildings, and miscellaneous equipment is zero. The salvage value for land is equal to the purchase price because land does not depreciate from use. The purchase price and salvage value for certain equipment and investments are shown in Table 5.

Capital Recovery Factor. Capital recovery factor is the amortization factor or annual payment whose present value at compound interest is 1. It is the function of the interest rate and years of life of the investment. *Interest Rate.* The interest rate of 7.40% used to calculate capital recovery cost is the USDA-ERS's ten year average of California's agricultural sector long-run rate of return to production assets from current income. It is used to reflect the long-term realized rate of return to these specialized resources that can only be used effectively in the agricultural sector. In other words, the next best alternative use for these resources is in another agricultural enterprise.

239

Acknowledgment. Appreciation is expressed to those cooperators who provided support for this study.

REFERENCES

American Society of Agricultural Engineers. 1994. *American Society of Agricultural Engineers Standards Yearbook.* Russell H. Hahn and Evelyn E. Rosentreter (ed.) St. Joseph, Missouri. 41st edition.

Boelje, Michael D., and Vernon R. Eidman. 1984. *Farm Management.* John Wiley and Sons. New York, New York

Klonsky, Karen, G. Steven Sibbett, Mark Freeman, and Pete Livingston. 1997. *Sample Costs to Establish A Manzanillo Olive Orchard And Produce Olives In The Southern San Joaquin Valley - 1997.* University of California, Cooperative Extension. Department of Agricultural and Resource Economics. Davis, CA.

Sibbett, G. Steven and Joseph Connell. 1993. *Producing Olive Oil in California.* Pub. 21516. University of California, Division of Agriculture and Natural Resources. Oakland, CA.

Statewide IPM Project. 1990. *UC Pest Management Guidelines, Olive. In* M. L. Flint (ed.) *UC IPM pest management guidelines.* Pub. 3339. IPM Education and Publ. University of California, Division of Agriculture and Natural Resources. Oakland, CA.

University of California. 1995. *Olive Production Manual.* Pub. 3353. University of California, Division of Agriculture and Natural Resources. Oakland, CA.

Table 1.

U.C. COOPERATIVE EXTENSION
SAMPLE COSTS PER ACRE TO ESTABLISH AN OLIVE ORCHARD
NORTH COAST OF CALIFORNIA - 1999

Labor Rate: $13.13/hr. machine labor
$9.85/hr. non-machine labor

Trees Per Acre: 180
Long Term Interest Rate: 7.4%

	Cost Per Acre					
Year	1st	2nd	3rd	4th	5th	6th
Fresh Tons Per Acre				0.5	1.0	2.0
Gallons Per Acre (19% oil & 90% extraction 1st press)				22.5	44.9	89.9
Planting Costs:						
Erosion Control Plan & Fees	$1,270					
Land Preparation - Erosion Control	200					
Land Preparation - Subsoil	125					
Land Preparation - Disc	50					
Trees: 180 Per Acre (1% in 2nd year)	724	$8				
Survey, Mark, Dig Holes & Plant	720	8				
Wrap Trees	224					
Weed Control - Pre-emergent Strip Spray	47					
TOTAL PLANTING COSTS	3,360	16				
Cultural Costs:						
Pruning And Suckering	11	17	$17	$180	$270	$360
Brush Disposal				21	21	21
Irrigate	38	78	158	258	258	258
Fertilizer - Nitrogen	12	13	16	18	22	22
Weed Control - Summer Residual Herbicide		47				
Weed Control - Spot Spray 3X	19	19	19	19	19	19
Weed Control - Mow Centers 3X	25	25	25	25	25	25
Weed Control - Dormant Residual Herbicide	25	35	11	11	11	11
Disease Control - Peacock Spot & Olive Knot				100	100	100
Pickup Truck Use	160	160	160	160	160	160
TOTAL CULTURAL COSTS	290	394	406	792	886	976
Harvest Costs:						
Hand Pick Olives				175	350	700
TOTAL HARVEST COSTS				175	350	700
Interest On Operating Capital @ 9.69%	363	17	22	38	39	32
TOTAL OPERATING COSTS/ACRE	4,013	427	428	1,005	1,275	1,708
Cash Overhead Costs:						
Office Expense	133	133	133	133	133	133
Liability Insurance	19	19	19	19	19	19
Management Fees	25	25	25	25	25	25
Property Taxes	173	174	173	181	181	181
Property Insurance	124	124	124	129	129	129
Investment Repairs	58	58	58	58	58	58
TOTAL CASH OVERHEAD COSTS	532	533	532	545	545	545
TOTAL CASH COSTS/ACRE	4,545	960	960	1,550	1,820	2,253
INCOME/ACRE FROM PRODUCTION				250	500	1,000
NET CASH COSTS/ACRE FOR THE YEAR	4,545	960	960	1,300	1,320	1,253
ACCUMULATED NET CASH COSTS/ACRE	4,545	5,505	6,465	7,765	9,085	10,338

241

UC COOPERATIVE EXTENSION
Table 1. continued

Year	Cost Per Acre					
	1st	2nd	3rd	4th	5th	6th
Fresh Tons Per Acre				0.5	1.0	2.0
Gallons Per Acre (19% oil & 90% extraction 1st press)				22.5	44.9	89.9
Capital Recovery Cost:						
Land @ $15,000/Producing Acre	1,110	1,110	1,110	1,110	1,110	1,110
Shop Building	97	97	97	97	97	97
Irrigation System	80	80	80	80	80	80
Shop Tools	19	19	22	22	22	22
Pruning Tools	5	5	5	5	5	5
Equipment	247	247	233	388	388	388
TOTAL CAPITAL RECOVERY COST	1,558	1,558	1,547	1,702	1,702	1,702
TOTAL COST/ACRE FOR THE YEAR	6,103	2,518	2,507	3,252	3,522	3,955
TOTAL COST/CASE FOR THE YEAR				229	124	70
INCOME/ACRE FROM PRODUCTION				250	500	1,000
TOTAL NET COST/ACRE FOR THE YEAR	6,103	2,518	2,507	3,002	3,022	2,955
TOTAL ACCUMULATED NET COST/ACRE	6,103	8,621	11,128	14,130	17,152	20,107

ESTABLISHMENT YEAR PROCESSING AND MARKETING COSTS

Year	Cost Per Acre					
	1st	2nd	3rd	4th	5th	6th
Fresh Tons Per Acre				0.5	1.0	2.0
Gallons Per Acre (19% oil & 90% extraction 1st press)				22.5	44.9	89.9
Cases Per Acre (12 - 500 ml bottles per case)				14.2	28.4	56.7
Processing and Marketing Costs:						
Press, Process, Bottle, Label & Cork				675	1,358	2,713
Market Olive Oil				312	625	1,247
TOTAL PROCESSING AND MARKETING COSTS				987	1,983	3,960

242

Table 2.

U.C. COOPERATIVE EXTENSION
WHOLE FARM ANNUAL EQUIPMENT, INVESTMENT, AND BUSINESS OVERHEAD COSTS
FOR PRODUCING OLIVE OIL
NORTH COAST OF CALIFORNIA - 1999

ANNUAL EQUIPMENT COSTS

Yr	Description	Price	Yrs Life	Salvage Value	Capital Recovery	- Cash Overhead - Insur-ance	Taxes	Total
99	55 HP 4WD Tractor	31,102	12	7,776	3,575	139	194	3,908
99	Mower - Flail 9'	7,372	10	1,304	976	31	43	1051
99	Pickup Truck - 1/2 Ton	18,200	7	6,904	2,636	90	126	2851
99	Weed Sprayer - 50 Gal	1,500	15	144	163	6	8	177
	TOTAL	58,174		16,128	7,351	265	372	7,988
	60% of New Cost *	34,904		9,677	4,411	159	223	4,793

* Used to reflect a mix of new and used equipment.

ANNUAL INVESTMENT COSTS

Description	Price	Yrs Life	Salvage Value	Capital Recovery	--------- Cash Overhead --------- Insur-ance	Taxes	Repairs	Total
INVESTMENT								
Buildings	15,000	20		1,460	53	75	300	1,889
Irrigation System	15,350	40		1,205	55	77	450	1,787
Land	300,000	50	300,000	22,200	2,139	3,000		27,339
Olive Orchard Establishment	116,475	56		8,780	415	582		9,778
Pruning Tools	200	3	20	71	1	1	50	122
Shop Tools	3,000	15	300	326	12	16	70	424
TOTAL INVESTMENT	450,025		300,320	34,042	2,675	3,752	870	41,339

ANNUAL BUSINESS OVERHEAD COSTS

Description	Units/ Farm	Unit	Price/ Unit	Total Cost
Liability Insurance	20	Acre	19	378
Management Fee	15	Acre	75	1,125
Office Expense	15	Acre	400	6,000

Table 3.

HOURLY EQUIPMENT COSTS
FOR PRODUCING OLIVE OIL
NORTH COAST - 1999

Yr	Description	Actual Hours Used	Capital Recovery	----------COSTS PER HOUR ---------- -- Cash Overhead -- Insur-ance	Taxes	---------- Operating ---------- Repairs	Fuel & Lube	Total Oper.	Total Costs/Hr.
99	55 HP 4WD Tractor	35.40	60.58	2.35	3.29	0.58	1.93	2.51	68.73
99	Mower - Flail 9'	17.20	34.08	1.09	1.51	3.00	0.00	3.00	39.68
99	Pickup Truck - 1/2 Ton	285.00	5.55	0.19	0.26	1.33	2.93	4.26	10.26
99	Weed Sprayer - 50 Gal	15.00	6.53	0.23	0.33	0.39		0.39	7.49

243

Table 4.

U.C. COOPERATIVE EXTENSION
COSTS PER ACRE TO PRODUCE OLIVE OIL
NORTH COAST - 1999

Labor Rate: $13.13/hr. machine labor
$9.85/hr. non-machine labor

Interest Rate: 9.69%
Yield per Acre: 71.0 Cases

Operation	Operation Time (Hrs/A)	Cash and Labor Costs per Acre: Labor Cost	Fuel,Lube & Repairs	Material Cost	Custom/ Rent	Total Cost	Your Cost
Cultural:							
Irrigate	0.80	8	0	250	0	258	
Weed Control - Spot Spray 3X	0.75	12	2	5	0	19	
Pruning & Sucker	0.00	0	0	0	360	360	
Weed Control - Mow Middles 3X	1.15	18	7	0	0	25	
Fertilizer - Nitrogen	1.00	10	0	13	0	22	
Brush Disposal	0.00	0	0	0	21	21	
Weed Control - Residual	0.25	4	1	18	0	23	
Disease Control - Peacock Spot	0.00	0	0	24	20	44	
Pickup Truck Use	8.00	126	34	0	0	160	
TOTAL CULTURAL COSTS	11.95	178	44	309	401	932	
Harvest:							
Hand Pick	0.00	0	0	0	875	875	
TOTAL HARVEST COSTS	0.00	0	0	0	875	875	
Process & Market:							
Press, Process, Bottle, Label & Cork	0.00	0	0	3,395	0	3,395	
Marketing	0.00	0	0	1,562	0	1,562	
TOTAL PROCESS & MARKET COSTS	0.00	0	0	4,957	0	4,957	
Interest on operating capital @ 9.69%						10	
TOTAL OPERATING COSTS/ACRE		178	44	5,267	1,276	6,774	
CASH OVERHEAD:							
Office Expense						133	
Liability Insurance						19	
Management Fee						25	
Property Taxes						189	
Property Insurance						134	
Investment Repairs						58	
TOTAL CASH OVERHEAD COSTS						558	
TOTAL CASH COSTS/ACRE						7,333	

CAPITAL RECOVERY COSTS (7.4% Interest Rate):

Investment:	Per producing Acre	-- Annual Cost -- Capital Recovery	
Land	15,000	1,110	1,110
Buildings	1,000	97	97
Irrigation System	1,023	80	80
Shop Tools	200	22	22
Pruning Tools	13	5	5
Olive Orchard Establisment	7,765	585	585
Equipment	1,905	233	233
TOTAL CAPITAL RECOVERY COSTS	26,907	2,132	2,132
TOTAL COSTS/ACRE			9,505

244

Table 5.

U.C. COOPERATIVE EXTENSION
COSTS AND RETURNS PER ACRE TO PRODUCE OLIVE OIL
NORTH COAST - 1999

Labor Rate: $13.13/hr. machine labor
$9.85/hr. non-machine labor

Interest Rate: 9.69%

	Quantity/Acre	Unit	Price or Cost/Unit	Value or Cost/Acre	Your Cost
GROSS RETURNS					
Olive Oil	71.00	Case	120.00	8,520	
TOTAL GROSS RETURNS FOR OLIVE OIL				8,520	
OPERATING COSTS					
Irrigation:					
Water	30.00	Acin	8.33	250.00	
Herbicide:					
Roundup Ultra	0.60	Pint	7.84	5.00	
Karmex DF	2.00	Lb	4.84	10.00	
Princep Caliber 90	2.00	Lb	4.22	8.00	
Custom:					
Prune Trees	180.00	Tree	2	360.00	
Shred Brush	1.00	Acre	21	21.00	
Hand Pick Fruit	2.50	Ton	350	875.00	
Fertilizer:					
46-0-0	45.00	Lb N	0.279	13.00	
Fungicide:					
Kocide	8.00	Lb	2.99	24.00	
Contract:					
Ground Application	1.00	Acre	20	20.00	
Processing & Marketing:					
Press & Process	2.50	Ton	350	875.00	
Bottling Charge	71.00	Case	3.5	249.00	
Bottle	71.00	Case	25	1,775.00	
Label & Cork	71.00	Case	7	497.00	
Marketing Charge	71.00	Case	22	1,562.00	
Labor (machine)	12.18	Hrs	13.13	160.00	
Labor (non-machine)	1.80	Hrs	9.85	18.00	
Fuel - Gas	20.00	Gal	1.02	20.00	
Fuel - Diesel	6.36	Gal	0.62	4.00	
Lube				4.00	
Machinery repair				16.00	
Interest on operatingcapital @ 9.69%				10.00	
TOTAL OPERATING COSTS/ACRE				6,774.00	
NET RETURNS ABOVE OPERATING COSTS				1,746.00	
CASH OVERHEAD COSTS:					
Office Expense				133.00	
Liability Insurance				19.00	
Management Fee				25.00	
Property Taxes				189.00	
Property Insurance				134.00	
Investment Repairs				58.00	
TOTAL CASH OVERHEAD COSTS/ACRE				558.00	
TOTAL CASH COSTS/ACRE				7,333.00	
CAPITAL RECOVERY COSTS (7.4% Interest Rate):					
Land				1,110	
Buildings				97	
Irrigation System				80	
Shop Tools				22	
Pruning Tools				5	
Olive Orchard Establishment				585	
Equipment				233	
TOTAL CAPITAL RECOVERY COSTS/ACRE				2,132	
TOTAL COSTS/ACRE				9,505	
NET RETURNS ABOVE TOTAL COSTS				-985	

245

Table 6.

U.C. COOPERATIVE EXTENSION
MONTHLY CASH COSTS PER ACRE TO PRODUCE OLIVE OIL
NORTH COAST OF CALIFORNIA - 1999

Beginning MAR 98 Ending FEB 99	MAR 98	APR 98	MAY 98	JUN 98	JUL 98	AUG 98	SEP 98	OCT 98	NOV 98	DEC 98	JAN 99	FEB 99	TOTAL
Cultural:													
Irrigate	18	23	33	38	51	46	28	18					258
Weed Control - Spot Spray	6	6		6									19
Pruning & Sucker		180	180										360
Weed Control - Mow Middle		8	8	8									25
Fertilizer - Nitrogen		22											22
Brush Disposal			21										21
Weed Control - Residual Herbicide								23					23
Disease Control - Peacock Spray										44			44
Pickup Truck Use	13	13	13	13	13	13	13	13	13	13	13	13	160
TOTAL CULTURAL COSTS	38	254	256	66	64	59	42	55	13	57	13	13	932
Harvest:													
Hand Pick										438	438		875
TOTAL HARVEST COSTS										438	438		875
Process & Market:													
Press, Process, Bottle, Label & Cork											3395		3395
Marketing											1562		1562
TOTAL PROCESS & MARKET COSTS											4,957		4,957
Interest on operating Capital 9.69% [1]	0	2	4	5	5	6	6	7	7	11	-44	0	10
TOTAL OPERATING COSTS/ACRE	38	256	260	71	70	65	48	61	20	506	5365	13	6774
CASH OVERHEAD:													
Office Expense	11	11	11	11	11	11	11	11	11	11	11	11	133
Liability Insurance	19												19
Management Fee	2	2	2	2	2	2	2	2	2	2	2	2	25
Property Taxes					94						94		189
Property Insurance					67						67		134
Investment Repairs	5	5	5	5	5	5	5	5	5	5	5	5	58
TOTAL CASH OVERHEAD COSTS	37	18	18	18	180	18	18	18	18	18	180	18	558
TOTAL CASH COSTS/ACRE	75	274	278	89	249	83	66	79	38	524	5544	31	7333

[1] Postharvest operation costs are discounted back to the time of the first harvest

246

Table 7.

U.C. COOPERATIVE EXTENSION
RANGING ANALYSIS
FOR PRODUCING OLIVE OIL
NORTH COAST OF CALIFORNIA - 1999

	YIELD(CASE/ACRE)						
	40	50	60	70	80	90	100
OPERATING COSTS/ACRE:							
Cultural Cost	932	932	932	932	932	932	932
Harvest, Process & Market Costs	3,286	4,107	4,929	5,750	6,572	7,393	8215
Interest on operating capital	28	22	17	11	5	0	-6
TOTAL OPERATING COSTS/ACRE	4,245	5,061	5,877	6,693	7,509	8,324	9140
TOTAL OPERATING COSTS/CASE	106	101	98	96	94	92	91
CASH OVERHEAD COSTS/ACRE	558	558	558	558	558	558	558
TOTAL CASH COSTS/ACRE	4,803	5,619	6,435	7,251	8,067	8,883	9699
TOTAL CASH COSTS/CASE	120	112	107	104	101	99	97
NON-CASH OVERHEAD COSTS/ACRE	2,132	2,132	2,132	2,132	2,132	2,132	2,132
TOTAL COSTS/ACRE	6,976	7,792	8,608	9,424	10,240	11,055	11,871
TOTAL COSTS/CASE	174	156	143	135	128	123	119

NET RETURNS PER ACRE ABOVE OPERATING COSTS FOR PRODUCING OLIVE OIL

PRICE (DOLLARS/CASE)	YIELD (CASES/ACRE)						
Olive Oil	40	50	60	70	80	90	100
80	-645	-561	-477	-393	-309	-224	-140
90	-245	-61	123	307	491	676	860
100	155	439	723	1,007	1,291	1,576	1,860
110	555	939	1,323	1,707	2,091	2,476	2,860
120	955	1,439	1,923	2,407	2,891	3,376	3,860
130	1,355	1,939	2,523	3,107	3,691	4,276	4,860
140	1,755	2,439	3,123	3,807	4,491	5,176	5,860

NET RETURNS PER ACRE ABOVE CASH COSTS FOR PRODUCING OLIVE OIL

PRICE (DOLLARS/CASE)	YIELD (CASES/ACRE)						
Olive Oil	40	50	60	70	80	90	100
80	-1,203	-1,119	-1,035	-951	-867	-783	-699
90	-803	-619	-435	-251	-67	117	301
100	-403	-119	165	449	733	1,017	1,301
110	-3	381	765	1,149	1,533	1,917	2,301
120	397	881	1,365	1,849	2,333	2,817	3,301
130	797	1,381	1,965	2,549	3,133	3,717	4,301
140	1,197	1,881	2,565	3,249	3,933	4,617	5,301

NET RETURNS PER ACRE ABOVE TOTAL COSTS FOR PRODUCING OLIVE OIL

PRICE (DOLLARS/CASE)	YIELD (CASES/ACRE)						
Olive Oil	40	50	60	70	80	90	100
80	-3,376	-3,292	-3,208	-3,124	-3,040	-2,955	-2,871
90	-2,976	-2,792	-2,608	-2,424	-2,240	-2,055	-1,871
100	-2,576	-2,292	-2,008	-1,724	-1,440	-1,155	-871
110	-2,176	-1,792	-1,408	-1,024	-640	-255	129
120	-1,776	-1,292	-808	-324	160	645	1,129
130	-1,376	-792	-208	376	960	1,545	2,129
140	-976	-292	392	1,076	1,760	2,445	3,129

247

Table 8.

U.C. COOPERATIVE EXTENSION
COSTS AND RETURNS / BREAKEVEN ANALYSIS
FOR PRODUCING OLIVE OIL
NORTH COAST OF CALIFORNIA - 1999

COSTS AND RETURNS - TOTAL ACREAGE

Crop	1. Gross Returns	2. Operating Costs	3. Net Returns Above Oper. Costs (1-2)	4. Cash Costs	5. Net Returns Above Cash Costs (1-4)	6. Total Costs	7. Net Returns Above Total Costs (1-6)
Olive Oil	8,520	6,774	1,746	7,373	1,147	9,505	-985

COSTS AND RETURNS - TOTAL ACREAGE

Crop	1. Gross Returns	2. Operating Costs	3. Net Returns Above Oper. Costs (1-2)	4. Cash Costs	5. Net Returns Above Cash Costs (1-4)	6. Total Costs	7. Net Returns Above Total Costs (1-6)
Olive Oil	127,800	101,616	26,184	110,594	17,206	142,581	-14,781

BREAKEVEN PRICES PER YIELD UNIT

CROP	Base Yield (Units/Acre)	Yield Units	Breakeven Price To Cover: Operating Costs	Breakeven Price To Cover: Cash Costs	Breakeven Price To Cover: Total Costs
			$ per Yield Unit		
Olive Oil	71	Case	95.41	103.84	133.88

BREAKEVEN YIELDS PER ACRE

CROP	Yield Units	Base Price ($/Unit)	Breakeven Yield To Cover: Operating Costs	Breakeven Yield To Cover: Cash Costs	Breakeven Yield To Cover: Total Costs
			Yield Units /Acre		
Olive Oil	Case	120	56.5	61.4	79.2

248

Table 9.

U.C. COOPERATIVE EXTENSION
COSTS AND RETURNS PER ACRE TO PRODUCE
OLIVES SOLD FOR OIL
NORTH COAST OF CALIFORNIA - 1999

Labor Rate: $13.13/hr. machine labor
$9.85/hr. non-machine labor

Interest Rate: 9.69%

	Quantity/Acre	Unit	Price or Cost/Unit	Value or Cost/Acre	Your Cost
GROSS RETURNS					
Olives Sold For Oil	2.50	Ton	500.00	1,250	
TOTAL GROSS RETURNS FOR OLIVE SOLD FOR OIL				1,250	
OPERATING COSTS					
Irrigation:					
Water	30.00	Acin	8.33	250	
Herbicide:					
Roundup Ultra	0.60	Pint	7.84	5	
Karmex DF	2.00	Lb	4.84	10	
Princep Caliber 90	2.00	Lb	4.22	8	
Custom:					
Prune Trees	80.00	Tree	2.00	360	
Shred Brush	1.00	Acre	21.00	21	
Hand Pick Fruit	2.50	Ton	350.00	875	
Fertilizer:					
46-0-0	45.00	Lb N	0.279	13	
Fungicide:					
Kocide	8.00	Lb	2.99	24	
Contract:					
Ground Application	1.00	Acre	20.00	20	
Labor (machine)	12.18	Hrs	13.13	160	
Labor (non-machine)	1.80	Hrs	9.85	18	
Fuel – Gas	20.00	Gal	1.02	20	
Fuel – Diesel	6.36	Gal	0.62	4	
Lube				4	
Machinery repair				16	
Interest on operatingcapital @ 9.69%				51	
TOTAL OPERATING COSTS/ACRE				1,857	
NET RETURNS ABOVE OPERATING COSTS				-607	
CASH OVERHEAD COSTS:					
Office Expense				133	
Liability Insurance				19	
Management Fee				25	
Property Taxes				189	
Property Insurance				134	
Investment Repairs				58	
TOTAL CASH OVERHEAD COSTS/ACRE				558	
TOTAL CASH COSTS/ACRE				2,415	
CAPITAL RECOVERY COSTS (7.4% Interest Rate):					
Land				1,110	
Buildings				97	
Irrigation System				80	
Shop Tools				22	
Pruning Tools				5	
Olive Orchard Establishment				585	
Equipment				233	
TOTAL CAPITAL RECOVERY COSTS/ACRE				2,132	
TOTAL COSTS/ACRE				4,588	
NET RETURNS ABOVE TOTAL COSTS				-3,338	

249

Table 10.

U.C. COOPERATIVE EXTENSION
RANGING ANALYSIS
OLIVES SOLD FOR OIL
NORTH COAST OF CALIFORNIA – 1999

COSTS PER ACRE AT VARYING YIELDS TO PRODUCE OLIVES SOLD FOR OIL

	YIELD (TON/ACRE)						
	1.75	2.00	2.25	2.50	2.75	3.00	3.25
OPERATING COSTS/ACRE:							
Cultural Cost	932	932	932	932	932	932	932
Harvest Cost	613	700	788	875	963	1,050	1,138
Interest on operating capital	51	51	51	51	51	51	51
TOTAL OPERATING COSTS/ACRE	1,595	1,682	1,770	1,857	1,945	2,032	2,120
TOTAL OPERATING COSTS/TON	911	841	787	743	707	677	652
CASH OVERHEAD COSTS/ACRE	558	558	558	558	558	558	558
TOTAL CASH COSTS/ACRE	2,153	2,240	2,328	2,415	2,503	2,590	2,678
TOTAL CASH COSTS/TON	1,230	1,120	1,035	966	910	863	824
NON-CASH OVERHEAD COSTS/ACRE	2,132	2,132	2,132	2,132	2,132	2,132	2,132
TOTAL COSTS/ACRE	4,326	4,413	4,501	4,588	4,676	4,763	4,851
TOTAL COSTS/TON	2,472	2,207	2,000	1,835	1,700	1,588	1,493

NET RETURNS PER ACRE ABOVE OPERATING COSTS FOR OLIVES SOLD FOR OIL

PRICE (DOLLARS/TON)	YIELD (TON/ACRE)						
Olives Sold For Oil	1.75	2.00	2.25	2.50	2.75	3.00	3.25
350	-982	-982	-982	-982	-982	-982	-982
400	-895	-882	-870	-857	-845	-832	-820
450	-807	-782	-757	-732	-707	-682	-657
500	-720	-682	-645	-607	-570	-532	-495
550	-632	-582	-532	-482	-432	-382	-332
600	-545	-482	-420	-357	-295	-232	-170
650	-457	-382	-307	-232	-157	-82	-7

NET RETURNS PER ACRE ABOVE CASH COSTS FOR OLIVES SOLD FOR OIL

PRICE (DOLLARS/TON)	YIELD (TON/ACRE)						
Olives Sold For Oil	1.75	2.00	2.25	2.50	2.75	3.00	3.25
350	-1,540	-1,540	-1,540	-1,540	-1,540	-1,540	-1,540
400	-1,453	-1,440	-1,428	-1,415	-1,403	-1,390	-1,378
450	-1,365	-1,340	-1,315	-1,290	-1,265	-1,240	-1,215
500	-1,278	-1,240	-1,203	-1,165	-1,128	-1,090	-1,053
550	-1,190	-1,140	-1,090	-1,040	-990	-940	-890
600	-1,103	-1,040	-978	-915	-853	-790	-728
650	-1,015	-940	-865	-790	-715	-640	-565

NET RETURNS PER ACRE ABOVE TOTAL COSTS FOR OLIVES SOLD FOR OIL

PRICE (DOLLARS/TON)	YIELD (TON/ACRE)						
Olives Sold For Oil	1.75	2.00	2.25	2.50	2.75	3.00	3.25
350	-3,713	-3,713	-3,713	-3,713	-3,713	-3,713	-3,713
400	-3,626	-3,613	-3,601	-3,588	-3,576	-3,563	-3,551
450	-3,538	-3,513	-3,488	-3,463	-3,438	-3,413	-3,388
500	-3,451	-3,413	-3,376	-3,338	-3,301	-3,263	-3,226
550	-3,363	-3,313	-3,263	-3,213	-3,163	-3,113	-3,063
600	-3,276	-3,213	-3,151	-3,088	-3,026	-2,963	-2,901
650	-3,188	-3,113	-3,038	-2,963	-2,888	-2,813	-2,738

250

Table 11.

U.C. COOPERATIVE EXTENSION
COSTS AND RETURNS / BREAKEVEN ANALYSIS
OLIVES SOLD FOR OIL
NORTH COAST OF CALIFORNIA - 1999

COSTS AND RETURNS - PER ACRE BASIS

Crop	1. Gross Returns	2. Operating Costs	3. Net Returns Above Oper. Costs (1-2)	4. Cash Costs	5. Net Returns Above Cash Costs (1-4)	6. Total Costs	7. Net Returns Above Total Costs (1-6)
Olives Sold For Oil	1,250	1,857	-607	2,456	-1,206	4,588	-3,338

COSTS AND RETURNS - TOTAL ACREAGE

Crop	1. Gross Returns	2. Operating Costs	3. Net Returns Above Oper. Costs (1-2)	4. Cash Costs	5. Net Returns Above Cash Costs (1-4)	6. Total Costs	7. Net Returns Above Total Costs (1-6)
Olives Sold For Oil	18,750	27,859	-9,109	36,837	-18,087	68,824	-50,074

BREAKEVEN PRICES PER YIELD UNIT

CROP	Base (Units/Acr	Yield Units	Operating Costs	Cash Costs	Total Costs
			$ per Yield Unit		
Olives Sold For Oil	2.5	Ton	742.91	982.32	1,835.31

BREAKEVEN YIELDS PER ACRE

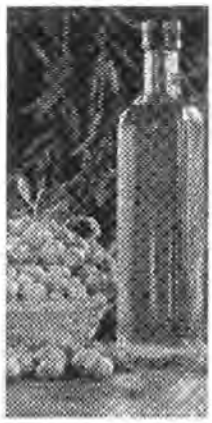
CROP	Yield Units	Base Price ($/Unit)	Operating Costs	Cash Costs	Total Costs
			Yield Units/Acre		
Olives Sold For Oil	Ton	500	3.7	4.9	9.2

251

SPANISH VARIETALS

Varieties

Olive tree cultivation originated in the Middle East more than 5,000 years ago and spread to the West throughout the Mediterranean area. From the wild olive trees that grew spontaneously, the first cultivators of the Iberian Peninsula began to choose those that had the best characteristics depending on the areas, productivity, adaptation to the soils, yield, etc. The olive trees in the Iberian peninsula are now, therefore, hardy trees that can withstand diverse climatic and orographic conditions and grow alongside other autochthonous plants, such as vines, almond trees or fig trees.



There are a surprising diversity of climates and microclimates in Spain resulting from its complex orography, as well as a great variety and richness of soils. These geographic conditions, together with the large number of olive varieties used in making our olive oils, mean that Spain can offer a wide range of aromas and flavours that are unrivalled by any other producing country. Oils with a very sweet and mild flavour can be found alongside others with great body and character, with a pleasant bitter or peppery flavour of different intensities. Spanish oils generally have an intense fruity aroma that reminds us of a green or ripe olives.

In Spain alone there are 260 olive tree cultivars. By way of example, the characteristics of some of the main varieties are described below:

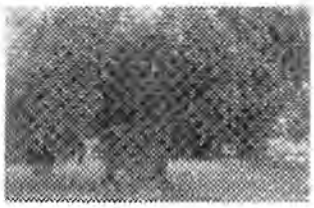
Picual	Lechin of Seville
Cornicabra	Verdial
Hojiblança	Empeltre
Arbequina	Picudo

PICUAL

General data and areas.

This is the most important variety in the world, representing 50% of Spain's olives and trees and, therefore, approximately 20% world-wide. Its geographic location is clearly linked to Andalusia, the main producing region in the world, and specifically to the provinces of Jaen, Cordoba and Granada. This cultivar is given different names depending on the producing area, but its main name, Picual (from the Spanish root "pico", meaning "peak"), comes from the shape of the drupe, as it is like a swollen teat ending in a point.

The tree.



It is very vigorous with rather short spreading branches and it tends to sprout superfluous shoots. Its crowns are vigorous with a tendency to close in on themselves. The foliage develops well and the young wood is greenish-grey. It starts to produce early and is highly productive, which is one of the reasons why plantations of this olive tree have increased so much. It is a type of tree that adapts to different climatic and soil conditions and can tolerate frosts, although it is not very resistant to droughts and very limy soils.

The leaf.

It is rather elongated and wider in the upper half.

The drupe.

It is generally medium-sized to thick and weighs about 3.2 grams. The flesh-to-pit ratio is about 5.6. It ripens between the second week of November and the third week of December. The oil yield is very good and can sometimes be up to 27%.

The oil.

From a physical-chemical point of view, it is excellent due to its fatty acid composition and the number of natural antioxidants it contains. Its high content of monounsaturated oleic acid, important to avoid cardiovascular diseases, and its low content of linoleic acid (an essential acid for the human diet, but if there are excessive quantities, the oil starts to oxidise and free radicals, which are harmful for certain organs in the human body, are formed) as well as its high content of polyphenols, make it *the most stable oil in the world*, with a long shelf life and it performs excellently when heated for cooking.

From an organoleptic point of view, we have to differentiate between the plains and the mountains, as their organoleptic profiles are very different. Oils from the plains have great body, are normally bitter, with a certain flavour of wood. Oils from the mountains are usually sweeter, although they have a "fresh" and pleasant flavour.

Back

CORNICABRA

General data and areas.

This cultivar is the second in importance in the number of cultivated hectares, but the third in production. It originated in Mora de Toledo, and its cultivation area covers the provinces of Toledo and Ciudad Real in the Community of Castilla la Mancha. Its name ("one-horned goat") comes from the characteristic horn-shape of its fruit.

The tree.

The Cornicabra olive is an old cultivar and is most likely a varietal population (family), with a large number of local ecotypes perfectly adapted to their area. This variety has average vigour, with medium-length branches, not very many shoots and the young wood is light grey with ochre tones.

The leaf.

It is long and symmetrical, light green on the top side and greenish-grey on the back.

The drupe.

The olive is elongated and rather bent, asymmetrical, plump and flat at the back, with a horn-shaped belly. It is of average size and weight (about 3 grams), but has a high oil yield, about 19% and a high flesh-to-pit ratio (5). The drupe ripens late, usually starting in the last week of October and finishing in the first week of January. As it is strongly resistant to being picked, mechanised harvesting is difficult.

The oil.

It is golden yellow with touches of light green indicating its fruitiness. When it is obtained from riper olives, at the end of the harvest, there are normally different flavours and textures that remind us of exotic fruit, like avocados. Cornicabra oils are fruity and have a noticeable balance between sweet at first, the bitterness of green leaves and a medium-intense peppery flavour. Their texture is smooth and velvety. They are stable oils because of their high content in monounsaturated fatty acids. The balanced composition of essential fatty acids, high content in oleic acid and minor components, which produce excellent aromas and flavours, make it especially appropriate for dietary purposes.

Back

HOJIBLANCA

General data and areas.

The name (from the Spanish for "hoja", leaf, and "blanca", white) comes from the colour of the leaf's back side, making the tree look bright, and, from a distance, silver. It is found in Andalusia, to be precise in the east of the province of Seville, the south of Cordoba and all of the north of the province of Malaga. It accounts for about 16% of the Andalusian olive groves. It is used both for black Californian style table olives, due to the firm texture of its flesh, and for oil production.

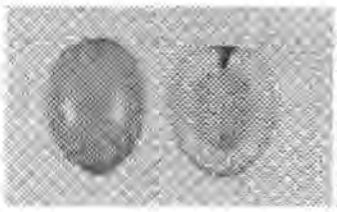
The tree.

Its vigour is average to good, with long branches that bear a lot of fruit, although they tend to droop. The crowns have an average density and a regular foliage cover, and the trees are characterised by the light greenish-grey colour of their young wood. They can withstand cold weather well, and adapt to limy soils perfectly.

The leaf.

It is partially fluted and elongated, although it is rather wide and the back side is silver.

The drupe.



Although the size fluctuates a great deal, the drupe is normally large to thick and weighs 4.3 grams on average. It is an almost perfect sphere with a high flesh-to-pit ratio, about 8. The drupe can also withstand cold weather well. It flowers in the first two weeks of May, with most blossoms at the beginning of the second week. It ripens quite late, between the end of November and the end of December, and once it is ripe it resists being picked, so harvesting is somewhat difficult. Its oil yield is low, with an average between 17-19%.

The oil.

From a physical-chemical point of view, it has a very balanced composition of fatty acids, with saturated acids that are relatively lower than in the rest of the oils of other varieties. Its composition is ideal for dietary purposes. As its stability is not very high and it oxidises easily, this oil should be kept out of the light and stored without excessive oxygenation.

From an organoleptic point of view, it has an enormous range of flavours, but the most common are sweet at the beginning of the tasting, with a fresh grassy fruity aroma, a slightly bitter flavour of green fruit and other fruits that sometimes recall a fruit cocktail, slightly peppery in the throat and a final almondy aftertaste.

Back

ARBEQUINA

General data and areas.

This is one of the best known Spanish cultivars. Although it has been planted in the provinces of Saragossa and Huesca in the community of Aragon, it originated in the locality of Arbeca (Lerida), where the name comes from, and it is widespread in the provinces of Tarragona and Lerida, both in the community of Catalonia. The quality of the oil has led these two producing areas to being recognised with two Designations of Origin: Siurana and Les Garrigues respectively. The tree is found in olive groves or mixed with other crops, mainly vines, and sometimes grows on the edges of plots.

The tree.

The plant, which is not very vigorous, can be used in intensive plantations. Its long shoots, non-spreading branches and the dark green colour of the young wood, make this olive tree look like a broomstick. The olive orchards of the arbequina cultivar give the landscape a very strange appearance and are very different from those seen in Andalusia, for example. This variety has an average flowering period in the first fortnight of May.

The leaf.

It is fluted with not very thick edges, becoming wider at the tip. It is an ochre-green colour on the top side and greenish-grey-yellow on the back.

The drupe.

It is short, oval and almost symmetrical, with a low flesh-to-pit ratio of 4.6, and as it is small, weighing about 1.9 grams, mechanical harvesting is difficult. However, it is a greatly appreciated cultivar as it starts producing early, with an average ripening period between the second week of December and the second week of January. It is highly productive and has a good oil yield, about 20.5%, which makes it one of the varieties with the highest oil extraction percentage.

The oil.

These oils have an exquisite flavour with traces of tomatoes and vegetable gardens, and the aroma reminds us of fresh artichokes. They are also fruity with a certain exotic aroma. A fresh apple smell, accompanied by a certain mildness and sweetness, identifies the oils, with a final aftertaste of green almonds. They are also very fresh and young oils which, because of their composition, are a little more delicate than other varieties as far as oxidation is concerned, which is why they must be kept in the dark at a low temperature to guarantee protecting them for a longer period of time. These oils have been appreciated for their quality for centuries, even though their production usually fluctuates greatly due to climatic conditions. Virgin olive oils of the arbequina cultivar are dense and pour well and vary greatly from one area to another, as well as within the same area, in successive years. When harvesting is started, the olives are very green and this characteristic is reflected in the organoleptic properties of the oils. The olives are not normally left to become completely ripe. To describe the average characteristics of these oils, we could say that they are fruity, slightly green and more or less bitter, peppery and sweet. They are, therefore, very balanced oils, with greener flavours (leaf), bitter and peppery at the beginning of the harvesting season, and sweeter at the end. We should also mention the almondy (green almond) aroma and flavour and the way they pour smoothly, which is a very pleasant sensation when tasting them.

Back

LECHIN OF SEVILLE

General data and areas.

This variety is distributed throughout the provinces of Seville, Cordoba, Cadiz, Malaga and Huelva. Its name (from the Spanish root "leche" meaning "milk") comes from the whitish colour of its pulp and its oily liquid (a mixture of vegetable water and oil).

The tree.

It is characterised by being very vigorous with large roots, an open appearance and a thick, dense crown. It is a rustic tree and tolerates cold weather, droughts and limy or bad quality soils. Although it is productive, its flowering period is average and there are a number of faults in the harvest due to blossom deterioration. Harvesting is expensive as the drupe is large and resists picking.

The leaf.

It is small, flat, short and a little wider in the middle. The top side is a bright yellowish-green and the back is grey-green.

The drupe.

The average olive weighs almost 3 grams and is ellipsoidal, a little convex at the back and slightly asymmetrical. The end is rounded and sometimes has a small teat. It is black when ripe. The oil content is medium to low, about 18%, and it has a high flesh-to-pit ratio of about 7.2 to 8.5. It ripens early.

The oil.

From a physical-chemical point of view, its composition makes it rather unstable as far as oxidation is concerned, and it loses its organoleptic properties relatively quickly.

From an organoleptic point of view, it is a balanced smooth-pouring oil, with an average, grassy aroma of olives. The flavour is bitter with an average peak intensity and leaves a green almond aftertaste in the mouth. It is very pleasant when recently produced.

Back

VERDIAL

The name verdial covers a series of local varieties spread throughout the South and the South-west of the peninsula. It has different names depending on its geographic origin.

I. VERDIAL OF BADAJOZ

General data and areas.

It is found throughout the province of Badajoz, in the community of Extremadura, to the borders with Portugal.

The tree.

As it is resistant to droughts, it is used as the root stock of other cultivars. It blossoms in the first fortnight of May.

The drupe.

It is very large and has an average weight of 5.7 grams, and is appreciated for its dual purposes: for oil (it has a high oil yield of almost 22%, because of its high flesh-to-pit ratio of about 7.2), and for table olives (it is large and easy to pickle). It ripens between the last third of November and almost the end of the year. Mechanical

harvesting is difficult as it ripens late and is highly resistant to being picked.

Back

II. VERDIAL OF VELEZ-MALAGA

General data and areas.

This is a typical variety of the Axarquía region, in the South-east of the province of Malaga.

The drupe.

It is larger than the other varieties that are processed to obtain oil, although it does not have a very high oil yield.

The oil.

The oils are fruity with a very sweet and pleasant flavour and do not taste bitter or peppery at all. Due to its composition, it has to be protected from heat, light and air to be best preserved. The virgin oil of this verdial cultivar is processed separately in Malaga, although it is also normally mixed with oils from the hojiblanca variety resulting in a perfect organoleptic blend with average stability.

Back

EMPELTRE

General data and areas.

The empeltre variety is characteristic of the community of Aragon, originating in the locality of Pedrola (in the province of Saragossa). It is cultivated in an area that extends from the provinces of Logroño and Teruel through the Ebro Valley to the province of Tarragona, and can even be found on the Balearic Islands.

The tree.

It is very vigorous, with upright branches and crowns that tend to be vertical. It ripens in the first third of May.

The leaf.

It is wider in the upper half. The top side is dark green and the back is silver scored by a green, prominent nerve.

The drupe.

The olive is elongated, asymmetrical and slightly plump at the back. It has an average volume (2.7 grams) with a flesh-to-pit ratio of 5.3 and an acceptable oil yield of 18.3%. It ripens early, between the first week of November and the first week of December. As it is not resistant to being picked, and its branches are upright, it is ideal for mechanical harvesting. However, there may be fruit setting problems and damage due to winter frosts. It is a variety greatly appreciated for its productivity and the excellent quality of its oil.

The oil.

258

The oils are a pale yellow colour in the majority of the cases, which is not due to harvesting when the olives are overripe. The oils have a mild fruity aroma and are very pleasant and very sweet tasting. They are never bitter or peppery and usually leave an aftertaste of almonds. As it is mild, it is ideal for blending.

Back

PICUDO

General data and areas.

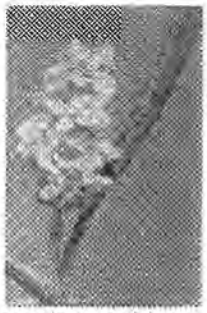
The olive is called picudo (which means "prominent peak") because of the shape of the drupe with a pointed and curved end and a noticeable teat. It has many other names, but the one it is given in Luque, a town in the south-east of Cordoba, where it is called "pajarero" (bird trapper) is curious. According to legend, because the oil is so sweet, when it is ripe, the birds peck at the fruit. This cultivar is widespread in the provinces of Cordoba, Granada, Malaga and Jaen, with the most plantations in the area of the Designation of Origin Baena, in the south-east of Cordoba.

The tree.

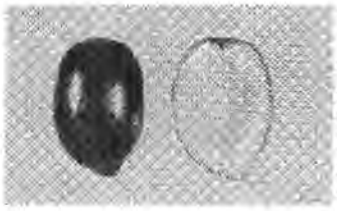
It is very vigorous and has an open appearance and a thick, dense crown. It takes root easily and adapts well to limy soils and prefers an excess of humidity to droughts. It can withstand cold weather and is considered a rustic tree.

The leaf.

It is large, long and wide. The top side is very dark green. You can tell this tree from a distance as it looks darker than the rest.



The drupe.



The drupe is black when ripe and it is characterised by being the second largest of those that are used to obtain oil. Its average weight is about 4.8 grams. The fruit-to-pit ratio is high, about 6.3. It ripens between the fourth week of November and the end of December and the oil yield is good, but not as good as the picual, with figures of around 20%. It is an excellent choice for table olives, both green and Californian style.

The oil.

Its composition of fatty acids is almost the same as the lechin cultivar. As far as oxidation is concerned, the oils are included in the delicate range. Its organoleptic characteristics are very good, with unbeatable balance and sweetness, with no hard flavours and they pour very smoothly and are light, sometimes reminding us slightly of exotic fruits, apples and almonds.

Back

259

GRADE STANDARDS & QUALITY

Olive Oil Buyer's Guide

World Olive Oil Market

Mediterranean countries account for 98% of world olive oil production. The total world supply determines to a great extent what world prices will be, whether or not individual producing countries have large or small crops.

North American Market

The US and Canada constitute the largest consuming market, outside the producing countries, but only import about 8% of the world supply.

Types of Olive Oil Sold in North America

- EXTRA VIRGIN OLIVE OIL is obtained from the fruit of the olive tree by mechanical means. It is immediately edible and has a maximum acidity of 1%. Its fruitiness may vary in intensity. Experts judge it to have perfect flavor characteristics.
- VIRGIN OLIVE OIL is obtained from the fruit of the olive tree by mechanical means. It is immediately edible and has a maximum acidity of 2%. Its fruitiness may vary in intensity. Experts judge it to have good flavor characteristics.
- OLIVE OIL is blend of refined olive oil and varying amounts of extra virgin or virgin olive oil to give it a characteristic olive flavor, color, and aroma.
- "LIGHT" OLIVE OIL is a blend of refined oil and sufficient extra virgin or virgin olive oil to give it a light color, taste and aroma.
- OLIVE POMACE OIL is a blend of refined olive pomace oil and varying amounts of extra virgin or virgin olive oil. A small amount of oil remains in the pomace or residue that is left after olives have been pressed. It can be recovered by solvent extraction, which is the method that is generally used to produce seed oils.

Quality Assurance

Every company in the olive oil trade must have quality assurance and testing procedures to make sure that the product it buys or sells is in compliance with the international standards for olive oil. As suppliers establish their names in the market, it is not necessary that every lot purchased from them be tested, but some testing is always called for to make sure that performance is consistent.

Exporting and importing countries test olive oil, some in a very aggressive manner - testing every lot - and others on a random basis. The North American Olive Oil Association (NAOOA) regularly collects and arranges for the testing of olive oils from supermarket shelves in the US and Canada to make sure that they conform to the international standards. It also collects and tests olive oils from food service distributors, club stores, and other outlets handling olive oils sold in the food service trade. Even bulk olive oils are collected by the NAOOA for testing.

Price As Indicator of Quality

Buyers in North America are well aware of the pricing for popular brands in the marketplace. Price differentials may be due to the way brands are brought to market, the quality of the oils, and the timing of promotions. Still, there are levels at which olive oils must be sold in order to cover the import cost, the cost of distribution, and normal overhead. These levels can vary within a certain range. When product is being sold consistently at less than fair market values it should be tested frequently.

The NAOOA does not judge what constitutes a legitimate or fair price for olive oil. If a buyer is suspicious about an oil because of its price or appearance, he may contact the NAOOA for guidance on how it should be tested.

Product Guarantee

Buyers are advised to require in their Purchase Orders a written guarantee that they will be reimbursed for the price of product found to be out of compliance with the International Standard for Olive Oil, plus the cost of any recall, legal fees, and loss of consumers' good will.

NAOOA's Olive Oil Testing Program

As a service to the industry the NAOOA arranges for the testing of olive oils under established procedures that provide lawful protection for all parties.

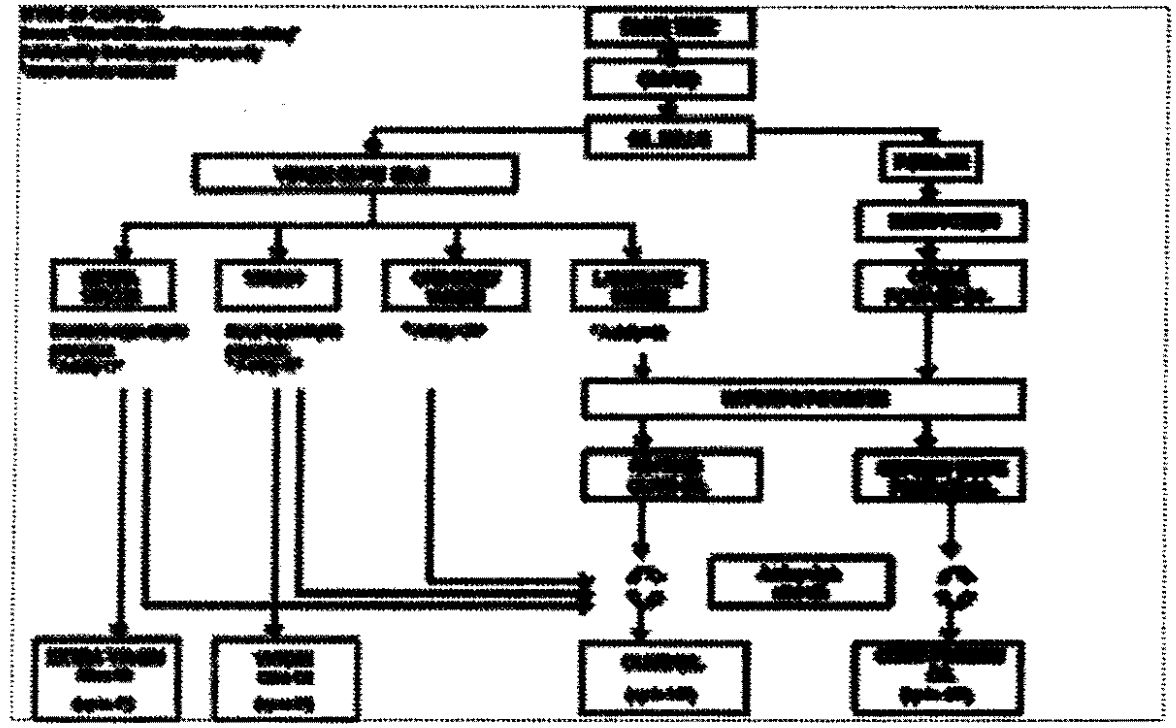
Buyers are encouraged to buy from members of the NAOOA, or from companies whose testing program is equivalent to the NAOOA program.

For more information on the NAOOA Testing Program and its membership write to the North American Olive Oil Association, 5 Ravine Drive, P.O. Box 545, Matawan, NJ 07747 or call 732-583-8188. Fax requests to 732-583-0798.

NORTH AMERICAN OLIVE OIL ASSOCIATION

February, 1998

261



TYPES OF OLIVE OIL

Virgin olive oils

(Regulation (EC) no. 1638/98 OJEC 219 of 28/7/98)

Oils only obtained from the fruit of the olive tree by mechanical processes or other physical processes, in conditions, especially thermal ones, that do not cause alterations in the oil which must not receive any treatment other than washing, decantation, centrifugation and filtering This category does not include oils that have been obtained by using solvents or by a re-esterification process or any other mixture with oils of different characteristics.

- ***Extra Virgin Olive Oil***

  Virgin olive oil whose free acidity, expressed as oleic acid, is not more than 1 gram per 100 grams and whose other characteristics are in accordance with those established for the category.

- ***Virgin Olive Oil***
  (In the production and wholesale marketing phase the term "fine" can be used)

  Virgin olive oil whose free acidity, expressed as oleic acid, is not more than 2 grams per 100 grams and whose other characteristics are in accordance with those established for the category.

- ***Ordinary Virgin Olive Oil***

  Virgin olive oil whose free acidity, expressed as oleic acid, is not more than 3.3 grams per 100 grams and whose other characteristics are in accordance with those established for the category.

- ***Lampante Virgin Olive Oil***

  Virgin olive oil whose free acidity, expressed as oleic acid, is more than 3.3 grams per 100 grams and whose other characteristics are in accordance with those established for the category.

PREVIOUS PAGE BLANK

262

Regulation (EEC) no. 356/92, OJEC 39 of 15/2/92

- ***Refined Olive Oil***

  Olive oil obtained by refining virgin olive oils, whose free acidity, expressed as oleic acid, cannot be more than 0.5 grams per 100 grams and whose other characteristics are in accordance with those established for the category.

- ***Olive Oil***

  Olive oil that consists of a mixture of refined olive oil and virgin olive oils, not including lampante, whose free acidity, expressed as oleic acid, cannot be more than 1.5 grams per 100 grams and whose other characteristics are in accordance with those established for the category.

Of the abovementioned oils, only **Extra Virgin Olive Oil**, **Virgin Olive Oil** and **Olive Oil** are marketed directly to the final consumer. **Ordinary Virgin** and **Lampante Oils** are only marketed wholesale.

Sometimes, due to unfavourable climate conditions or deficiencies in the production process, virgin olive oils have a high degree of acidity or a defective flavour, colour or aroma. These oils, called **Lampante Virgin Oils**, must go through a refining process to correct these defects. This process reduces the degree of acidity and eliminates the defective colour and aroma. This is how **Refined Olive Oil** is obtained, a product that maintains the chemical structure of olive oil but does not have any aroma or flavour. This product is not marketed, but a certain quantity of **Extra Virgin, Virgin or Ordinary Virgin Olive Oil** is added to the oil to give it aroma and flavour. This is how the category called **"Olive Oil"** is obtained.

TYPES OF OLIVE-POMACE OIL

(Regulation (EEC) no. 356/92, OJEC 39 of 15/2/92)

- ***Crude olive-pomace oil***

  Oil obtained by treating olive pomace with solvents, not including oils obtained from re-esterification processes and from any mixture of oils with other properties, and whose other characteristics are in accordance with those established for the category.

- ***Refined olive-pomace oil***

  Oil obtained by refining crude olive-pomace oil, whose free acidity, expressed as oleic acid, cannot be more than 0.5 grams per 100 grams and whose other characteristics are in accordance with those established for the category.

- ***Olive-pomace oil***

  Oil that consists of a mixture of refined olive-pomace oil and virgin olive oils, not including lampante, whose free acidity, expressed as oleic acid, cannot be more than 1.5 grams per 100 grams and whose other characteristics are in accordance with those established for the category.

Olive pomace is the solid residue or paste obtained from the olive oil production process (during pressing or centrifugation). This residue is treated with solvents to extract the oil it contains to obtain **Crude Olive-Pomace Oil**. As it is not edible, it has to undergo a refining process, which is similar to the lampante virgin olive oil process, to obtain **Refined Olive-Pomace Oil**. This oil, with no flavour, aroma or colour, is improved with edible virgin olive oils (not including lampante) to obtain the oil called **"Olive-Pomace Oil"**.

United States
Department of
Agriculture

Agricultural
Marketing
Service

Fruit and
Vegetable
Division

Processed
Products
Branch

United States Standards for Grades of Olive Oil

Effective date March 22, 1948

264

This is the first issue of the United States standards for Grades of Olive Oil published in the **FEDERAL REGISTER** of February 20, 1948, to become effective March 22, 1948.

Voluntary U.S. grade standards are issued under the authority of the Agricultural Marketing Act of 1946, which provides for the development of official U.S. grades to designate different levels of quality. These grade standards are available for use by producers, suppliers, buyers, and consumers. As in the case of other standards for grades of processed fruits and vegetables, these standards are designed to facilitate orderly marketing by providing a convenient basis for buying and selling, for establishing quality control programs, and for determining loan values.

The standards also serve as a basis for the inspection and grading of commodities by the Federal inspection service, the only activity authorized to approve the designation of U.S. grades as referenced in the standards, as provided under the Agricultural Marketing Act of 1946. This service, available as on-line (in-plant) or lot inspection and grading of all processed fruit and vegetable products, is offered to interested parties, upon application, on a fee-for-service basis. The verification of some specific recommendations, requirements, or tolerances contained in the standards can be accomplished only by the use of on-line inspection procedures. In all instances, a grade can be assigned based on final product factors or characteristics.

In addition to the U.S. grade standards, grading manuals or instructions for inspection of several processed fruits and vegetables are available upon request for a nominal fee. These manuals or instructions contain detailed interpretations of the grade standards and provide step-by-step procedures for grading the product.

Grade standards are issues by the Department after careful consideration of all data and views submitted, and the Department welcomes suggestions which might aid in improving the standards in future revisions. Comments may be submitted to, and copies of standards and grading manuals obtained from:

Chief, Processed Products Branch
Fruit and Vegetable Division, AMS
U.S. Department of Agriculture
P.O. Box 96456, Rm. 0709, So. Bldg.
Washington, D.C. 20090-6456

United States Standards for Grades of Olive Oil

Section		Page No.
§52.1531	Product description.	2
§52.1532	Grades of olive oil.	2
§52.1533	Recommended fill of container.	2
§52.1534	Ascertaining the grade.	2
§52.1535	Ascertaining the rating for each factor.	3
§52.1536	Free fatty acid content.	3
§52.1537	Absence of defects.	4
§52.1538	Odor.	4
§52.1539	Flavor.	5
§52.1540	Ascertaining the grade of a lot.	5
§52.1541	Score sheet for olive oil.	6

Authority: Agricultural Marketing Act of 1946, Secs. 203, 205, 60 Stat. 1087, as amended, 1090, as amended (7 U.S.C. 1622, 1624).

Note: Compliance with the provisions of these standards shall not excuse failure to comply with the provisions of the Federal Food, Drug, and Cosmetic Act, or with applicable State laws and regulations.

§52.1531 Product description.

Olive oil is the edible oil obtained from the fruit of the olive tree (Olea europaea L.); is clarified, has a specific gravity of 0.910 to 0.915 at 25° C./25° C.; has an iodine number (Hanus) of 79 to 90; has a refractive index of 1.4668 to 1.4683 at 25° C.; and is packed in containers suitable for preservation of the product.

§52.1532 Grades of olive oil.

(a) **U.S. Grade A or U.S. Fancy** is the quality of olive oil that possesses the typical greenish to light yellow color of olive oil; possesses a free fatty acid content, calculated as oleic, of not more than 1.4 percent; is free from defects; and is of such quality with respect to odor and flavor as to score not less than 90 points when scored in accordance with the scoring system outlined in this subpart.

(b) **U.S. Grade B or U.S. Choice** is the quality of olive oil that possesses the typical greenish to light yellow color of olive oil; possesses a free fatty acid content, calculated as oleic, of not more than 2.5 percent; is reasonably free from defects; possesses a reasonably good typical odor; possesses a reasonably good typical flavor; and scores not less than 80 points when scored in accordance with the scoring system outlined in this subpart.

(c) **U.S. Grade C or U.S. Standard** is the quality of olive oil that possesses the typical greenish to light yellow color of olive oil; possesses a free fatty acid content, calculated as oleic, of not more than 3.0 percent; is fairly free from defects; possesses a fairly good typical odor; possesses a fairly good typical flavor; and scores not less than 70 points when scored in accordance with the scoring system outlined in this subpart.

(d) **U.S. Grade D or Substandard** is the quality of olive oil that fails to meet the requirements of U.S. Grade C or U.S. Standard.

§52.1533 Recommended fill of container.

The recommended fill of container is not incorporated in the grades of the finished product since fill of container, as such, is not a factor of quality for the purposes of these grades. It is recommended that each container of olive oil be filled with olive oil as full as practicable without impairment of quality.

§52.1534 Ascertaining the grade.

(a) The grade of olive oil may be ascertained by considering, in addition to the requirements of the respective grade, the following factors: Free fatty acid content, absence of defects, odor, and flavor.

(b) The relative importance of each factor is expressed numerically on a scale of 100. The maximum number of points that may be given for each factor is:

Factors	Points
Free fatty acid content	30
Absence of defects	30
Odor	20
Flavor	20
Total Score	100

§52.1535 Ascertaining the rating for each factor.

The essential variations within each factor are so described that the value may be ascertained for each factor and expressed numerically. The numerical range within each factor is inclusive (for example, **27 to 30 points** means 27, 28, 29, or 30 points).

§52.1536 Free fatty acid content.

(a) **General.** The free fatty acid content shall be determined in accordance with the method described in the current **Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists.**

(b) **"A" classification.** Olive oil that possesses a free fatty acid content of not more than 1.4 percent, calculated as oleic acid, may be given a score of 27 to 30 points.

(c) **"B" classification.** If the olive oil possesses a free fatty acid content of more than 1.4 percent, but not more than 2.5 percent, calculated as oleic acid, a score of 24 to 26 points may be given. Olive oil that falls into this classification shall not be graded above U.S. Grade B or U.S. Choice, regardless of the total score for the product (this is a limiting rule).

(d) **"C" classification.** If the olive oil possesses a free fatty acid content of more than 2.5 percent but not more than 3.0 percent, calculated as oleic acid, a score of 21 to 23 points may be given. Olive oil that falls into this classification shall not be graded above U.S. Grade C or U.S. Standard, regardless of the total score for the product (this is a limiting rule).

(e) **"SStd" classification.** Olive oil that contains more than 3.0 percent free fatty acid, calculated as oleic, may be given a score of 0 to 20 points, and shall not be graded above U.S. Grade D or Substandard, regardless of the total score for the product (this is a limiting rule).

§52.1537 Absence of defects.

(a) **General.** The factor of absence of defects refers to the degree of freedom from cloudiness at 60° F. due to stearin, and from sediment.

(b) **"A" classification.** Olive oil that is free from defects may be given a score of 27 to 30 points. **Free from defects** means that the olive oil is entirely free from the defects mentioned and that no water or other liquid immiscible with the olive oil is present.

(c) **"B" classification.** If the olive oil is reasonably free from defects, a score of 24 to 26 points may be given. Olive oil that falls into this classification shall not be graded above U.S. Grade B or U.S. Choice, regardless of the total score for the product (this is a limiting rule). **Reasonably free from defects** means that the olive oil is reasonably free from defects mentioned and that no water or other liquid immiscible with the olive oil is present.

(d) **"C" classification.** If the olive oil is fairly free from defects, a score of 21 to 23 points may be given. Olive oil that falls into this classification shall not be graded above U.S. Grade C or U.S. Standard, regardless of the total score for the product (this is a limiting rule). **Fairly free from defects** means that the defects mentioned may be present but may not impair the quality of the olive oil and that no water or other liquid immiscible with the olive oil is present.

(e) **"SStd" classification.** Olive oil that fails to meet the requirements of paragraph (d) of this section may be given a score of 0 to 20 points and shall not be graded above U.S. Grade D or Substandard, regardless of the total score for the product (this is a limiting rule).

§52.1538 Odor.

(a) **General.** The factor of odor refers to a typical olive oil odor and the degree of freedom from strong green olive odor, from musty, moldy, butyric, zapatera, rancid, or any other off-odors.

(b) **"A" classification.** Olive oil that possesses a good typical odor may be given a score of 18 to 20 points. **Good typical odor** means that the olive oil has a typical olive oil odor and is practically free from off-odors of any kind.

(c) **"B" classification.** If the olive oil possesses a reasonably good typical odor, a score of 16 or 17 points may be given. **Reasonably good typical odor** means that the olive oil has a typical olive oil odor and is reasonably free from off-odors of any kind.

269

(d) **"C" classification.** If the olive oil possesses a fairly good typical odor, a score of 14 or 15 points may be given. **Fairly good typical odor** means that the olive oil has a typical olive oil odor and is fairly free from off-odors of any kind. Olive oil that falls into this classification shall not be graded above U.S. Grade C or U.S. Standard, regardless of the total score for the product (this is a limiting rule).

(e) **"SStd" classification.** Olive oil that fails to meet the requirements of paragraph (d) of this section and that possesses a definite musty, moldy, butyric, or zapatera odor or any other definitely objectionable odor may be given a score of 0 to 13 points and shall not be graded above U.S. Grade D or Substandard, regardless of the total score for the product (this is a limiting rule).

§52.1539 Flavor.

(a) **General.** The factor of flavor refers to the typical olive oil flavor and the degree of freedom from strong green olive flavors, from musty, moldy, butyric, zapatera, rancid, or any other off-flavors.

(b) **"A" classification.** Olive oil that possesses a good typical flavor may be given a score of 18 to 20 points. **Good typical flavor** means that the olive oil has a typical olive oil flavor and is practically free from off-flavors of any kind.

(c) **"B" classification.** If the olive oil possesses a reasonably good typical flavor, a score of 16 or 17 points may be given. **Reasonably good typical flavor** means that the olive oil has a typical olive oil flavor and is reasonably free from off-flavors of any kind.

(d) **"C" classification.** If the olive oil possesses a fairly good typical flavor, a score of 14 or 15 points may be given. **Fairly good flavor** means that the olive oil has a typical olive oil flavor and is fairly free from off-flavors of any kind. Olive oil that falls into this classification shall not be graded above U.S. Grade C or U.S. Standard, regardless of the total score for the product (this is a limiting rule).

(e) **"SStd" classification.** Olive oil that fails to meet the requirements of paragraph (d) of this section and that possesses a definite musty, moldy, butyric, zapatera, or rancid flavor or any other definitely objectionable flavor may be given a score of 0 to 13 points and shall not be graded above U.S. Grade D or Substandard, regardless of the total score for the product (this is a limiting rule).

§52.1540 Ascertaining the grade of a lot.

The grade of a lot of olive oil covered by these standards is determined by the procedures set forth in the **Regulations Governing Inspection and Certification of Processed Fruits and Vegetables, Processed Products Thereof, and Certain Other Processed Food Products** (7 CFR 52.1 through 52.83).

§52.1541 Score sheet for olive oil.

The following score sheet may be used to summarize the factors determining the various grades:

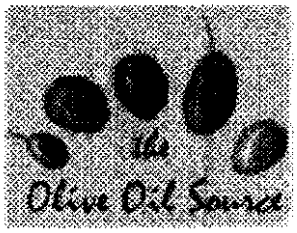
Size and kind of container . . . . . .
Container code or markings . . . . . .
Label . . . . . .
Contents (liquid measure) . . . . . .

Factors	Score Points		
Free fatty acid content	30	"A"	27-30
		"B"	24-26 1/
		"C"	21-23 1/
		"D"	0-20 1/
Absence of defects	30	"A"	27-30
		"B"	24-26 1/
		"C"	21-23 1/
		"D"	0-20 1/
Odor	20	"A"	18-20
		"B"	16-17
		"C"	14-15 1/
		"D"	0-13 1/
Flavor	20	"A"	18-20
		"B"	16-17
		"C"	14-15 1/
		"D"	0-13 1/
Total Score	100		

U.S. Grade . . . . . .

1/ Indicates limiting rule

Recodified in the Federal Register of December 9, 1953 (18 F.R. 8014)
Section 52.1540 amended May 22, 1957 (22 F.R. 3535)



U.S. Olive Growers
International Growers
Newsletter & Events
Olive Oil Sales

- Olive Oil by the case
- Bulk Oil by the gallon
- Olive Oil for Soap Making
- Private Labeling

Books
Health & Recipes

- Ask the Dr.
- Recipes
  - Curing olives
  - Flavored Oils
  - Tuscan Dishes
  - Baking with Olive Oil
  - Recipe Links
- Tours & Map
- Cosmetics
- Tasting
- Making Olive Oil
- Buying Fresh Olives

Equipment & Containers

- Mills and Presses
  - Mills and presses home
  - Enoroasi
  - Centrisys
  - Alfa Laval
  - Pieralisi
  - First Press - home olive oil press
- Complete catalog
- Glass Bottles
- Bottle Spouts
- Plastic Containers
- Stainless Tanks Big
- Stainless Fustis
- Pneumatic Pickers
- Shakers & Harvesters
- Pneumatic Saws & Pruners
- Filters & Bottlers

Industry Resources

- Growing Olives - all topics
  - Olive Varietals and botany
  - Setting up an orchard
  - Propagating olive trees
  - Plant - spacing
  - Irrigating
- Making Olive Oil
  - Olive Mills and Presses explained
  - Tips to making perfect olive oil
  - Regulations
  - Mills & Presses for sale
  - Olive Waste Disposal
- Suppliers to the Industry
  - Olive Tree Sources
  - Farming consultants
  - Olive Oil Mills
  - Fresh Olives
  - Bulk / Private label
  - Bottlers/Bottles
  - Box/Pallet
  - Label Design
  - Advertising / Web Design
  - Farming Supplies
  - Fresh olives
  - Testing Laboratories
- Links & Associations
  - California and U.S.Associations
  - International Associations
- Want Ads
- Olive People
- Industry Regulations
- Today's Food Industry News

Olive Facts

Olive Oil Labeling

Links

- United States Food and Drug Administration
- Olive oil is the edible oil expressed from the sound, mature fruit of the olive tree - FDA
- The Food Label: U. S. Food and Drug Administration FDA Backgrounder May 1999

Brief Labeling Guide for Olive Oil

From the North American Olive Oil Association
5 Ravine Drive P.O. Box 545 Matawan, NJ 07747 Tel: 734.583.8188 F

Almost all food labeling regulations are enforced by the US Food and Drug Administration (FD Requirement that is enforced by the US Customs Service, but even that regulation can be enforc the United States today is on nutrition. Olive oil companies must comply with regulations on:

- Nutritional labeling
- Net weight declarations
- Metric declarations
- Country of origin marking requirements
- Labeling in two languages
- Product descriptions

Nutritional Labeling Regulations

Nutritional Labeling Regulations became effective August 6, 1994.

Nutrient Declarations

21 CFR Section 101.9 sets out and explains all the nutrients that need to be declared on the foo are a few exemptions for products that have no significant food value, i.e. less than 5 calories pe For the latter, there must be a telephone number or an address on the label that the consumer c

FDA has specified that the following nutrients must be declared on a label: calories. Total fat, sa

Sodium, total carbohydrate, dietary fiber, sugars, protein, vitamin A, vitamin C, calcium and Iron amounts* of seven or more of these nutrients, such as olive oil, only the following nutrients mu – total fat – saturated fat – total carbohydrate – protein – sodium, and nutrients present in more

> *An "insignificant amount" is defined as an amount that allows a declaration o that for total carbohydrates, dietary fiber, and protein, it shall be an amount th one gram."

Additionally, the serving size and the number of servings per container must be declared. The s measure and in grams or milliliters (if product is fluid). For olive oil the serving size would be 1 a declaration to the simplified format, such as "monounsaturated fat", then the statement "Not on the next page.

Calories must be expressed to the nearest 5calorie increment up to and including 50 calories. an Amounts less than 5 calories may be expressed as zero (0). In determining Caloric Content, one factors (i.e., the Atwater method) given in Table 13 "Energy Value of Foods-Basis and Derivati Handbook No. 74. This book is available from: Division of Nutrition, (Center for Food Safety SW, Washington, DC 20204.

Calories from Fat must be expressed to the nearest 5caloric increment up to and including 50 c calories. Amounts less than 5 calories may be expressed as zero.

Calories from saturated fat are not required. This declaration is voluntary Total Fat (total lipid declared in grams to the nearest ½ gram increments below 5 grams and to the nearest gram incr expressed as zero (0)

Saturated fat (sum of all fatty acids containing no double bonds) may be declared as 0 if there i

272

History of the Olive
Olive Varietals
Olive Recipes
Olive Tasting
Olive Production Statistics
How is olive oil made?
Definitions
Questions
Olive Chemistry
Olive Waste Disposal

Olive Art
Olive Fly
About Us
Archives - Search
Site Map
email

Polyunsaturated fat is a voluntary declaration unless there is a declaration for monounsaturate cholesterol on the label.

Monounsaturated fat is also a voluntary declaration, unless there is a declaration of polyunsaturat cholesterol on the label.

Cholesterol need not be declared if there is less than 2 milligrams per serving. If it is not declar source of cholesterol". For 2-5 milligrams, the declaration must be: "less than 5 milligrams." Ov 5-milligram increment. Alternatively, if the cholesterol is less than 2 milligrams per serving, it can

Sodium must be declared. Under 5 milligrams per serving, the declaration is zero. Between 5 an increment. Over 140 milligrams the declaration is the nearest 10 gram increment.

Carbohydrate or Total Carbohydrate must be declared to the nearest gram. Under 1/2 gram, t

Protein must be declared to the nearest gram. Under 1/2 gram, the declaration is zero. Under I Footnotes: Most olive oil companies are expected to include information on their labels on poly cholesterol. Therefore, they will have to include the following footnotes on their label: "Not a si vitamin C, calcium, and iron." "Percent Daily Values are based on a 2,000 calorie diet."

Format for Nutritional Label

There are two illustrations below indicating the format for the nutritional label for olive oil In th based on the actual amount of each nutrient before rounding. The percentage of daily value for t actual fat in a tablespoon, namely, 13.6 grams. In the second illustration the percentage of daily v amount declared on the label, i.e. 14 grams. FDA states that "The percent shall be calculated by each nutrient or the actual amount of each nutrient (i.e., before rounding) by the Daily Recomm Recommended Value for total fat is 65 grams, and for saturated fat, it is 20 grams. %When one When one divides 14 grams by 65 grams, the result is 22%. Likewise for the saturated fat, when grams, by 20 grams the result is 9%. When one divides the declared saturated fat of 2 grams by 2 exceed the fat content declared by 20% because of normal product variation. A 9% declaration 2.15 grams. A 10% declaration would cover all oils with a saturated fat content up to 2.4 grams. variation to expect in the product by conducting laboratory tests. While the rule clearly states tha on the basis of actual amounts of the nutrients or on the rounded declarations, the preamble to greatest consistency in their label declarations, e.g. between grams declared and percentage of da of total fat (65 grams) and saturated fat (20 grams) are not declared on the Olive Oil Labels belo relevant. Serving Size Declaration/Servings Per Container

Illustration 1.

Nutrition Facts	
Serving Size 1 Tbsp (15mL) Servings Per Container	
Amount Per serving	
Calories 120	Fat Cal. 120
	% Daily Value *
Total Fat 14g	21 %
Saturated Fat 2g	9%
Polyunsaturated Fat 1.5g	
Monounsaturated Fat 10g	
Cholesterol 0mg	0%
Sodium 0mg	0%
Total Carbohydrate 0g	0%
Protein 0g	
Not a significant source of dietary fiber, sugar, vitamin A, Vitamin C, calcium and iron.	
Percent Daily Values are based on a 2,000 calorie diet	

Illustration 2.

Nutrition Facts Serving Size 1 Tbsp (15mL) Servings Per Container	
Amount Per Serving	
Calories 120	Fat Cal. 120
	% Daily Value *
Total Fat 14g	22%
Saturated Fat 2g	10%
Polyunsaturated Fat 1.5g	
Monounsaturated Fat 10g	
Cholesterol 0mg	0%
Sodium 0mg	0%
Total Carbohydrate 0g	0%
Protein 0g	
Not a significant source of dietary fiber, sugars, vitamin A, vitamin C, calcium and iron.	
*Percent Daily values are based on a 2,000 calorie diet.	

Serving size is 1 tablespoon(15ml). One tablespoon is actually 14.786 ml. Either 15 ml or 14.786 in a container, and the number of servings are rounded to the nearest whole number. In using t number of servings per container will be as follows: 1 liter -68, 500 ml 34, 250 ml - 17, in three li calculates serving sizes on the basis of 15 ml per tablespoon, there will be one or more fewer ser

Nutrient Content Claims

21 CFR, Section 101.54 sets limitations on the use of terms that describe the nutrient value of a source of', etc. If there is no Recommended Daily Intake (RDI) or Daily Recommended Value (descriptive term like "high in" to describe the nutrient on the label. For example, there is no RD describe olive oil as being "high in", or a "good source of" monounsaturated fat.

Light or Lite

21 CFR, Section 101.56 deals with the nutrient content claims for "light" or "lite". "Light" or "lit describes some physical or organoleptic attribute of the food such as texture or color and it is st texture") that it clearly conveys the nature of the product; and the attribute (e.g. color or texture) type size as the word "light" and in immediate proximity thereto. If a manufacturer can demonst through common use, with a particular food to reflect a physical or organoleptic attribute (e.g. li to the point where it has become part of the statement of identify, such use of the term "light" s subject to the requirements for such claims." It does not appear that any company could substa through common use, with olive oil to reflect a physical or organoleptic attribute in the manner

Sodium Content Claims

Olive Oil can make a "Sodium Free" claim. "Sodium Free" means that the food contains less tha statement must accompany the claim: "See nutrition information for fat content." If the food is "A sodium free food."

Salt Free, No Salt Added

Olive Oil can make a "Salt Free" claim. Salt is not sodium. It is sodium chloride. A "salt free" cla free food.

Nutrient Content Claims for Fat, Fatty Acid, and

Content of Foods

21 CFR, Section 101.62 sets out the regulations on nutrient content claims for fat, fatty acid and make any claims relative to fat content. It cannot claim to be "low in saturated fat" because the r must have less than 1 gram of saturated fat per serving and the saturated fat only accounts for 1 cannot claim to be "high in monounsaturated fat", because nutrient content claims can only be monounsaturated fat. FDA seems to limit nutrient content claims on fatty acids to such terms a similar expressions. Although it permits truthful and non-misleading graphic devices to amplify supplementary information must be consistent with the requirements for nutrient content claim

274

Cholesterol Content Claim

Olive oil can make "Cholesterol Free" and "No Cholesterol" claims provided that the food cont discloses the level of total fat in a serving in immediate proximity to the claim in type size no less disclosure statement is not necessary if the cholesterol free claim is only made on the informatio of a cholesterol free claim on the principal display panel would be

A Cholesterol Free Food
Contains 14 g of fat per serving
See nutrition information for fat content.

Implied Nutrient Content Claims

21 CFR, Section 101.65 differentiates between implied nutrient claims and claims that do not im rules applies the same guidelines for implied nutrient claims as pertain to transparent nutrient co nutrient content claims" being made by any olive oil company.

Appropriate Claims

For non-nutrient content claims, FDA only requires that they be accurate. "100% Pure" and "All they are accurate they are permitted. The North American Olive Oil Association agreed not to u name of the product. FDA has decided to maintain its current policy not to restrict the use of th substances, and flavors as provided in 21 CFR § 10 1.22. Additionally, the agency will maintain it that nothing artificial or synthetic (including all color additives regardless of source) has been inc not normally be expected to be in the food. Thus, olive oil companies in the US may use the ter olive oil is cold pressed or naturally cold pressed is appropriate on the label of Extra Virgin Oliv states that the term "cold pressed" is to be restricted to "virgin olive oil", and the members of th subject to the terms of the Agreement.

Health Claims

FDA will promulgate regulations authorizing a health claim only when it determines that there is qualified by scientific training and experience to evaluate such claims, and that the claim is suppo evidence. Health claims are not allowed on any product that contains more than 13 grams of fat olive oil product.

Net Weight Declaration

The net weight or net contents declaration must be in the bottom 30% of the principal display p information appearing on the principal display panel must be clear and conspicuous. In no case of an inch. Exemptions to this type size requirement are not relevant to olive oil containers or la use the words "net" or "net contents" before the weight declaration or they may simply use the for sizes under one pint, but are optional for sizes one pint and over. The largest unit of measur Either can be first. For example, one can state: Net I pt 0.9 fl oz (500 mL), or it can be reversed following declarations would be acceptable: Net 1 gallon (3.785 L) or 3.785 L (1 gallon) Net 3 qt (I L) or I L (1 qt 1.8 fl oz) Net 1 pt 0.9 fl oz (500 mL) or 500 mL (1 pt 0.9 fl oz) Note that the w

Country of Origin Marking Requirement

Country of Origin Marking Requirements are set out in 19 CFR 134.11. Unless excepted by law, U.S.C. 1304), requires that every article of foreign origin (or its container) imported into the Unit legibly, indelibly, and permanently as the nature of the article (or container) will permit, with the 134.35 exempts from the country of origin marking requirements those products that are substa If they are substantially changed in another country then that country is the country of origin. 19 representation appears on a label that would mislead the ultimate purchaser as to the actual coun proximity, the actual country(s) of origin preceded by "Made in", "Product of", or other words o imported from one country contains extra virgin oils from several countries, then each of those origin declaration. It is also permissible to list the country from which the product was imported where the extra virgin olive oils were produced. A country of origin declaration could appear as f Contains extra virgin olive oils from _____, _____ and _____

Product of _____, _____, _____

Words of similar meaning can also be used to declare the country of origin but it is not sufficien

"Imported from _____ or "Packed in _____

The origins of extra virgin olive oils that are blended in the United States to produce a new extra The importer of bulk extra virgin olive oil must certify to US Customs that if it packs the oil, it sells the oil to a packer, it will inform the packer of its obligation to put the country of origin on extra virgin olive oil to produce 'olive oil' appears to be a substantial change. The country where 'Olive oil' is not refined olive oil, nor is it extra virgin olive oil. Refined olive oil is not a consume colorless, and odorless. Extra virgin olive oil is a premium product that is immediately consuma contain any refined olive oil. It has distinctive compositional, nutritional and sensory characterist declarations may change as a result of the international harmonization project being carried out United States is participating in this project. It is unlikely that the project will be completed befor origin do not control advertising messages. Advertising is subject to the jurisdiction of the Feder restricting references to foreign countries in advertising. Statements, however, must be truthful.

Labeling in Two Languages

When labels contain words or statements in a language other than English, FDA regulations spe be in both languages. 21 CFR § 101.15 (c) (2) reads in part: "If the label contains any representat other information required by or under authority of the act [FD&C Act] to appear on the label s Nutritional Label both languages can be used in the one "Nutritional Facts" box. It is a little cro

Lot Codes

All olive oil products should be marked with a lot code. If the government determines that there to be withdrawn from the marketplace, the only way to limit the extent of the recall is by isolatin codes, there is no way to limit the recall The "Agreement to Monitor Olive Oils and Olive Pom member companies of the Signatory Associations, that is, essentially all packers of olive oil, to us pomace oil.

Product Descriptions

Product descriptions are regulated by Section 403 (a) (1) of the FDA&C Act which states that: " labeling is false or misleading in any particular .." Not only the product description must be acc the label must be accurate and consistent with the product description. Contradictory informati display panel describes a product as Pure Olive Oil, and the information panel describes it as "N contradictory and misleading. Use of the product descriptions "Extra Fine Olive Oil" and "Extr conceivable that these terms could be used on a label as advertising superlatives as long as they a product descriptions they do not conform with the international standards for olive oil. Use of t ing Extra Virgin Olive Oil" is confusing and misleading. It conveys the false notion that the con Pomace Oil". By definition, Olive Pomace Oil contains virgin olive oil, be it extra virgin or not, a virgin olive oil is misleading. Use of the product description "Pure Olive Oil with Extra Virgin O stating that the "Pure Olive Oil" is enriched with "Extra Virgin Olive Oil", as opposed to simply be considered the characterizing ingredient. In that case, the percentage of extra virgin olive oil definition, is a blend of refined olive oil and virgin olive oil. It cannot contain rectified oil, that is, the principal display panel that its product is Pure Olive Oil, and then on the information panel product is misbranded. All the words in the product description "Olive Pomace Oil" should be smaller or less conspicuous. Designating Extra Virgin Olive Oil as: "Highest Grade"; Virgin Oli "Lowest Grade, chemically washed, Second Press" is confusing and misleading to the consumer classifications. It is also inaccurate to described Pure Olive Oil as "chemically washed" because it product. To describe Pure Olive Oil as Second Press is inaccurate as well. A claim that olive oil and dangerous. It subjects the packer to greater liability for law suits stemming from fire damage consumer overheating olive oil.

Name and Place of Business

The name and place of business of the manufacturer, packer, or distributor of olive oil must be on the principal display panel or on the information panel. Street address, city, state and zip cod omitted if it is shown in a current city directory or telephone directory. The address can be in the address abroad.

Ingredient Statement

No ingredient statement is required on an olive oil because the product has no ingredient other t

276

Any Brand

Olive Oil

A Cholesterol and Sodium Free Food

Contains 14g of fat per serving
See nutritional information
for fat content

1 QT. 1.8 FL OZ. (1 LITER)

Principal

Bold Type - size of the type to be reasonably
the label. It shall be parallel to the base of th

If this claim is made on the label, the disclos

Country of Origin may appear on the princip

Net Contents must be easily readable in bold
to the background. It must appear in the bot
the label.

Type size requirements differ depending on t
or less the type is to be at least one sixteenth
inches -not less than one-eight inch type; ov
three-eighth inch type; over 100 square inche
quarter inch type

A Cholesterol and Sodium Free Food

Nutrition Facts
Serving Size 1 Tbsp (15mL)
Servings Per Container
Amount Per Serving

Calories 120	**Fat Cal. 120**
	% Daily Value
Total Fat	**21%**
Saturated Fat 2g	**9%**
Polyunsaturated Fat 1.5g	
Monounsaturated Fat 10g	
Cholesterol 0mg	**0%**
Sodium 0mg	**0%**
Total Carbohydrate 0g	**0%**

Not a significant source of dietary fiber, sugars, vitamin A, vitamin C, calcium and iron

Product of Italy

Any Company

Anywhere, 00000

[BARCODE]

Info

Claim can be made at top without any expl

Required Nutritional Statement is inset

Country of Origin must appear close to the
readable and in type size at least as big as t

Name of U.S. distributor or packer abroad

Bar Code is not required by government. It
before all government required information

Copyright ©January 08, 2001 [The Olive Oil Source]. All rights reserved.
voice - 415-461-6267 Fax 415-461-1015 390 Vista Grande, Greenbrae, Ca 94904
www.oliveoilsource.com

CODEX STANDARD FOR OLIVE OIL, VIRGIN AND REFINED, AND FOR REFINED OLIVE-POMACE OIL

CODEX STAN 33-1981 (Rev. 1-1989) [1]

1. SCOPE

This Standard applies to virgin olive oil, refined olive oil, refined olive-pomace oil, blends of refined olive oil and virgin olive oil and blends of refined olive-pomace oil and virgin olive oil.

2. DESCRIPTION

2.1 ***Olive oil*** is the oil obtained from the fruit of the olive tree (*Olea europaea sativa* Hoffm. et Link) without having been subjected to manipulation or any treatment not authorized by sub-sections 2.2 and 2.3 of this Standard.

2.2 ***Virgin olive oil*** is the oil obtained from the fruit of the olive tree by mechanical or other physical means under conditions, particularly thermal, which do not lead to alteration of the oil. Virgin olive oil is an oil which is suitable for consumption in the natural state.

2.3 ***Refined olive oil*** is the oil obtained from virgin olive oil, the acid content and/or organoleptic characteristics of which render it unsuitable for consumption in the natural state, by means of refining methods which do not lead to alterations in the initial glyceridic structure.

2.4 ***Refined olive-pomace oil*** is the oil obtained from "olive pomace" by extraction by means of solvents and made edible by means of refining methods which do not lead to alteration in the initial glyceridic structure.

3. ESSENTIAL COMPOSITION AND QUALITY FACTORS [2]

3.1 Identity characteristics (under normal ecological conditions) [3]

3.1.1 GLC ranges of fatty acid composition (% m/m of methyl esters)

Lauric acid	(C 12:0)	Not present in discernible amounts

[1] Formerly CAC/RS 33-1970.

[2] The limits of essential composition and quality factors of virgin olive oils show very widely spaced minimum and maximum values, since they take account of the oil characteristics of all producing countries. Characteristics and limits of physical and chemical indices and values, and of fatty acid composition for the various grades of virgin olive oils produced in each olive-growing area, determined at the outset and close of the olive oil production year, are published yearly in each producing country's "National Olive Oil Index File".

[3] Samples falling outside the GLC fatty acid ranges are not in compliance with the Standard. Supplementary non-mandatory criteria may be employed if it is considered necessary to confirm that a sample is in compliance with the standard.

PREVIOUS PAGE BLANK

Myristic acid	(C 14:0)	< 0.1
Palmitic acid	(C 16:0)	7.5 - 20.0
Palmitoleic acid	(C 16:1)	0.3 - 3.5
Heptadecanoic acid	(C 17:0)	< 0.5
Heptadecenoic acid	(C 17:1)	< 0.6
Stearic acid	(C 18:0)	0.5 - 5.0
Oleic acid	(C 18:1)	55.0 - 83.0
Linoleic acid	(C 18:2)	3.5 - 21.0
Linolenic acid	(C 18:3)	< 1.5
Arachidic acid	(C 20:0)	< 0.8
Behenic acid	(C 22:0)	< 0.3
Erucic acid	(C 22:1)	Not present in discernible amounts
Lignoceric acid	(C 24:0)	< 1.0

3.1.2 Physical and chemical indices

3.1.2.1 Relative density (20°C/water at 20°C)

Virgin olive oil	)	
Refined olive oil	)	0.910 - 0.916
Refined olive-pomace oil	)	

3.1.2.2 Refractive index (n_D^{20})

Virgin olive oil	)	1.4677 - 1.4705
Refined olive oil	)	
Refined olive-pomace oil		1.4680 - 1.4707

3.1.2.3 Saponification value (mg KOH/g oil)

Virgin olive oil	)	184 - 196
Refined olive oil	)	
Refined olive-pomace oil		182 - 193

3.1.2.4 Iodine value (Wijs)

Virgin olive oil	)	75 - 94
Refined olive oil	)	
Refined olive-pomace oil		75 - 92

3.1.2.5 Unsaponifiable matter (using light petroleum)

Virgin olive oil	)	not more than 15 g/kg [4]
Refined olive oil	)	
Refined olive-pomace oil		not more than 30 g/kg [5]

3.1.2.6 Bellier index

Virgin olive oil	not more than 17 [6]
Refined olive oil	
Refined olive-pomace oil	not applicable

3.1.2.7 Semi-siccative oil test

Virgin olive oil	)	
Refined olive oil	)	negative
Refined olive-pomace oil	)	

3.1.2.8 Olive-pomace oil test

Virgin olive oil	)	negative
Refined olive oil	)	
Refined olive-pomace oil		not relevant

3.1.2.9 Cottonseed oil test

Virgin olive oil	)	
Refined olive oil	)	negative
Refined olive-pomace oil	)	

3.1.2.10 Teaseed oil test

Virgin olive oil	)

[4] A characteristic feature of the unsaponifiable matter in olive oil is its content of squalene, which is higher than that of the other vegetable oils. Another distinctive feature is that its sterols are composed of practically pure beta-sitosterol.

[5] The unsaponifiable matter of olive-pomace oil contains more alcoholic compounds than that of virgin or refined olive oils, and its iodine value is therefore lower than that normally noted in virgin or refined olive oils, and its melting point is higher.

[6] Should this index be higher than 17, the content of arachidic, behenic and lignoceric acid shall be given.

Refined olive oil	)	negative
Refined olive-pomace oil	)	

3.1.2.11 Sesameseed oil tests

Virgin olive oil	)	
Refined olive oil	)	negative
Refined olive-pomace oil	)	

3.1.2.12 Sterol content (% of the sum of beta-sitosterol, campesterol and stigmasterol) [7]

		Beta-sitosterol	Campesterol	Cholesterol
Virgin olive oil	)			
Refined olive oil	)	= 93	= 4.0	= 0.5
Refined olive-pomace oil	)			

3.1.2.13 Saturated fatty acids at position 2

	Maximum level
Virgin olive oil	1.5% m/m
Refined olive oil	1.8% m/m
Blends of refined olive oil and virgin olive oil	1.8% m/m
Refined olive-pomace oil	2.2% m/m
Blends of refined olive-pomace oil and virgin olive oil	2.0% m/m

The saturated fatty acids at position 2 means the sum of the palmitic (16:0) and stearic (18:0) acids expressed as a percentage (m/m) of the total fatty acids at position 2.

3.2 Quality characteristics

3.2.1 Colour, odour and taste

Virgin olive oil: Clear oil, of a yellow to green colour, with specific odour and taste, free from odours or tastes indicating alteration or pollution of oil.

Refined olive oil: Clear oil, limpid without sediment, of clear yellow colour without specific odour or taste and free from odours or tastes indicating alteration or pollution of the oil.

Refined olive-pomace oil: Clear oil, limpid, without sediment, of a yellow to yellow-brown colour, without specific odour or taste and free from odours or tastes indicating alteration or pollution of the oil.

[7] Beta-sitosterol as determined by the method of analysis specified by the Standard includes Δ-5 avenasterol since this is not separated from beta-sitosterol by the column packing material SE30.

Blends: The colour, odour and taste shall be intermediate between those of two types blended.

3.2.2 Free acidity

	Acidity maximum % m/m expressed as oleic acid	Acid value maximum mg KOH/g oil
Virgin olive oil	3.3	6.6
Refined olive oil	0.3	0.6
Refined olive-pomace oil	0.3	0.6
Blends	1.5	3.0

3.2.3 Peroxide value (in milliequivalents peroxide oxygen/kg oil)

Virgin olive oil	= 20
Refined olive oil	= 10
Refined olive-pomace oil	= 10
Blends	= 20

3.2.4 Specific extinction in ultra-violet ($E^{1\%}_{1cm}$)

	$E^{1\%}_{1cm}$ maximum at 232 nm	$E^{1\%}_{1cm}$ maximum at 270 nm	$\Delta E^{1\%}_{1cm}$ maximum variation at near 270 nm
Virgin olive oil	3.50	0.30	[8]
Refined olive oil	-	1.10	0.16
Refined olive-pomace oil	6.00	2.00	0.20
Blends of refined olive oil and virgin olive oil	-	0.90	0.15
Blends of refined olive- pomace oil and virgin olive oil	5.50	1.70	0.18

4. FOOD ADDITIVES

			Maximum level
4.1	Virgin olive oil	Nnone permitted	
4.2	Refined olive oil) Refined olive-pomace oil)	Alpha-tocopherol for the purpose of restoring natural tocopheral lost in processing	200 mg/kg total alpha-tocopherol in the final product

[8] Oils having a specific extinction at 270 nm exceeding 0.30 may still be regarded as virgin oils if, after passage of the sample through activated alumina, their specific extinction at 270 nm is less than 0.11.

Blends)

5. CONTAMINANTS

5.1 Matter volatile at 105°C

Virgin olive oil	≤ 0.2% m/m
Refined olive oil	≤ 0.1% m/m
Refined olive-pomace oil	≤ 0.1% m/m
Blends	≤ 0.1% m/m

5.2 Insoluble impurities

Virgin olive oil	≤ 0.1% m/m
Refined olive oil	≤ 0.05% m/m
Refined olive-pomace oil	≤ 0.05% m/m
Blends	≤ 0.05% m/m

5.3 Soap Test

Refined olive oil	)	
Refined olive-pomace oil	)	negative
Virgin olive oil	)	
Virgin olive oil	)	not applicable
Blends	)	

6. HYGIENE

It is recommended that the product covered by the provisions of this Standard be prepared in accordance with the appropriate Sections of the General Principle of Food Hygiene recommended by the Codex Alimentarius Commission (Ref. No. CAC/RCP 1-1969, Rev. 2-1985).

7. LABELLING

The provisions of the General Standard for the Labelling of Prepackaged Foods (CODEX STAN 1-1985 (Rev. 1-1991)) shall apply.

7.1 The name of the food

7.1.1 All products designated as "olive oil" shall conform to the provisions of this Standard for virgin olive or refined olive oil and shall be either virgin olive oil, refined olive oil or a blend of refined olive oil and virgin olive oil.

7.1.2 All products designated as "virgin olive oil" shall conform to the provisions for virgin olive oil.

7.1.3 All products designated as "refined olive oil" shall conform to the provisions for refined olive oil.

7.1.4 All products designated as "refined olive-pomace oil" shall conform to the provisions for refined-pomace oil.

7.1.5 Refined olive-pomace oil shall in no case be described as "olive oil" but shall always be designated as "refined olive-pomace oil".

7.1.6 Blends of refined olive-pomace oil and virgin olive oil shall be described as "olive-pomace oil".

7.2 Labelling of non-retail containers [9]

The labelling of non-retail containers shall comply with the section 5.3 of the Guidelines on Labelling Provisions in Codex Standards.

8. METHODS OF ANALYSIS AND SAMPLING

See Codex Alimentarius Volume 13.

[9] Applicable only to outer containers for a number of prepackaged foods only.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS METHODS

(AOAC)

41.1.19

AOAC Official Surplus Method 920.161★
Soluble Acids in Oils and Fats

Final Action
Surplus 1965

26.030

Place flask used in **920.160(B)** and its contents on H_2O bath and evap. the alcohol. Add quantity of 0.5*N* HCl equiv. to quantity of KOH used for saponification of sample in **920.160(B)** and 1 mL more (quantity of 0.5*N* HCl to be added = titrn for blank – titrn for sample + 1 mL), and place flask on steam bath until sepd fatty acids form clear layer on upper surface of liquid. Fill flask to neck with hot H_2O and cool contents in ice-H_2O until cake of fatty acids hardens thoroly. Pour liquid contents of flask thru filter into 1 L flask, refill flask with hot H_2O, and set on steam bath until fatty acids collect at surface. Cool by immersing in ice-H_2O and again filter liquid into the 1 L flask. Repeat this treatment with hot H_2O 3 times, cooling and collecting washings in the 1 l flask after each treatment. Titr. combined washings with 0.1*N* NaOH, **936.16(C)** and **(D)**, using phthln. Subtract 5 (corresponding to excess of 1 mL 0.5*N* acid) from mL 0.1*N* NaOH used and multiply by 0.0088 to obtain wt sol. acids as butyric acid. Calc. % sol. acids.

© AOAC INTERNATIONAL

41.1.14

AOAC Official Method 920.159
Iodine Absorption Number of Oils and Fats
Wijs Method
Final Action

A. Reagents

(*Caution: See Appendix B*, safety notes on pipets, acetic acid, and chlorine.)

Wijs iodine solution.—(*1*) Dissolve 13 g resublimed I_2 in 1 L CH_3COOH, and pass in dried (through H_2SO_4) Cl_2 until original $Na_2S_2O_3$ titration of solution is not quite doubled. (Characteristic color change at end point indicates proper amount of Cl_2. Convenient method is to reserve some of original I_2 solution, add slight excess of Cl_2 to bulk of solution, and bring to desired titer by readditions of reserved portion.) Or: (*2*) Dissolve 16.5 g ICl in 1 L CH_3COOH.

Store in amber bottle sealed with paraffin until ready for use. Wijs solutions are sensitive to temperature, moisture, and light. Store in dark at <30°. Determine I/Cl ratio as follows:

Iodine content.—Pipet 5 mL Wijs solution into 500 mL Erlenmeyer containing 150 mL saturated Cl_2-H_2O and some glass beads. Shake, heat to bp, and boil briskly 10 min. Cool, add 30 mL H_2SO_4 (1 + 49) and 15 mL 15% KI solution, and titrate immediately with 0.1*N* $Na_2S_2O_3$.

Total halogen content.—Pipet 20 mL Wijs solution into 500 mL Erlenmeyer containing 150 mL recently boiled and cooled H_2O and 15 mL 15% KI solution. Titrate immediately with 0.1*N* $Na_2S_2O_3$.

$$I/Cl = 2X/(3B - 2X)$$

where X = mL 0.1*N* $Na_2S_2O_3$ required for I_2 content and B = mL required for total halogen content. I/Cl ratio must be 1.10 ± 0.1.

B. Determination

Use sample weight calculated as 26/expected Iodine value, or from Table **920.159**.

Weigh melted and filtered sample into clean, dry, 500 mL glass-stoppered flask containing 20 mL CCl_4. With pipet, add 25 mL I_2 solution, draining pipet definite time. Excess of I_2 should be 50–60% of amount added, that is, 100–150% of amount absorbed. Swirl, and let bottle stand in dark 30 min at 25 ± 5°. Let samples with I_2 values >150 (linseed and perilla) stand 1 h.

Add 20 mL 15% KI solution and 100 mL recently boiled and cooled H_2O. Titrate the I_2 with 0.1*N* $Na_2S_2O_3$, **942.27** (*see* A.1.13), added gradually, shaking constantly until yellow solution turns almost colorless. (Vigorous magnetic stirring is convenient.) Add few drops starch indicator, (mix ca 1 g soluble starch with enough cold H_2O to make thin paste, add 100 mL boiling H_2O, and boil ca 1 min while stirring) and continue titration until blue entirely disappears. Toward end of reaction, stopper bottle and shake violently so that any I_2 remaining in solution in CCl_4 may be taken up by KI solution.

Conduct 2 determinations on blanks in same manner as sample, but without fat. Slight variations in temperature appreciably affect titer of I_2 solution, as CH_3COOH has high coefficient of expansion. It is essential, therefore, that blanks and determinations on sample be made at same time. $Na_2S_2O_3$ equivalent of I_2 absorbed by sample taken = mL standard $Na_2S_2O_3$ solution required by blank (B) – mL used in determination (S). Calculate % by weight of I_2 absorbed as in **920.158B** (*see* 41.1.13) and report as I_2 number, Wijs method.

Table 920.159 Sample Weights

Iodine Value	g Sample[a]	Accuracy, mg
3	10.58–8.46	± 0.5
10	3.17–2.54	0.2
20	1.59–1.27	0.2
40	0.79–0.63	0.2
80	0.40–0.32	0.2
120	0.26–0.21	0.1
160	0.20–0.16	0.1
200	0.16–0.13	0.1

[a] For 100 and 150% excess, respectively.

Reference: JAOAC **48**, 127(1965).

Copyright 1998 AOAC INTERNATIONAL

AOAC Official Method 993.20
Iodine Value of Fats and Oils
Wijs (Cyclohexane–Acetic Acid Solvent) Method
First Action 1993
IUPAC–AOCS–AOAC Method

(Applicable to determination of iodine value for fats and oils which do not contain conjugated double bonds.)

(*Caution*: Wijs solution causes severe burns; vapors can cause lung and eye damage. Use of fume hood is recommended. *See* "Appendix: Laboratory Safety" for procedures on safe handling of acids and organic solvents [cyclohexane].)

Method Performance:

See Table **993.20A** for method performance data.

A. Principle

Fat or oil sample is mixed with iodine monochloride solution to halogenate double bonds in fat or oil. Excess iodine monochloride is reduced to free iodine in presence of potassium iodide, and free iodine is measured by titration with sodium thiosulfate using starch as indicator.

Iodine value (IV), calculated as cg iodine absorbed per g of sample (% iodine absorbed), is a measure of unsaturation of fats and oils.

B. Apparatus

(a) *Glass-stoppered iodine flasks.*—500 mL.

(b) *Glass-stoppered volumetric flasks.*—1000 mL, for preparing standard solutions.

(c) *Volumetric dispensers.*—*(1)* 25 mL, for Wijs and 15% potassium iodide (KI) solutions. *(2)* 2 mL, for starch solution. *(3)* 50 mL, for H_2O.

(d) *Repeater pipet.*—20 mL, with filling flask, for cyclohexane.

(e) *Analytical balance.*—Accurate to ±0.0001 g.

(f) *Filters.*—Ashless, coarse grade (Whatman No. 541 is suitable).

(g) *Hot air oven.*—Capable of maintaining 100° within ±1.5°.

C. Reagents

(a) *Potassium iodide (KI) solution.*—15%. Dissolve 15 g KI in 100 mL H_2O.

(b) *Wijs iodine solution.*—*(1)* Dissolve 13 g resublimed I in 1 L acetic acid, and pass in dried (through H_2SO_4) Cl until original $Na_2S_2O_3$ titration of solution is not quite doubled. (Characteristic color change at end point indicates proper amount of Cl. Convenient method is to reserve some of original I solution, add slight excess of Cl to bulk of solution, and bring to desired titer by readditions of reserved portion.) Or: *(2)* Dissolve 16.5 g ICl in 1 L acetic acid.

Determine I/Cl ratio as follows:

(1) Iodine content.—Pipet 5 mL Wijs solution into 500 mL Erlenmeyer flask containing 150 mL saturated Cl–H_2O and some glass beads. Shake, heat to boiling, and boil briskly 10 min. Cool, add 30 mL H_2SO_4 solution (1 + 49) and 15 mL 15% KI solution, and titrate immediately with 0.1N $Na_2S_2O_3$.

(2) Total halogen content.—Pipet 20 mL Wijs solution into 500 mL Erlenmeyer flask containing 150 mL recently boiled and cooled H_2O and 15 mL 15% KI solution. Titrate immediately with 0.1N $Na_2S_2O_3$.

$$I/Cl = 2X/(3B - 2X)$$

where X = mL 0.1N $Na_2S_2O_3$ required for I content and B = mL required for total halogen content. If I/Cl ratio is not 1.10 ± 0.1, add I or Cl to correct ratio.

Standardized Wijs solution may be obtained from commercial suppliers (specify without carbon tetrachloride).

Store in amber bottle sealed with paraffin until ready for use. Wijs solutions are sensitive to temperature, moisture, and light. Store in dark at <30°.

(c) *Soluble starch solution.*—Mix paste of 1 g starch with small amount cold H_2O. While stirring, add 200 mL boiling H_2O. Test for sensitivity: place 5 mL starch solution in 100 mL H_2O and add 0.05 mL 0.1N iodine solution; deep blue color produced must be discharged by 0.05 mL 0.1*N* sodium thiosulfate solution. (*Note*: 1% starch solution, commercially available, is suitable.)

(d) *Potassium dichromate ($K_2Cr_2O_7$).*—Finely grind and dry to constant weight (ca 110°) before using in **D**.

(e) *Sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$) solution.*—0.1*N*. Standardize as in **D**.

(f) *Acids.*—*(1) Hydrochloric acid (HCl).*—Concentrated, sp gr 1.19. *(2) Acetic acid ($C_2H_4O_2$).*—Glacial. *(3) Sulfuric acid (H_2SO_4).*—Concentrated.

Table 993.20A Method Performance for Determination of Iodine Value by Wijs Method using Carbon Tetrachloride Solvent or Cyclohexane-Acetic Acid (1 + 1) Solvent

	Mean Value		s_r		s_R		RSD_r, %		RSD_R, %	
Sample	CTC[a]	CHX[b]	CTC	CHX	CTC	CHX	CTC	CHX	CTC	CHX
Sunflower	133.6	132.9	1.4	1.7	3.4	2.3	1.1	1.3	2.6	1.7
Refined palm	53.1	53.0	0.1	0.2	0.3	0.4	0.2	0.3	0.5	0.7
Crude fish	109.1	108.5	0.7	0.5	1.7	1.1	0.6	0.5	1.6	1.0
Tung	164.5	163.1	2.0	1.4	3.1	2.5	1.2	0.9	1.9	1.5
Tallow (beef)	47.2	46.9	0.2	0.2	0.5	0.4	0.5	0.5	1.0	0.8
Crude palm	52.5	52.6	0.3	0.4	0.4	0.5	0.5	0.8	0.8	1.0
Used frying	37.7	37.7	0.1	0.2	0.2	0.3	0.4	0.5	0.4	0.9
Palm kernel	18.2	18.3	0.01	0.01	0.03	0.04	0.1	0.1	0.2	0.2
Olive	82.3	82.2	0.2	0.5	0.6	0.8	0.3	0.6	0.7	0.9
HSBO[c]-1	102.6	102.3	0.5	0.8	1.8	1.9	0.5	0.8	1.7	1.8
HSBO-2	74.7	74.8	0.5	0.4	1.0	0.6	0.6	0.5	1.3	0.8
HFO[d]	73.0	72.8	0.4	0.4	0.7	0.6	0.6	0.6	0.9	0.8

[a]Carbon tetrachloride.
[b]Cyclohexane–acetic acid (1 + 1).
[c]Hydrogenated soybean oil.
[d]Hydrogenated fish oil.

Copyright 1998 AOAC INTERNATIONAL

(g) *Cyclohexane.*—[*Note:* Erratic results may result if cyclohexane is old, i.e., contains oxidizable matter; see (h).]

(h) *Cyclohexane–acetic acid solvent.*—Mix cyclohexane, (g), and acetic acid, (f)(*2*), 1 + 1 (volume/volume). Verify absence of oxidizable matter in solvent by shaking 10 mL solvent with 1 mL saturated aqueous $K_2Cr_2O_7$ solution and 1 mL H_2SO_4, (f)(*3*). No green color should appear.

D. Standardization of Sodium Thiosulfate Solution

Accurately weigh 0.16–0.22 g dried, finely ground $K_2Cr_2O_7$, C(d), to nearest 0.0001 g into 500 mL flask, dissolve in 25 mL H_2O, add 5 mL HCl, C(f)(*1*), and 20 mL KI solution, C(b), and rotate to mix. Let stand 5 min. Add 100 mL H_2O. Titrate with sodium thiosulfate solution, C(e), shaking continuously until yellow color has *almost* disappeared. Add 1–2 mL starch indicator solution, C(c), and continue adding thiosulfate solution slowly until blue color just disappears.

$Na_2S_2O_3$ solution normality, N =
[20.394 × wt $K_2Cr_2O_7$]/mL sodium thiosulfate

E. Determination

Melt test sample, if not already liquid (do not exceed sample melting point by >10°). Pass test sample through double layer of filter paper to remove any solid impurities and traces of H_2O (filtration may be performed in air oven, ca 100°, but should be completed within 5 min ± 30 s). Sample must be absolutely dry. (*Note:* All glassware must be absolutely clean and completely dry.)

Let filtered test sample cool to 68–71°. Immediately weigh amount of test sample indicated in Table 993.20B into clean, dry 500 mL flask, B(a).

Prepare at least 2 blank determinations to run with each sample group.

Add 15 mL cyclohexane–acetic acid solvent, C(h), to each test sample and swirl to ensure that sample is completely dissolved.

Dispense 25 mL Wijs solution into flask containing test sample, stopper flask, and swirl to mix. Immediately set timer for 1.0 or 2.0 h, depending on iodine value of sample (IV <150, 1.0 h; IV ≥150, 2.0 h) and store flasks in dark at 25 ± 5° for duration of reaction.

Remove flasks from dark, add 20 mL KI solution, C(b), and mix. Add 150 mL H_2O and gradually titrate with 0.1*N* standard $Na_2S_2O_3$ solution, D, with constant and vigorous shaking or mechanical stirring. Continue titrating until yellow color has *almost* disappeared. Add 1–2 mL starch indicator solution to flasks and continue titrating until blue color has just disappeared. (*Note:* If reaction is not terminated by addition of KI and H_2O within 3 min past 1.0 or 2.0 h reaction time, sample must be discarded. The sample must be titrated within 30 min of reaction termination; if not, the analysis is invalid.)

Table 993.20B Sample Weights

I Value	Sample, g	Accuracy, mg
3	10.58–8.46	0.5
10	3.17–2.54	0.2
20	1.59–1.27	0.2
40	0.79–0.63	0.2
80	0.40–0.32	0.2
120	0.26–0.21	0.2
160	0.20–0.16	0.2
200	0.16–0.13	0.2

F. Calculation

$$\text{Iodine value (IV)} = [(B - S) \times N \times 12.69]/\text{wt of sample}$$

where B = titration of blank (mL); S = titration of sample (mL); N = normality of $Na_2S_2O_3$ solution.

References: Pure & Appl. Chem. 62, 2339(1990); J. AOAC Int. 77, 674(1994).

Copyright 1998 AOAC INTERNATIONAL

Determination of the Iodine Value of Oils and Fats: Summary of Collaborative Study

DAVID FIRESTONE
U.S. Food and Drug Administration, Division of Pesticide and Industrial Chemicals, 200 C St, SW, Washington, DC 20204

Two collaborative studies were conducted using the Wijs method for determining the iodine value in a wide range of vegetable and animal oils and fats. The results obtained when using carbon tetrachloride were compared to those obtained when using a substitute solvent mixture of cyclohexane and glacial acetic acid. The values reported for the iodine values indicate that the cyclohexane and acetic acid mixture can be used in place of carbon tetrachloride without loss of precision. The method has been adopted first action by AOAC INTERNATIONAL as an IUPAC/AOCS/AOAC method.

Eleven laboratories from 9 countries participated in a collaborative study conducted in 1989. The test samples represented a range of lipid materials, including 7 vegetable oils consisting of olive oil, refined palm kernel oil, crude and refined palm oil, tung oil, sunflower seed oil, and hydrogenated soybean oil; 3 animal fats consisting of crude and hydrogenated fish oil; and tallow. Each of the 11 materials was provided as blind duplicates. The participants were required to determine the iodine value once only using carbon tetrachloride, and once only using a mixture of cyclohexane and glacial acetic acid (1 + 1).

Eighteen laboratories from 11 countries participated in a second collaborative study conducted in 1990 (1). The test samples included 3 materials in blind duplicates (hydrogenated soybean oils at 2 levels of hydrogenation, and hydrogenated fish oil). Solvents used in the second study were the same as those used in the 1989 study.

Submitted for publication December 12, 1992.

The recommendation was approved by the Committee on Food Nutrition and was adopted by the Official Methods Board of the Association. See "Changes in Official Methods of Analysis" (1994) *J. AOAC Int.* 77, Jan/Feb issue, and "Official Methods Board Actions" (1993) *The Referee*, 17, July issue.

D. Firestone is the AOAC INTERNATIONAL General Referee on Fats and Oils.

993.20 Iodine Value of Fats and Oils—Wijs (Cyclohexane–Acetic Acid Solvent) Method

IUPAC/AOCS/AOAC Method

First Action 1993

(Applicable to determination of iodine value for fats and oils that do not contain conjugated double bonds.)

Method Performance:

See Table **993.20A** for method performance data.

[*Caution*: Wijs solution causes severe burns; vapors can cause lung and eye damage. Use of fume hood is recommended. *See Appendix: Laboratory Safety* for procedures on safe handling of acids and organic solvents (cyclohexane).]

A. Principle

Fat or oil sample is mixed with iodine monochloride solution to halogenate double bonds in fat or oil. Excess iodine monochloride is reduced to free iodine in presence of potassium iodide, and free iodine is measured by titration with sodium thiosulfate using starch as indicator.

Iodine value (IV), calculated as centigrams (cg) iodine absorbed per g sample (% iodine absorbed), is a measure of unsaturation of fats and oils.

B. Apparatus

(a) *Glass stoppered iodine flasks.*—500 mL.

(b) *Glass stoppered volumetric flasks.*—1000 mL, for preparing standard solutions.

(c) *Volumetric dispensers.*—*(1)* 25 mL, for Wijs and 15% potassium iodide (KI) solutions. *(2)* 2 mL, for starch solution. *(3)* 50 mL, for H_2O.

(d) *Repeater pipet.*—20 mL, with filling flask, for cyclohexane.

(e) *Analytical balance.*—Accurate to ±0.0001 g.

(f) *Filters.*—Ashless, coarse grade (Whatman No. 541 is suitable).

(g) *Hot air oven.*—Capable of maintaining 100° within ±1.5°.

C. Reagents

(a) *Potassium iodide (KI) solution.*—15%. Dissolve 15 g KI in 100 mL H_2O.

(b) *Wijs iodine solution.—(1)* Dissolve 13 g resublimed I in 1 L acetic acid, and pass in dried (through H_2SO_4) Cl until original $Na_2S_2O_3$ titration of solution is not quite doubled. (Characteristic color change at end point indicates proper amount of Cl. Convenient method is to reserve some of original I solution, add slight excess of Cl to bulk of solution, and bring to desired titer by readditions of reserved portion.) Or: (2) Dissolve 16.5 g ICl in 1 L acetic acid.

Determine I/Cl ratio as follows:

Iodine content.—Pipet 5 mL Wijs solution into 500 mL erlenmeyer flask containing 150 mL saturated Cl–H_2O and some glass beads. Shake, heat to boiling, and boil briskly 10 min. Cool, add 30 mL H_2SO_4 solution (1 + 49) and 15 mL 15% KI solution, and titrate immediately with 0.1N $Na_2S_2O_3$.

Total halogen content.—Pipet 20 mL Wijs solution into 500 mL erlenmeyer flask containing 150 mL recently boiled and cooled H_2O and 15 mL 15% KI solution. Titrate immediately with 0.1N $Na_2S_2O_3$.

$$\frac{I}{Cl} = \frac{2X}{3B - 2X}$$

where X = mL 0.1N $Na_2S_2O_3$ required for I content and B = mL required for total halogen content. If I/Cl ratio is not 1.10 ± 0.1, add I or Cl to correct ratio.

Standardized Wijs solution may be obtained from commercial suppliers (specify without carbon tetrachloride).

Store in amber bottle sealed with paraffin until ready for use. Wijs solutions are sensitive to temperature, moisture, and light. Store in dark at <30°.

(c) *Soluble starch solution.*—Mix paste of 1 g starch with small amount cold H_2O. While stirring, add 200 mL boiling H_2O. Test for sensitivity: place 5 mL starch solution in 100 mL H_2O and add 0.05 mL 0.1N iodine solution; deep blue color produced must be discharged by 0.05 mL 0.1N sodium thiosulfate solution. (*Note*: 1% starch solution, commercially available, is suitable.)

(d) *Potassium dichromate ($K_2Cr_2O_7$).*—Finely grind and dry to constant weight (ca 110°) before using in *D*.

(e) *Sodium thiosulfate ($Na_2S_2O_3 5H_2O$) solution.*—0.1N. Standardize as in *D*.

(f) *Acids.—(1) Hydrochloric acid (HCl).*—Concentrated, sp gr 1.19. *(2) Acetic acid ($C_2H_4O_2$).*—Glacial. *(3) Sulfuric acid (H_2SO_4).*—Concentrated.

(g) *Cyclohexane.*—(*Note*: Erratic results may result if cyclohexane is old, i.e., contains oxidizable matter; *see* (h).)

(h) *Cyclohexane–acetic acid solvent.*—Mix cyclohexane, (g), and acetic acid, (f)*(2)*, 1 + 1 (v/v). Verify absence of oxidizable matter in solvent by shaking 10 mL solvent with 1 mL saturated aqueous $K_2Cr_2O_7$ solution and 1 mL H_2SO_4, (f)*(3)*. No green color should appear.

D. Standardization of Sodium Thiosulfate Solution

Accurately weigh 0.16–0.22 g dried, finely ground $K_2Cr_2O_7$, *C*(d), to nearest 0.0001 g into 500 mL flask, dissolve in 25 mL H_2O, add 5 mL HCl, *C*(f)*(1)*, and 20 mL KI solution, *C*(b), and rotate to mix. Let stand 5 min.

Add 100 mL H_2O. Titrate with sodium thiosulfate solution, *C*(e), shaking continuously until yellow color has *almost* disappeared. Add 1–2 mL starch indicator solution, *C*(c), and continue adding thiosulfate solution slowly until blue color just disappears.

$$Na_2S_2O_3 \text{ solution normality, } N = \frac{20.394 \times \text{Wt } K_2Cr_2O_7}{\text{mL sodium thiosulfate}}$$

E. Determination

Melt test sample, if not already liquid (do not exceed sample melting point by >10°). Pass test sample through double layer of filter paper to remove any solid impurities and traces of H_2O (filtration may be performed in air oven, ca 100°, but should be completed within 5 ± 0.5 min). Sample must be absolutely dry. (*Note*: All glassware must be absolutely clean and completely dry.)

Let filtered test sample cool to 68–71°. Immediately weigh amount of test sample indicated in Table 993.20B into clean, dry 500 mL flask, *B*(a).

Prepare at least 2 blank determinations to run with each sample group.

Add 15 mL cyclohexane–acetic acid solvent, *C*(h), to each test sample and swirl to ensure that sample is completely dissolved.

Dispense 25 mL Wijs solution into flask containing test sample, stopper flask, and swirl to mix. Immediately set timer for 1.0 or 2.0 h, depending on iodine value of sample (IV <150, 1.0 h; IV ≥150, 2.0 h) and store flasks in dark at 25 ± 5° for duration of reaction.

Remove flasks from dark, add 20 mL KI solution, *C*(b), and mix. Add 150 mL H_2O and gradually titrate with 0.1N standard $Na_2S_2O_3$ solution, *D*, with constant and vigorous shaking or mechanical stirring. Continue titrating until yellow color has *almost* disappeared. Add 1–2 mL starch indicator solution to flasks and continue titrating until blue color has just disappeared. (*Note*: If reaction is not terminated by addition of KI and H_2O within 3 min past 1.0 or 2.0 h reaction time, sample must be discarded. The sample must be titrated within 30 min of reaction termination; if not, the analysis is invalid.)

F. Calculation

$$\text{Iodine value (IV)} = \frac{(B - S) \times N \times 12.69}{\text{Wt of sample}}$$

where B = titration of blank (mL); S = titration of sample (mL); N = normality of $Na_2S_2O_3$ solution.

Ref.: *Pure & Appl. Chem.* **62**, 2339(1990); JAOAC **77**, 674 (1994)

Reference

(1) Pocklington, W.D. (1990) *Pure Appl. Chem.* **62**, 2340–2343

Table 993.20A Method performance for determination of iodine value by wijs method using carbon tetrachloride solvent or cyclohexane–acetic acid (1 + 1) solvent

	Mean value		s_r		s_R		RSD_r, %		RSD_R, %	
Sample	CTC[a]	CHX[b]	CTC	CHX	CTC	CHX	CTC	CHX	CTC	CHX
Sunflower	133.6	132.9	1.4	1.7	3.4	2.3	1.1	1.3	2.6	1.7
Refined palm	53.1	53.0	0.1	0.2	0.3	0.4	0.2	0.3	0.5	0.7
Crude fish	109.1	108.5	0.7	0.5	1.7	1.1	0.6	0.5	1.6	1.0
Tung	164.5	163.1	2.0	1.4	3.1	2.5	1.2	0.9	1.9	1.5
Tallow (beef)	47.2	46.9	0.2	0.2	0.5	0.4	0.5	0.5	1.0	0.8
Crude palm	52.5	52.6	0.3	0.4	0.4	0.5	0.5	0.8	0.8	1.0
Used frying	37.7	37.7	0.1	0.2	0.2	0.3	0.4	0.5	0.4	0.9
Palm kernel	18.2	18.3	0.01	0.01	0.03	0.04	0.1	0.1	0.2	0.2
Olive	82.3	82.2	0.2	0.5	0.6	0.8	0.3	0.6	0.7	0.9
HSBO[c]-1	102.6	102.3	0.5	0.8	1.8	1.9	0.5	0.8	1.7	1.8
HSBO-2	74.7	74.8	0.5	0.4	1.0	0.6	0.6	0.5	1.3	0.8
HFO[d]	73.0	72.8	0.4	0.4	0.7	0.6	0.6	0.6	0.9	0.8

[a] Carbon tetrachloride.
[b] Cyclohexane–acetic acid (1 + 1).
[c] Hydrogenated soybean oil.
[d] Hydrogenated fish oil.

Table 993.20B Sample Weights

I value	Sample, g	Accuracy, mg
3	10.58–8.46	± 0.5
10	3.17–2.54	± 0.2
20	1.59–1.27	± 0.2
40	0.79–0.63	± 0.2
80	0.40–0.32	± 0.2
120	0.26–0.21	± 0.2
160	0.20–0.16	± 0.2
200	0.16–0.13	± 0.2

41.1.01

AOAC Official Method 981.11
Oils and Fats
Preparation of Sample
Procedure

Use clear sediment-free liquid sample directly after inverting the container several times. If liquid sample contains sediment, release all sediment from walls of container and distribute uniformly throughout the oil for determination of moisture and volatile matter. For determinations in which results might be affected by possible presence of H_2O (e.g., iodine value), dry sample as follows: Add anhydrous Na_2SO_4 in proportion of 1–2 g per 10 g sample, and hold in oven at 50°. Stir vigorously and filter to obtain clear filtrate. Prepare solid sample for determination of moisture and volatile matter as directed in **926.12** (*see* 41.1.02). For other determinations, melt sample in drying oven at a temperature at least 10° above mp. If clear, proceed directly. If turbid or contains sediment, filter sample inside oven. For determinations which might be affected by the presence of moisture (e.g., iodine value), heat portion of sample in drying oven at 50°, or 10° above mp, adding 1–2 g anhydrous Na_2SO_4 per 10 g sample. Mix sample thoroughly and filter in drying oven. To retard rancidity, keep oils and fats in cool place and protect from light and air.

Reference: JAOAC **64**, 429(1981).

Copyright 1998 AOAC INTERNATIONAL

AOAC Official Method 993.25
Polymerized Triglycerides in Oils and Fats
Gel-Permeation Liquid Chromatographic Method
First Action 1993
Final Action 1996
IUPAC–AOCS–AOAC Method

(Applicable to determination of 3% [weight/weight] polymerized triglycerides in vegetable fats and oils: heated, not heated, and used for frying.)

(*Caution*: Tetrahydrofuran and toluene are highly flammable and toxic by ingestion and inhalation. Use in a fume hood at all times.)

Method Performance:
See Table 993.25 for method performance data.

Table 993.25 Method Performance for Determination of Polymerized Triglycerides in Vegetable Oils and Fats, Gel Permeation Liquid Chromatographic Method

Sample No.[a]	Mean, %	s_r	s_R	RSD_r, %	RSD_R, %
1	3.6	0.1	0.5	3.3	12.5
2	5.2	0.1	0.3	2.3	6.7
3	9.7	0.3	0.4	3.2	4.6
4	10.0	0.2	1.2	2.2	12.2
5	22.0	0.2	1.5	0.8	6.8

[a] 1 = reference material (oil with known polymer content); 2 = frying oil with low polymer content; 3 = frying oil with an average polymer content; 4 = frying oil with an average polymer content and presenting resolution difficulties between monomers and polymerized triglycerides; and 5 = exhausted frying oil greatly damaged.

A. Principle

Oil and fat samples are dissolved in tetrahydrofuran; polymerized triglycerides in samples are separated on basis of molecular size by gel-permeation liquid chromatography (GPLC) and quantified by refractive index detection.

B. Apparatus

(a) *GPLC system.—(1) Solvent reservoir.*—Equipped with mobile-phase line filter (pore size 1 µm). *(2) LC pump.*—Pulseless, with flow 0.7–1.5 mL/min. *(3) Injection port.*—With low dead volume, equipped with 10 µL sample loop.

(b) *Syringe.*—50–100 µL, compatible with injector.

(c) *GPLC column.*—7.8 mm id × 300 mm, stainless steel, packed with spherical polystyrene-divinylbenzene microporous particles, ca 5 µm particle diameter, 100 pore size (PL Gel, Phenomenex, Torrance, CA, or Polymer Laboratories, Ltd, Stretton, Shropshire, UK, is suitable). (*Note*: Store column in toluene.)

(d) *Detector.*—Refractive index detector with minimum sensitivity at full-scale, 1.10×10^{-4} refraction index units; capable of being maintained at a few degrees above ambient temperature (if detector is not equipped with heater, use recirculating, constant-temperature water bath).

(e) *Recorder.*—Suitable to display and quantify peak areas; response time, 1.5 s (90% response with 100% signal); chart speed, 0.1–10 mm/min; sensitivity, 1–100 mV; accepts signal output from detector (d).

(f) *Calculator and/or integrator.*

(g) *Round-bottom flask.*—250 mL, with heating mantle capable of maintaining flask contents at 180 2°.

(h) *Filters.*—1 µm pore size, medium porosity, polytetrafluoroethylene (Teflon TFE) or cellulose ester filter (commercial LC disposable syringe filter units are suitable).

C. Reagents

(a) *Mobile phase.*—Tetrahydrofuran (peroxide-free, nonstabilized).

(b) *Storage solution.*—Toluene.

(c) *Oil standard.*—Heat ca 185 mL refined soybean oil in flask, B(g), to 180 2° until polymerized triglyceride level is 10–15%, as analyzed in F.

(d) *Sodium sulfate.*—Anhydrous.

D. Preparation of GPLC System

To condition GPLC column, change solvent stepwise from toluene, C(b) (solvent B), to tetrahydrofuran, C(a) (solvent A). Pump 10 mL portions of solvents at 1 mL/min as follows: step 1, 25% A in B; step 2, 50% A in B; step 3, 75% A in B; step 4, 100% A. Continue to pump tetrahydrofuran for 12 h to stabilize column.

Connect column to injection valve and wash with ca 30 mL tetrahydrofuran. Connect column to detector sample cell. Fill reference cell with tetrahydrofuran. Adjust flow to 0.8–1 mL/min and wait for baseline stablization (typically ca 15 min).

(*Note*: Reverse stepwise wash of column for storage in toluene.)

E. System Suitability

Analyze oil standard as in F, adjusting detector sensitivity to achieve chromatogram with monomeric triglyceride peak (peak 3) more than half chart width (*see* Figure 993.25).

Use chromatogram to check efficiency and resolution power of column. Performance should be at least as good as shown in Figure 993.25.

Calculate theoretical plate number, n, and resolution, R, as follows:

$$n = 16\,[dR/w]^2$$

$$R = [\Delta/w]$$

where dR = retention distance from start of chromatogram to peak maximum for triglycerides, in mm; w = width of triglycerides peak at baseline, measured between tangents and baseline, in mm; and Δ = distance between peak maxima of triglycerides peak and neighbor peak of polymerized triglycerides (peaks 3 and 2, respectively, in Figure 993.25), in mm.

Choose analysis conditions such that n is 6000 and R is 1.

F. Determination

If sample is not completely melted at ambient temperature, heat sample until no more than 10° above its melting temperature and mix thoroughly.

Accurately weigh 0.2 0.01 g prepared sample into conical flask. Add 15 mL purified tetrahydrofuran, which has been collected after last peaks have eluted from column during a chromatographic run. Swirl flask, and leave until fat is completely dissolved. Add ca 50 mg sodium sulfate, C(d), to dissolved sample, shake, and wait ca 2 min. Filter through 1 µm pore size filter, B(h). When sample

Copyright 1998 AOAC INTERNATIONAL

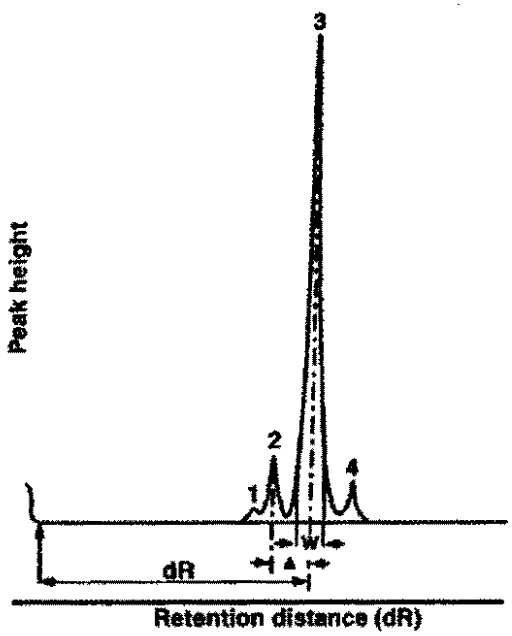


Figure 993.25—Typical gel permeation liquid chromatogram of oil standard (heated soyban oil) for calculation of resolution and theoretical place number. 1 and 2, polymerized triglycerides; 3, triglyceriedes; 4, free fatty acids

contains suspended particles, avoid blockage of porous fritted filter at column top by filtering sample through 1 µm pore size filter.

Take 50–100 µL prepared sample solution, by syringe, fill injection loop, and inject sample onto GPLC column. Elute sample with tetrahydrofuran at flow rate, 0.5–1 mL/min (at 1 mL/min, analysis takes ca 10 min).

G. Results

Chromatogram (*see* Figure **993.25**) shows main peak (monomeric triglycerides, MW ca 900) and 1 or more smaller peaks with shorter retention time (polymerized triglycerides, dimers, and oligomers). For samples in which complex degradation phenomena (possibly hydrolysis) may have occurred, peak pattern preceding triglyceride peak may be less well defined.

H. Calculation

Use area normalization to calculate percentage of polymerized triglycerides, assuming that all sample components are eluted.

For cases with a valley between triglycerides peak and highest peak of polymerized triglycerides, and lowest point of valley is lower than 75% height of highest peak of polymerized triglycerides, calculate polymerized triglycerides content, PT (in % *A/A*) as follows

$$PT = [A_{PT}/\Sigma A] \times 100$$

where A_{PT} = sum of areas of polymerized triglycerides peaks; ΣA = sum of areas of all peaks. Report PT to 1 decimal place.

For calculating A_{PT}, 2 cases are possible: *(1)* Good resolution between peaks ($R > 1$). Calculate individual and total areas by general integration methods (manual and electronic). *(2)* Poor resolution between peaks ($R < 1$). Assume that all components eluting before ($dR - w/2$) are polymerized triglycerides. With manual technique, determine triglyceride peak area by triangulation. With electronic integration, adjust integrator carefully (backward horizontal integration) to integrate all surfaces included between curve and baseline.

For cases with no valley between triglyceride peak and highest peak of polymerized triglycerides, or lowest point of valley is higher than 75% of highest peak of polymerized triglycerides, calculate polymerized triglycerides as follows:

$$PT = [A'_{PT}/\Sigma A] \times 100$$

where: A'_{PT} = area above baseline measured from start until retention distance ($dR - w/2$), described in E; ΣA = sum of areas of all peaks. Report result to 1 decimal place. [*Note:* As hydrolysis products increase in used frying oils, peak pattern preceding triglycerides peak becomes less clear or even disappears. In this case, determine polymerized triglyceride content by assuming that all components eluting before ($dR - w/2$) are polymerized triglycerides.]

References: Pure and Appl. Chem. **63**, 1163(1991); IUPAC Standard Methods for Analysis of Oils, Fats and Derivatives, 7th Ed., Blackwell Scientific Publications, 1987, Method 2.508; J. AOAC Int. **77**, 957(1994).

Revised March 1997

© AOAC INTERNATIONAL

Copyright 1998 AOAC INTERNATIONAL

41.1.41

AOAC Official Method 986.19
Triglycerides in Fats and Oils
Gas Chromatographic Method
First Action 1986
Final Action 1992

IUPAC-AOAC Method

A. Principle

Triglyceride groups having same C number are separated by direct gas chromatography of solution of oil or fat, under temperature-programmed conditions, and identified by reference to standard triglyceride solution. Triglycerides having same C number are not determined individually. Content is determined by peak area ratio.

B. Apparatus and Reagents

(a) *Gas chromatograph.*—With facilities for on-column injection, oven temperature programming up to 350°, and, preferably, electronic integration. All-glass system is preferable.

(b) *Column.*—Glass, ca 0.5–0.6 m long, and 2–4 mm id, filled with 3% (or less) methyl polysiloxane on acid-washed silanized support (OV-1 is suitable). Carrier gas flow 50 mL/min. He is recommended as carrier gas but N_2 may be used with some loss in resolution. Condition column prior to use at 350° for ≥36 h with carrier gas flow rate of ca 5 mL/min. *Note:* Equivalent results may be obtained with use of short (≤6 m) column.

(c) *Triglycerides.*—Purity 99%. Prepare standard solution in CH_2Cl (or diisopropyl ether) containing ca 10 mg/mL each of tricaprin, tricaprylin, trilaurin, trimyristin, tripalmitin, and tristearin.

C. Determination of Triglycerides Correction Factors

With 2 µL microsyringe, inject ca 1 µL triglycerides standard solution with injection and detector temperatures set at ca 375° and initial oven temperature ca 220°. Immediately commence programming oven temperature to increase at rate of ca 4–5°/min (but not >5°) and continue analysis until temperature reaches ca 350°. Maintain this temperature until all triglycerides have eluted from column.

Assume that trilaurin is completely recovered from column and calculate correction factor, f_i, for each remaining triglyceride from

$$f_i = (C_{si}/C_L) \times (A_L/A_{si})$$

where A_L = peak area for trilaurin; A_{si} = peak area for standard triglyceride i; C_L = concentration (mg/mL) of trilaurin; C_{si} = concentration (mg/mL) of standard triglyceride i.

Determine f from ≥2 injections of standard solution. Plot graph of average values for f for each triglyceride against corresponding C number. Correction factor >1.1 is unsatisfactory. Decrease stationary phase loading or increase carrier gas flow rate to achieve acceptable correction factors.

Plot values of retention time for each standard triglyceride peak against corresponding C number. Straight line should be obtained from which expected retention times for other triglycerides can be determined.

D. Preparation of Sample Solution

Warm sample as necessary to completely liquefy. Homogenize liquid sample by gently shaking container. Prepare 50 mg/mL solution of sample in CH_2Cl (or diisopropyl ether). For example, transfer ca 1.25 g liquid sample to 25 mL graduated flask using pipet and dissolve sample (while still liquid) in 2–3 mL solvent. Dilute with same solvent, and mix. If sample is known to contain significant amounts of mono- or diglycerides or free fatty acids, remove these according to 965.35 (*see* 41.1.61) before proceeding with analysis as described for determination of triglycerides correction factors.

E. Determination

Identify each peak by using identification graph. Determine peak areas of each group of triglycerides. Calculate corrected peak areas by using correction factors determined either by calculation or by interpolation from graph of correction factors obtained for standard triglycerides. Determine quantity of each group of triglycerides having same C number expressed as percentage relative to total triglycerides content by formula:

$$(A_{TGi}/A_T) \times 100$$

where A_{TGi} = corrected peak area of triglycerides group i; A_T = total corrected peak area of triglycerides groups contained in sample ($A_T = \Sigma A_{TGi}$).

Difference between results of 2 determinations carried out on same day by same analyst using same apparatus for same test material and for triglycerides present >10% should not exceed 1% absolute. For triglycerides present at levels of <10%, difference should not exceed 0.5% absolute. Triglycerides present at <5% are determined less accurately.

Reference: Pure Appl. Chem. 57, 1515(1985).

Copyright 1998 AOAC INTERNATIONAL

AOAC Official Method 940.28
Fatty Acids (Free) in Crude and Refined Oils
Titration Method
Final Action

National Cottonseed Products Association-AOAC Method

(a) *In crude oils.*—Weigh 7.05 g well-mixed oil into 250 mL flask or 4 oz bottle. Add 50 mL alcohol, previously neutralized by adding 2 mL phenolphthalein solution and enough 0.1*N* NaOH to produce faint permanent pink. Titrate with 0.25N NaOH, **936.16** (*see* A.1.12), with vigorous shaking until permanent faint pink appears and persists ≥1 min. Report as % free fatty acids expressed as oleic acid; mL 0.25*N* NaOH used in titration corresponds to this %.

(b) *In refined oils.*—To ca 50 mL alcohol in clean, dry 150 mL flask, add few drops of the oil and 2 mL phenolphthalein. Place flask in H_2O at 60–65° until warm, and add enough 0.1*N* NaOH to produce faint permanent pink. Weigh 56.4 g oil into the neutralized alcohol and titrate with 0.1*N* NaOH, **936.16** (*see* Appendix A), occasionally warming and violently shaking mixture until same faint permanent pink appears in supernate alcohol. Multiply mL 0.1*N* NaOH by 0.05 and report as % free fatty acids expressed as oleic acid.

Free fatty acids may also be expressed in terms of acid value (mg KOH necessary to neutralize 1 g sample). Acid value = % free fatty acids (as oleic) × 1.99.

Copyright 1998 AOAC INTERNATIONAL

AOAC Official Method 965.33
Peroxide Value of Oils and Fats

Titration Method
First Action 1965
Final Action 1969
AOCS-AOAC Method

(*Note*: Conduct analysis in diffuse daylight or in artificial light shielded from direct light source.)

A. Reagents

(a) *Acetic acid-chloroform solution.*—Mix 3 volumes CH_3COOH with 2 volumes $CHCl_3$, USP.

(b) *Potassium iodide solution, saturated.*—Dissolve excess KI in freshly boiled H_2O. Excess solid must remain. Store in dark. Test daily by adding 0.5 mL to 30 mL CH_3COOH-$CHCl_3$, (a); then add 2 drops 1% starch solution, (mix ca 1 g soluble starch with enough cold H_2O to make thin paste, add 100 mL boiling H_2O, and boil ca 1 min while stirring). If solution turns blue, requiring >1 drop 0.1*N* $Na_2S_2O_3$ to discharge color, prepare fresh solution.

(c) *Sodium thiosulfate standard solutions.*—0.1 and 0.01*N*. Prepare and standardize as in **942.27** (*see* A.1.13). For 0.01*N*, dilute 0.1*N* with freshly boiled and cooled H_2O.

B. Determination

(a) *Fats and oils.*—Weigh 5.00 ± 0.05 g sample into 250 mL glass-stoppered Erlenmeyer. Add 30 mL CH_3COOH-$CHCl_3$, (a), and swirl to dissolve. Add 0.5 mL saturated KI solution, (b), from Mohr pipet, let stand with occasional shaking 1 min, and add 30 mL H_2O. Slowly titrate with 0.1*N* $Na_2S_2O_3$ with vigorous shaking until yellow is almost gone. Add ca 0.5 mL 1% starch solution, and continue titration, shaking vigorously to release all I_2 from $CHCl_3$ layer, until blue just disappears. If <0.5 mL 0.1*N* $Na_2S_2O_3$ is used, repeat determination with 0.01*N* $Na_2S_2O_3$.

Conduct blank determination daily (must be ≤0.1 mL 0.1*N* $Na_2S_2O_3$). Subtract from sample titration.

Peroxide value (milliequivalent peroxide/kg sample) = $S \times N \times 1000$/g sample, where S = mL $Na_2S_2O_3$ (blank corrected) and N = normality $Na_2S_2O_3$ solution.

(b) *Margarine.*—Melt sample by heating with constant stirring on hot plate at low heat, or heat in air oven at 60–70°. (Avoid excessive heat and long exposure >40°.) When completely melted, hold in warm place until aqueous portion and most of solids have settled. Decant oil into clean beaker and filter through Whatman No. 4, or equivalent paper. Do not reheat unless necessary to obtain clear filtrate. Proceed as in (a).

References: J. Am. Oil Chem. Soc. **26**, 345(1949).
AOCS Method Cd 8–53. JAOAC **48**, 175(1965).

Revised: March 1998

© 1998 AOAC INTERNATIONAL

Spray plate with 10% molybdophosphoric acid. After evaporation of alcohol, heat plate in 120–130° drying oven. Fraction 1 (nonpolar) should be free of polar substances (*see* Figure **982.27**).

References: Fette Seifen Anstrichm **80,** 106(1978). JAOAC **64,** 1329(1981); **65,** 375(1982). IUPAC 2.507, 7th ed. Pure Appl. Chem. **54,** 242(1982).

Copyright 1998 AOAC INTERNATIONAL

AOAC Official Method 982.27
Polar Components in Frying Fats
Chromatographic Method
First Action 1982
Final Action 1984

IUPAC-AOAC Method

A. Principle

Method assesses deterioration of used frying fats, and is applicable to all fats and oils. Polar components are those components of fats determined by column chromatography under conditions specified, and include polar substances such as monoglycerides, diglycerides, free fatty acids that occur in unused fats, as well as polar transformation products formed during frying of foodstuffs and/or during heating. Nonpolar components are mostly unaltered triglycerides. Frying fats are separated by column chromatography on silica gel into nonpolar and polar components. Polar components are determined indirectly by subtracting concentration of nonpolar components. Quality of separation can be checked by thin layer chromatography.

B. Apparatus

(a) *Column.*—Glass, 2.1 cm id × 45 cm, with Teflon stopcock and ground-glass joint.

(b) *TLC plates.*—Precoated silica gel (without fluorescence indicator), 20 × 20 cm, layer thickness = 0.25 mm.

C. Reagents

(a) *Adsorbent.*—Silica gel 60, particle size 0.063–0.200 mm (70–230 mesh ASTM), Merck No. 7734, or equivalent, adjust to H_2O content of 5% as follows: Dry silica gel ≥4 h in porcelain dish in 160° oven; cool in desiccator to room temperature. Adjust H_2O content to 5%, e.g., weigh 152 g silica gel and 8 g H_2O in 500 mL round-bottom flask with ground-glass stopper and mechanically shake 1 h.

(b) *Eluting solvent mixture.*—Petroleum ether (bp 40–60°)-ether (87 + 13).

(c) *Sea-sand.*—Analytical reagent grade; purified by acid and calcined.

(d) *Spray reagent.*—Molybdophosphoric acid, 10% in alcohol.

D. Preparation of Sample

Warm semi-liquid and solid samples to temperature slightly above mp and mix thoroughly; avoid overheating. Remove visible impurities by filtration; if H_2O is present, use hydrophobic filter.

E. Preparation of Column

Fill column with ca 30 mL petroleum ether-ether (87 + 13). Place wad of cotton wool in bottom of column and remove air by pressing with glass rod.

In 100 mL glass beaker, prepare slurry of 25 g silica gel and ca 80 mL petroleum ether-ether (87 + 13) and pour slurry into column through 8 cm glass funnel. Rinse beaker, funnel, and sides of column with same solvent. Open stopcock and drain solvent to 10 cm above silica gel. Level silica gel by tapping column.

Add ca 4 g sea-sand through funnel into column. Drain solvent to sand layer.

F. Chromatography

To determine polar components by difference, only nonpolar fraction is used. However, if separation is controlled by TLC, both polar and nonpolar fractions are required. Separation may also be controlled by checking recovery of sample, but for samples containing substantial amounts of polar material, recovery may be incomplete because small amounts of highly polar material, generally 1–2%, are not eluted under conditions specified.

Accurately weigh 2.5 ± 0.1 g (to 0.001 g) sample into 50 mL volumetric flask, and dissolve in ca 20 mL petroleum ether-ether (87 + 13) while warming slightly. Let cool to room temperature and dilute to volume with same solvent. Using volumetric pipet, transfer 20 mL sample aliquot to column, without disturbing surface.

Dry two 250 mL round-bottom flasks in 103 ± 2° oven, cool to room temperature, and accurately weigh to 0.001 g. Place one flask under column, open stopcock, and let sample solution drain to level of sand layer. Elute nonpolar components with 150 mL petroleum ether-ether (87 + 13) contained in 250 mL dropping funnel. Adjust flow rate so that 150 mL passes through column within 60–70 min. After elution, wash any substance adhering to outlet of column into round-bottom flask with petroleum ether-ether (87 + 13).

In same manner, elute polar components into second 250 mL round-bottom flask with 150 mL ether. Discard silica gel.

Remove solvent from each fraction with a rotary evaporator and ≤60° H_2O bath or with N_2 stream in 250 mL flask on steam bath. Avoid losses due to foaming. If rotary evaporator is used, shortly before end of evaporation, introduce N_2 into system. Cool residue to ambient temperature and introduce N_2 into flask. Weigh flasks.

G. Calculations

Calculate polar components, as % (weight/volume) by formula:

$$\text{Polar components, \%} = [(E - A)/E] \times 100$$

where A = g nonpolar fraction; E = g sample in 20 mL aliquot (ca 1 g). Report result to 1 decimal place.

H. Check of Column Chromatography Efficiency by Thin Layer Chromatography

Dilute polar and nonpolar fraction (1 + 9) in $CHCl_3$. Apply 2 µL spots using capillary dispensing pipet. Develop plate with petroleum ether-ether-CH_3COOH (70 + 30 + 2) in tank lined with filter paper for ca 35 min (ca 17 cm). Remove plate and let solvent evaporate.

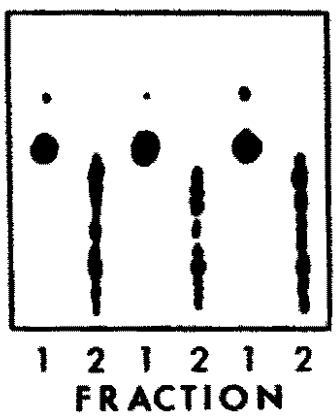


Figure 982.27—Evaluation of efficiency of fractionation by TLC separation of polar and non-polar fraction; Fraction 1 contains non-polar components, Fraction 2 contains polar components

Copyright 1998 AOAC INTERNATIONAL

AMERICAN OIL CHEMISTS SOCIETY METHODS

(AOCS)

AOCS Official Method Ca 5a-40
Reapproved 1997

Free Fatty Acids

DEFINITION
This method determines the free fatty acids existing in the sample.
SCOPE
Applicable to all crude and refined vegetable oils, marine oils and animal fats.

APPARATUS

1. Oil sample bottles—115 or 230 mL (4 or 8 oz), or 250-mL Erlenmeyer flasks.

REAGENTS

1. Ethyl alcohol, 95%—USSD formulas 30 and 3A are permitted (see Notes, 1). The alcohol must give a definite, distinct and sharp end point with phenolphthalein and must be neutralized with alkali to a faint, but permanent pink color just before using.
2. Phenolphthalein indicator solution—1% in 95% alcohol.
3. Sodium hydroxide solution—accurately standardized. See AOCS Specification H 12-52. See Table 1 for the appropriate normality of the sodium hydroxide solution, depending on the expected free fatty acid concentration range in the sample.

Table 1
Free fatty acid range, alcohol volume and strength of alkali[a]

FFA range (%)	Sample (g)	Alcohol (mL)	Strength of alkali
0.00 to 0.2	56.4 ± 0.2	50	0.1 N
0.2 to 1.0	28.2 ± 0.2	50	0.1 N
1.0 to 30.0	7.05 ± 0.05	75	0.25 N
30.0 to 50.0	7.05 ± 0.05	100	0.25 or 1.0 N
50.0 to 100	3.525 ± 0.001	100	1.0 N

[a]FFA, free fatty acid; N, normality.

PROCEDURE

1. Samples must be well mixed and entirely liquid before weighing; however, do not heat the sample more than 10°C over the melting point.
2. Use Table 1 to determine the sample weight for various ranges of fatty acids. Weigh the designated sample size into an oil sample bottle or Erlenmeyer flask (see Notes, 2).
3. Add the specified amount of hot neutralized alcohol and 2 mL of indicator.
4. Titrate with standard sodium hydroxide, shaking vigorously until the appearance of the first permanent pink color of the same intensity as that of the neutralized alcohol before the addition of the sample. The color must persist for 30 seconds.

CALCULATIONS

1. The percentage of free fatty acids in most types of fats and oils is calculated as oleic acid, although in coconut and palm kernel oils it is frequently expressed as lauric acid and in palm oil in terms of palmitic acid.

(a) Free fatty acids as oleic, % =

$$\frac{\text{mL of alkali} \times N \times 28.2}{\text{mass, g of sample}}$$

(b) Free fatty acids as lauric, % =

$$\frac{\text{mL of alkali} \times N \times 20.0}{\text{mass, g of sample}}$$

(c) Free fatty acids as palmitic, % =

$$\frac{\text{mL of alkali} \times N \times 25.6}{\text{mass, g of sample}}$$

2. The free fatty acids are frequently expressed in terms of acid value instead of percentage free fatty acids. The acid value is defined as the number of milligrams of KOH necessary to neutralize 1 g of sample. To convert percentage free fatty acids (as oleic) to acid value, multiply the percentage free fatty acids by 1.99.

PRECISION

Precision data for refined, bleached and deodorized oils are shown in Table 2. Precision data for crude oils are shown in Table 3.

Table 2
The average, expected between-laboratory variation (standard deviation of reproducibility, S_R) for the determination of free fatty acids in refined, bleached and deodorized oils[a]

Approximate FFA value (%)	0–0.05	0.05–0.1	0.1–1.0	1.0–2.0
S_R	0.007	0.010	0.046	0.073
RSD (CV, %)	33.93	12.73	9.90	4.75
R (95%) = 2.8 × S_R	0.02	0.03	0.13	0.20

[a]Values obtained from the AOCS Smalley Laboratory Proficiency Program.

Table 3
The average, expected between-laboratory variation (standard deviation of reproducibility, S_R) for the determination of free fatty acids in crude oils[a]

Approximate FFA value (%)	0.1–1.0	1.0–2.0
S_R	0.077	0.156
RSD (CV, %)	14.57	9.84
R (95%) = 2.8 × S_R	0.22	0.44

[a]Values obtained from the AOCS Smalley Laboratory Proficiency Program.

NOTES

1. Isopropanol, 99%, may be used as an alternate solvent with crude and refined vegetable oils.
2. Cap bottle and shake vigorously for 1 min if oil has been blanketed with carbon dioxide gas.

REFERENCES

See *J. Assoc. Off. Anal. Chem. 59:*658 (1976) regarding the ruggedness of this method.

AOCS Official Method Cd 8b-90
Reapproved 1997

Peroxide Value
Acetic Acid–Isooctane Method

DEFINITION
This method determines all substances, in terms of milliequivalents of peroxide per 1000 grams of sample, that oxidize potassium iodide under the conditions of the test. The substances are generally assumed to be peroxides or other similar products of fat oxidation.

SCOPE
Applicable to all normal fats and oils, including margarine. This method is highly empirical, and any variation in the test procedure may result in erratic results. Because this method gives erratic results at peroxide values ≥70, this method should not be used with the AOM test, AOCS Official Method Cd 12-57, with which peroxide values ≥70 may be encountered.

APPARATUS

1. Pipet—0.5 mL, or other suitable volumetric apparatus capable of dispensing 0.5 mL of saturated potassium iodide (KI) solution.
2. Erlenmeyer flasks—with glass stoppers, 250 mL.

REAGENTS

1. Acetic acid–isooctane solution—3:2, v/v, prepared by mixing 3 volumes of reagent-grade glacial acetic acid (see Notes, *Caution*) with 2 volumes of reagent-grade isooctane (see Notes, *Caution*).
2. Potassium iodide (KI) solution—saturated, prepared fresh each day analysis is performed by dissolving an excess of KI in recently boiled distilled water. Make certain the solution remains saturated during use, as indicated by the presence of undissolved KI crystals. Store in the dark when not in use. Test the saturated KI solution by adding 2 drops of starch solution to 0.5 mL of the KI solution in 30 mL of the acetic acid–isooctane solution. If a blue color is formed that requires more than 1 drop of 0.1 N sodium thiosulfate solution to discharge, discard the KI solution and prepare a fresh solution.
3. Sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$) solution—0.1 N, accurately standardized vs. potassium dichromate primary standard as follows:
   (a) Sodium thiosulfate solution 0.1 N, prepared by dissolving 24.9 g of sodium thiosulfate in distilled water and diluting to 1 L.
   (b) The potassium dichromate (see Notes, *Caution*) primary standard should be finely ground, dried at 105°C for 2 hr and cooled in a desiccator. Weigh 0.16–0.22 g of potassium dichromate into a 500-mL flask or bottle by difference from a weighing bottle. Dissolve in 25 mL of water, add 5 mL of concentrated hydrochloric acid (35–37%), 20 mL of potassium iodide solution (15% solution, 15 g KI in 100 mL water) and rotate to mix. Allow to stand for 5 min and then add 100 mL of distilled water. Titrate with sodium thiosulfate solution, shaking continuously until yellow color has almost disappeared. Add 1–2 mL of starch indicator and continue the titration, adding the thiosulfate solution slowly until the blue color just disappears. The strength of the sodium thiosulfate solution is expressed in terms of its normality.

   Normality of $Na_2S_2O_3$ solution =

$$\frac{20.394 \times \text{mass, g of } K_2Cr_2O_7\text{, g}}{\text{mL of sodium thiosulfate}}$$

4. Sodium thiosulfate solution—0.01 N, accurately standardized. This solution may be prepared by accurately pipetting 100 mL of 0.1 N sodium thiosulfate into a 1000-mL volumetric flask and accurately diluting to volume with recently boiled distilled water.
5. Starch indicator solution—tested for sensitivity, prepared by making a paste with 1 g of starch (see Notes, I) and a small amount of cold distilled water. Add, while stirring, to 200 mL of boiling water and boil for a few seconds. Immediately remove from heat and cool. Salicylic acid (1.25 g/L) may be added to preserve the indicator. If long storage is required, the solution must be kept in a refrigerator at 4–10°C. Fresh indicator must be prepared when the end point of the titration from blue to colorless fails to be sharp. If stored under refrigeration, the starch solution should be stable for about 2–3 weeks.

   Test for sensitivity—Place 5 mL of starch solution in 100 mL of water and add 0.05 mL of freshly prepared 0.1 N KI solution and one drop of a 50 ppm chlorine solution made by diluting 1 mL of a commercial 5% sodium hypochlorite (NaOCl) solution to 1000 mL. The deep blue color produced must be discharged by 0.05 mL of 0.1 N sodium thiosulfate.
6. Sodium lauryl sulfate (SDS)—≥ 98% [Aldrich Chemical (W. Milwaukee, WI, USA) or Mallinckrodt (Paris, KY, USA)]. Prepare 10% solution by dissolving 10 g SDS in 100 mL water.

PROCEDURE FOR FATS AND OILS

1. Weigh 5.00 ± 0.05 g of sample into a 250-mL Erlenmeyer flask with glass stopper and add 50 mL of the 3:2 acetic acid–isooctane solution. Swirl to dissolve the sample. Add 0.5 mL of saturated KI solution using a suitable volumetric pipet.

2. Allow the solution to stand for exactly 1 min, thoroughly shaking the solution at least three times during the 1 min, and then immediately add 30 mL of distilled water.
3. Titrate with 0.1 N sodium thiosulfate, adding it gradually and with constant and vigorous agitation (see Notes, 2). Continue the titration until the yellow iodine color has almost disappeared. Add 0.5 mL of 10% SDS (Reagents, 6), and then add about 0.5 mL of starch indicator solution. Continue the titration with constant agitation, especially near the end point, to liberate all of the iodine from the solvent layer. Add the thiosulfate solution dropwise until the blue color just disappears (see Notes, 3 and 4).
4. Conduct a blank determination of the reagents daily. The blank titration must not exceed 0.1 mL of the 0.1 N sodium thiosulfate solution.

PROCEDURE FOR MARGARINE

1. Melt the sample by heating with constant stirring on a hot plate set at low heat, or by heating in an air oven at 60–70°C. Avoid excess heating and particularly prolonged exposure of the oil to temperatures above 40°C.
2. When completely melted, remove the sample from the hot plate or oven and allow to settle in a warm place until the aqueous portion and most of the milk solids have settled to the bottom.
3. Decant the oil into a clean beaker and filter through a Whatman no. 4 paper (or equivalent) into another clean beaker. Do not reheat for filtration unless absolutely necessary. The sample must be clear and brilliant.
4. Proceed as directed in Procedure for Fats and Oils, paragraphs 1–4.

CALCULATIONS

1. Peroxide value (milliequivalents peroxide/1000 g sample) =

$$\frac{(S - B) \times N \times 1000}{\text{mass of sample, g}}$$

Where—

B = volume of titrant, mL of blank
S = volume of titrant, mL of sample
N = normality of sodium thiosulfate solution

NOTES

Caution

Isooctane is flammable and a fire risk. Explosive limits in air are 1.1–6.0%. It is toxic by ingestion and inhalation. A properly operating fume hood should be used when working with this solvent.

Acetic acid in the pure state is moderately toxic by ingestion and inhalation. It is a strong irritant to skin and tissue. The TLV in air is 10 ppm.

Potassium dichromate is toxic by ingestion and inhalation. There is sufficient evidence in humans for the carcinogenicity of chromium [+6], in particular, lung cancer. It is a strong oxidizing agent and a dangerous fire risk when in contact with organic chemicals.

NUMBERED NOTES

1. "Potato Starch for Iodometry" is recommended, because this starch produces a deep blue color in the presence of the iodonium ion. "Soluble Starch" is not recommended because a consistent deep blue color may not be developed when some soluble starches interact with the iodonium ion. The following are suitable starches: Soluble Starch for Iodometry, Fisher S516-100; Soluble Potato Starch, Sigma S-2630; Soluble Potato Starch for Iodometry, J. T. Baker 4006-04.
2. There is a 15–30 sec delay in neutralizing the starch indicator for peroxide values 70 meq/kg and higher. This delay is due to the tendency of isooctane to float on the surface of an aqueous medium, and the time necessary to adequately mix the solvent in large volumes of aqueous titrant, thereby liberating the last traces of iodine. Based on collaborative study results (References, 1, 2), the recommendation is to use 0.1 N titrant for peroxide value ranges (10–150 meq/kg). Erratic results reported for this method, especially at higher peroxide values, appear to be related to the isooctane floating on the surface of the aqueous layer (References, 3). Rapid mechanical stirring (e.g., with magnetic stirrer) and/or use of a surfactant, such as sodium lauryl sulfate (Reagents, 6), is highly recommended.
3. If the titration is less than 0.5 mL using 0.1 N sodium thiosulfate, repeat the determination using 0.01 N sodium thiosulfate, using vigorous agitation and/or surfactant for the reason stated in Notes, 2.
4. The test should be carried out in diffuse daylight or in artificial light shielded from a direct light source (References, 4).

REFERENCES

1. Brooks, D.D. and D.L. Berner, *Isooctane as an Alternative Solvent for Peroxide Value Determination. Study I*, poster presentation, AOCS National Meeting, Baltimore, MD, April 23, 1990.
2. Collaborative study results published in *INFORM 1*:884 (1990).
3. Brooks, D.D., S.K. Brophy, B. Hayden and G.R. Goss, *Alternative Solvents for Peroxide Value Determination*, poster presentation, AOCS National Meeting, Cincinnati, OH, May 10, 1989.
4. *J. Assoc. Off. Anal. Chem. 75*:507 (1992).
5. The International Standards Organization (ISO) successfully completed an international collaborative study of this method in 1996. The results were reported at the meeting of the ISO Commission on Oils and Fats in London in April 1996 in meeting document N576.

306

AOCS Official Method Cd 18-90

Reapproved 1997

p-Anisidine Value

DEFINITION

The p-anisidine value is defined by convention as 100 times the optical density measured at 350 nm in a 1-cm cuvette of a solution containing 1.00 g of the oil in 100 mL of a mixture of solvent and reagent according to the method described.

SCOPE

This method determines the amount of aldehydes (principally 2-alkenals and 2,4-dienals) in animal and vegetable fats and oils, by reaction in an acetic acid solution of the aldehydic compounds in an oil and the p-anisidine (see Notes, 1), and then measuring the absorbance at 350 nm.

APPARATUS

1. Test tubes—10 mL min. with either ground-glass stoppers or Teflon™-lined screw caps.
2. Volumetric flasks—25 mL.
3. Automatic pipet or automatic burette.

   Note—Any pipette and/or burette capable of delivering exactly 1 mL and 5 mL is satisfactory.
4. Spectrophotometer suitable for observation at 350 nm.
5. Glass cuvettes—1.00 ± 0.01 cm, the two cuvettes of each pair must be identical.

REAGENTS

1. Isooctane (2,2,4-trimethylpentane)—optically clear (see Notes, *Caution* and 2).
2. Glacial acetic acid—analytical reagent quality (see Notes, 3).
3. p-Anisidine—analytical reagent quality (see Notes, *Caution* and 4) 0.25 g/100 mL solution in glacial acetic acid (Reagents, 2) (see Notes, 5).

PROCEDURE

Note—The sample should be perfectly clear and dry (see Notes, 3).

1. Weigh 0.5–4.0 ± 0.001 g of the sample into a 25-mL volumetric flask. Dissolve and dilute to volume with isooctane.
2. Measure the absorbance (Ab) of the solution at 350 nm in a cuvette with the spectrophotometer, using the reference cuvette filled with solvent as a blank.
3. Pipet exactly 5 mL of the fat solution into one test tube (Apparatus, 1) and exactly 5 mL of the solvent into a second test tube. By means of an automatic pipet (Apparatus, 3) add exactly 1 mL of the p-anisidine reagent (Reagents, 3) to each tube, and shake (see Notes, 6).
4. After exactly 10 min measure the absorbance (As) of the solvent in the first test tube in a cuvette (Apparatus, 5) at 350 nm, using the solution from the second test tube as a blank in the reference cuvette.

CALCULATIONS

The p-anisidine value (p-A.V.) is given by the formula

$$\text{p-A.V.} = \frac{25 \times (1.2\text{As} - \text{Ab})}{\text{m}}$$

Where—

As = absorbance of the fat solution after reaction with the p-anisidine reagent (Reagents, 3)

Ab = absorbance of the fat solution

m = mass of the test portion, g

PRECISION (see References, 2)

	Crude Rapeseed Oil		Refined Palm Oil	
	Sample 1	Sample 2	Sample 1	Sample 2
No. of labs	20	20	20	20
Mean value	2.0	2.0	2.3	2.3
Repeatability, CV, %	4.0	5.8	4.8	4.6
Reproducibility, CV, %	35	37	30	31

NOTES

Caution

Isooctane is flammable and a fire risk. Explosive limits in air are 1.1–6.0%. It is toxic by ingestion and inhalation. A properly operating fume hood should be used when working with this solvent.

Acetic acid in the pure state is moderately toxic by ingestion and inhalation. It is a strong irritant to skin and tissue. The TLV in air is 10 ppm.

p-Anisidine is an irritant and should be handled with care, preferably in a fume hood. p-anisidine is an aromatic amine, a class of toxic and possibly carcinogenic chemicals. o-Anisidine is a carcinogen in rats and mice, causing urinary carcinomas or papillomas. [Fourth Annual Report on Carcinogens, NTP 85-002, 1985, p. 2; *Chem. Res. Toxicol.* 4:474 (1991)]. The TLV is 0.1 ppm.

NUMBERED NOTES

1. In the presence of acetic acid, p-anisidine reacts with aldehydic compounds in oils or fats. The intensity of color of the yellowish reaction products formed depends not only on the amount of aldehydic compounds present but also on their structure. It has been found that a double bond in the carbon chain conjugated with the carbonyl double bond increases the molar absorbance four to five times. This means that 2-alkenals and dienals, especially, will contribute substantially to the value found.

2. In most cases n-hexane can be substituted for isooctane as a solvent. However, oils containing high amounts of oxidized fatty acids will not dissolve completely in n-hexane. For such oils isooctane should be used as the solvent. The absorbance of the solvent used (isooctane or n-hexane), measured in a 1.00-cm cuvette between 300 and 380 nm, must be nil or nearly nil. The commercial product can be freed from absorptive material by percolating it through a glass column (3–5 cm i.d., and 100 cm long) filled with silica gel.
3. The reaction between p-anisidine and aldehydes involves the formation of water. Hence, the presence of moisture in any of the reagents or in the sample leads to incomplete reaction and, consequently, low values. Since glacial acetic acid is highly hygroscopic, it is essential to check its moisture content by a Karl Fischer determination. If the content exceeds 0.1 percent, the acetic acid must be discarded.
4. In storage, p-anisidine tends to darken as a result of oxidation. The p-anisidine crystals, which should be cream colored, should be stored at 0–4°C in a dark bottle. The crystals should not be exposed to strong light and should be used before any color change is observed. A discolored reagent can be reduced and decolorized in the following way. Dissolve 4.0 g of p-anisidine in 100 mL of water at 75°C. Add 0.2 g of sodium sulphite and 2.0 g of active carbon and stir for 5 min. Then filter through a double filter paper. If carbon passes through, repeat filtration. Cool the filtered solution to about 0°C, allow to stand at this temperature for at least 4 hr, or, preferably, overnight. Filter off the crystallized p-anisidine and wash with a small amount of water at a temperature of about 0°C. After drying in a vacuum desiccator, transfer the crystals into a brown glass bottle. If stored in the dark and at low temperature, the crystals obtained should not darken appreciably for 1 yr.
5. Reagent solutions having an absorbance greater than 0.200 when measured in a 1.00-cm cuvette at 350 nm against isooctane or n-hexane as a blank should be discarded.
6. The mixture should be completely homogenized with minimum shaking and then allowed to react for 10 min before proceeding with the absorbance measurement (References, 4).

REFERENCES

1. IUPAC, *Standard Methods for the Analysis of Oils, Fats and Derivatives*, 7th ed., Method Number 2.504 Determination of the p-anisidine value (p-A.V.), Blackwell Scientific Publications, Boston, MA and Oxford, UK (1987).
2. FOSFA International Collaborative Study #P15, May 1986, Document No. 384, ISO/TC 34/SC 11, February 12, 1987.
3. *JAOCS 51*:17 (1974).
4. Hamilton, R.J., and S. Hamilton, *Lipid Analysis*, Oxford University Press, New York, 1992, pp. 45–47.

308

AOCS Official Method Cd 12-57
Reapproved 1997

Fat Stability, Active Oxygen Method

DEFINITION

This method measures the time (in hours) required for a sample of fat or oil to attain a predetermined peroxide value under the specific conditions of the test. The length of this period of time is assumed to be an index of resistance to rancidity. The exact relationship between peroxide value and such qualities as shelf-life, actual rancidity and oxidative stability has not been firmly established.

SCOPE

Applicable to all normal fats and oils of animal and vegetable origin intended for human consumption (see Notes, 1). Not applicable to fatty acids.

The Oil Stability Index (OSI) method, AOCS Official Method Cd 12b-92, may be used as an alternate method. In November, 1993, the AOCS Commercial Oils and Fats Analysis Technical Committee unanimously recommended that the AOM be declared an obsolete method. It is retained for reference purposes only.

GENERAL PRECAUTIONS

This procedure is highly empirical and close attention to detail is required if reproducible results are expected. The greatest source of error results from the wrong air flow from capillary tubes. The air flow should not be assumed to be correct just because the proper sized capillary tubes are used. The air pressure on the inlet side of the capillary tubes affects the air flow on the discharge side. The air flow on the discharge side of the capillary tubes should be checked frequently to be certain it is at the required flow of 2.33 mL per tube per second. Other likely sources of error are inadequate cleanliness and inefficient temperature control. All equipment must be scrupulously clean. Do not use chromic acid or other acid cleaning agents, because it is difficult to remove the final traces of them. Even distilled water is a potential source of error if it contains traces of heavy metals, particularly copper. The bath or heater must be calibrated by checking the temperature of an actual sample in each sample tube opening under the specified test conditions including aeration. After the heater has been shown to be satisfactory in this respect, the actual temperature during operation must be measured by a thermometer in a sample tube containing the recommended quantity of oil. The oil in this tube should be changed often enough to prevent gelation.

APPARATUS (see Figs. 1–3)

1. Constant temperature bath or heater (see Notes, 2)—which will maintain all samples at a temperature of 97.8 ± 0.2°C.
2. Air-distributing manifold constructed of stainless steel, nickel, aluminum or glass. The capillaries must be calibrated to permit the same flow (± 10%) through each outlet when the total flow is adjusted to 2.33 mL per tube per second.
3. Air purification train (see Figure 1)—
   (a) Air inlet tube—from compressed air source equipped with stainless steel needle valve.
   (b) Air-washing column—consisting of hydrometer cylinder 50 mm o.d., 375 mm high, containing distilled water.
   (c) Air-washing column—consisting of hydrometer cylinder 50 mm o.d., 375 mm high, containing 2% potassium dichromate ($K_2Cr_2O_7$) in 1% sulfuric acid (H_2SO_4). Fill to a depth of about 25 cm and replace solution after 72 hr of continuous operation.
   (d) Air-washing column—consisting of hydrometer cylinder 50 mm o.d., 375 mm high, containing distilled water. Fill to a depth of about 25 cm. Replace with fresh distilled water at the first appearance of a yellow color.
   (e) Water cooled condenser—Allihn 5 bulb type, 300-mm jacket.
   (f) Trap—wide mouth 500-mL bottle containing glass wool.
   (g,h) Pressure regulating columns—hydrometer cylinders 50 mm o.d., 375 mm high, containing distilled water. Fill to a depth of 20 cm. Pressure regulation may also be obtained through the use of a suitable pressure regulating valve.
   (i) Manifold—for air distribution, see Figure 1, i.
4. A source of low-pressure, clean, oil-free, compressed air—an individual air compressor of the pistonless diaphragm may be used. Purified industrial compressed type air may also be used.
5. Thermometer—accurately calibrated vs. an NIST certified thermometer, which will indicate the temperature to within ± 0.1°C in the range of 95–110°C. ASTM no. 40°C is satisfactory.
6. A gas flow meter—preferably of the calibrated conical tube type.
7. Test tubes—Pyrex™, 25 × 200 mm. For convenience these tubes may be calibrated and etched at the 20-mL level. Each tube is provided with a two-hole neoprene stopper and aeration tube as shown in Figure 2. An all-glass assembly may also be used.

309

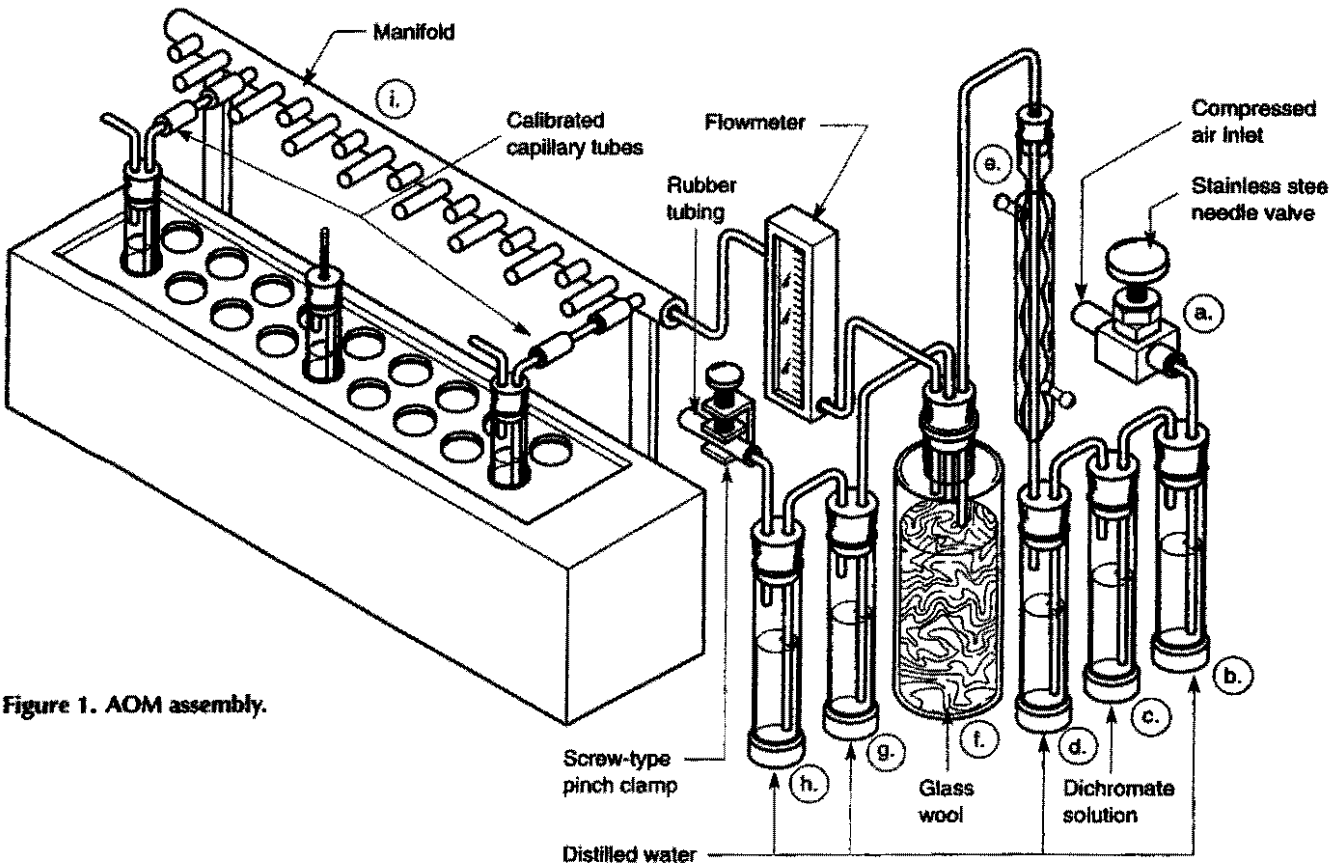


Figure 1. AOM assembly.

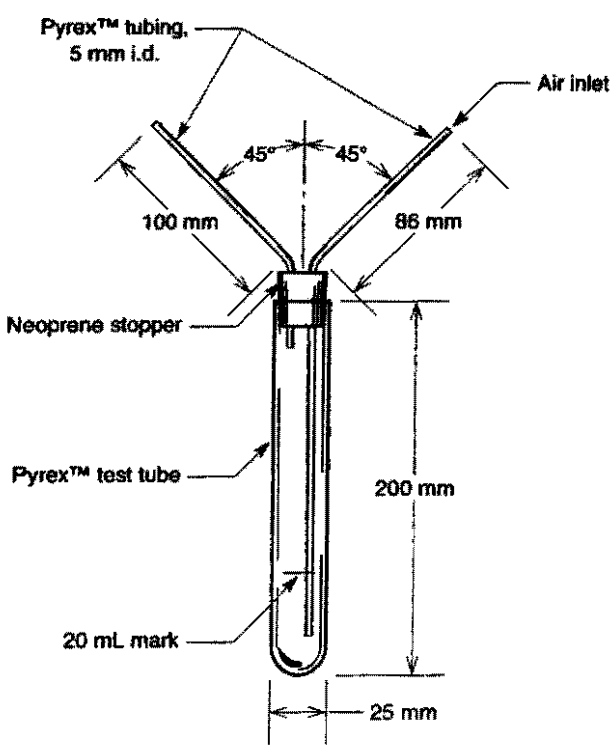


Figure 2. AOM sample tube assembly.

8. Tube-cleaning bath—consisting of a stainless steel pan or sink, heated with steam coils (no copper tubing) or gas and of sufficient area to permit laying the required number of aeration and test tubes on the bottom. Disposable test tubes may also be used. For best accuracy and precision, the disposable test tubes should be cleaned before use.
9. Test tube brush—fan tip, nylon.
10. Tongs—stainless steel or nickel plated, suitable for handling tubes in hot detergent solution.
11. Apparatus as described in AOCS Method Cd 8-53 for determining peroxide value.

REAGENTS

1. Petroleum ether—AOCS Specification H 2-41 (see Notes, *Caution*).
2. Acetone—ACS reagent grade (see Notes, *Caution*).
3. Detergent for cleaning glassware which will leave no contaminating residue. Alconox has been tested and is satisfactory, but equivalent detergents may also be used.
4. Reagents as described in AOCS Method Cd 8-53 for peroxide value. Observe the caution statements cited in Notes of Method Cd 8-53 regarding the reagents used in the peroxide value determination (see Notes, 6).

CLEANING SAMPLE AND AERATION TUBES

1. When recycling test tubes, melt and drain off as much of the fat from previous determinations as possible. Wash off the remaining fat with a suitable solvent. Petroleum ether is satisfactory, if the cleaning is done immediately after the preceding determination and the fat involved is of 100 iodine value or less. Otherwise, acetone must be used.

310

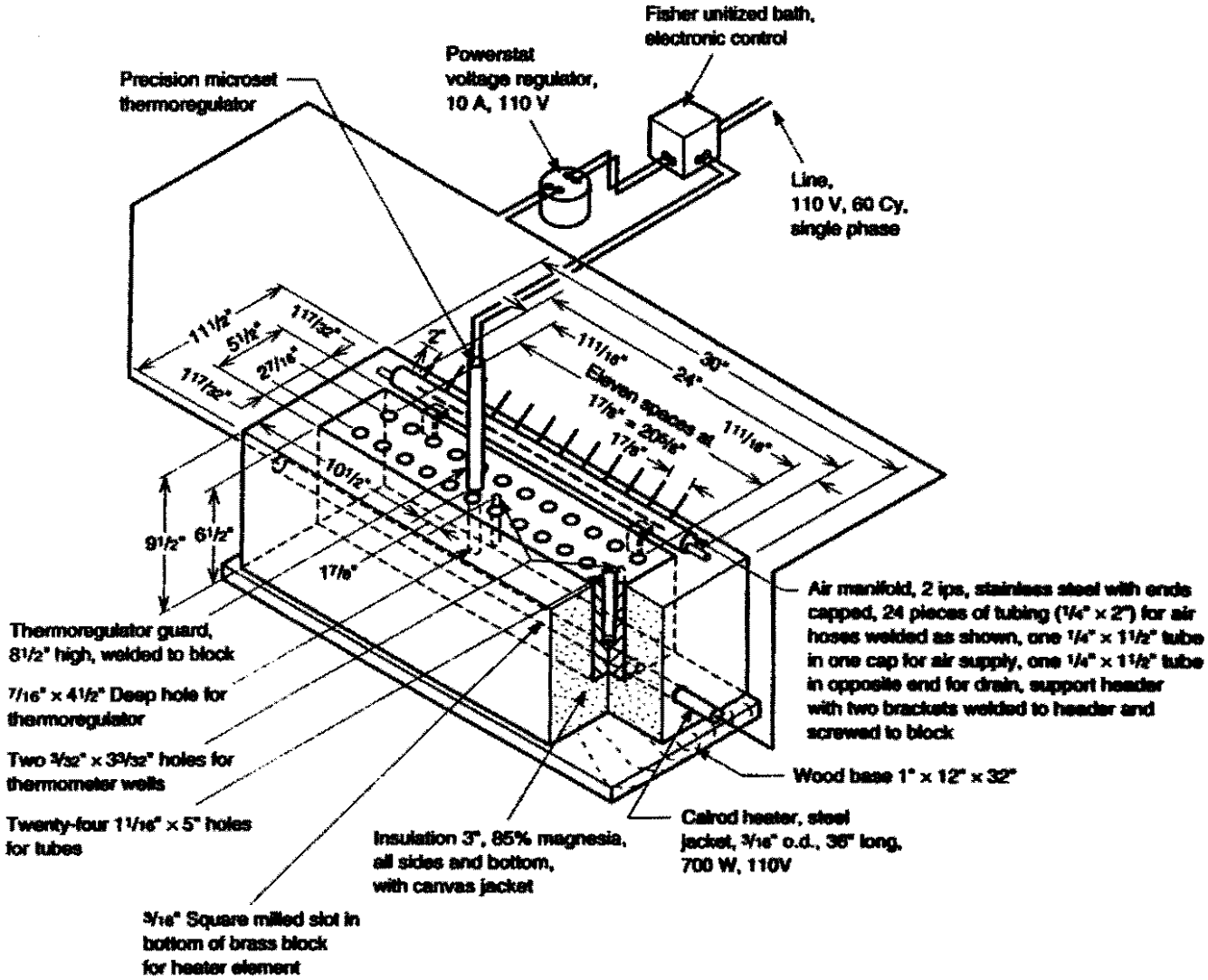


Figure 3. AOM aluminum heating block assembly. Blueprints are available from AOCS headquarters at the prevailing blue print copying charge.

2. Prepare a 1% solution of detergent and heat almost to boiling in the cleaning bath. Rinse each tube with the hot detergent solution, brushing briefly with a nylon brush. Then place the test tubes in the hot detergent solution in such a manner that the tubes are full and completely covered, and so that no air bubbles are trapped within. Place the fat-free aeration tubes with stoppers in the hot detergent bath in such a manner that they are completely covered and so that no air bubbles are trapped within. Boil all test tubes and aeration tubes with stoppers vigorously for 30 min. Brush each test tube vigorously with a nylon brush, rinsing twice in the hot detergent solution. Rinse thoroughly with tap water followed by distilled water and place upright in a test tube rack. Fill with distilled water and soak at least 1 hr. Rinse the aeration tubes thoroughly in tap water followed by distilled water and place upright in a clean 2-L beaker of distilled water in such a manner that the long straight tube and the stopper are covered. Allow to soak for at least 1 hr. At the end of the soaking period, rinse both test tubes and aeration tubes once again with fresh distilled water, drain on clean filter paper and dry in an oven at 100–105°C. Arrange washing and rinsing schedules so that test tubes and aeration tubes are dried at the same time. Assemble as soon as dry and store in a dust-free location.

SAMPLING

1. Due to peculiarities inherent in this procedure, special precautions are necessary in obtaining and transporting samples. Where packaged fats are involved, the sample should consist of an unopened package if possible. Where this is impractical, samples must be removed from large containers or processing equipment with clean sampling devices of stainless steel, aluminum, nickel or glass only.
2. Samples of solid fat should be taken at least 5 cm from the walls of large containers and 2.5 cm from the walls of small containers. If liquid oil is poured from a container, the pouring spout or lip should first be thoroughly cleaned using a clean cloth moistened with acetone.
3. After removal from packages or processing equipment,

samples should be transported or stored only in glass containers cleaned as described in Cleaning Sample and Aeration Tubes. Under no circumstances should sample containers have plastic or enameled tops or covers with paper or waxed liners.

4. Samples should be protected from contact with heat and air as much as possible.

PROCEDURE

1. Unless already completely liquid, the sample should be melted at a temperature no higher than 10°C above its melting point. Pour 20 mL into each of two or more sample tubes (see Notes, 3). Pour carefully into the center of the tube so that none of the fat comes in contact with the top of the tube and subsequently the stopper.
2. Insert the aeration tube assembly and adjust so that the end of the air delivery tube is 5 cm (2 in.) below the surface of the sample.
3. Place the tube and sample in a container of vigorously boiling water for a period of 5 min. At the end of this time, remove the tube from the water, wipe dry and transfer immediately to the constant temperature heater maintained at 97.8 ± 0.2°C. Connect the aeration tube to the capillary on the manifold, having previously adjusted the air flow rate and immediately record the starting time.
4. The figure to be reported as the AOM stability value is the time (to the nearest hour) required for the sample to attain a peroxide value of 100 milliequivalents (meq) (see Notes, 4) and should be the average of two samples. The result should be determined as follows—
   (a) A short time before the end point is reached (see Notes, 5), determine the peroxide value according to AOCS Official Method Cd 8-53 with the exception that the sample weight is 1 g instead of 5 g. If this determination indicates that the peroxide value is between 75 and 175 meq, another peroxide value determination should be made immediately, using a 5-g sample. If the peroxide value so obtained should be above 175 meq, the sample must be discarded and another determination started (see Notes, 5).
   (b) If the pilot determination indicates a peroxide value below 75 meq, estimate when a value of 75 meq will be reached and at that time make another determination on a 5-g sample.
   (c) Make a second determination on a 5-g sample from the same tube exactly 1 hr after the first.
   (d) This should give two peroxide values between 75 and 225 meq. Use rectangular coordinate paper to plot these two values against their respective aeration times in hours. The AOM stability value is the time in hours at which a straight line connecting these two points crosses the 100 meq coordinate. Repeat this procedure on the duplicate sample and report the average of the results.

NOTES

Caution

Petroleum ether and acetone are extremely flammable solvents. Avoid open flames and static electricity. The explosive limits in air for petroleum ether are 1–6%. A fume hood should be used at all times when using these solvents.

NUMBERED NOTES

1. This method was designed for oils and fats normally consumed by humans, including those containing approved antioxidants within established limits of concentration. The procedure up to the determination of the end point (Procedure, 4) may be suitable for evaluating or comparing fats for animal feeds, inedible greases, soapstocks and fats containing new antioxidants. However, there is insufficient data available regarding the peroxide value vs. time curves for such materials to permit establishment of a definite end point.
2. In view of existing baths and heaters available, the AOCS Committee does not feel justified in specifying any one particular type. One that has been found to have certain distinct advantages is an aluminum block, utilizing a thermostatically controlled electric heating unit, described in *JAOCS 33*:628 (1956). The aluminum block, which is pictured in this method (see Fig. 3), is not available commercially and must be hand-crafted. An 8-unit oxidation bath, modified to conform to the AOM test, but without air purification train and air pump, Model Number k12200/Special, is available from: Koehler Instrument Company, Inc., 1595 Sycamore Ave., Bohemia, NY 11716, USA, phone (516) 589-3800, fax (516) 589-3815. An automated unit, using conductivity to determine the end point, is available under the trade name Rancimat from Brinkmann Instruments, Inc.
3. At least two samples must be run for each determination. If the product is an ordinary material so that not more than two 1-g samples will be required to establish the 75–175 meq range, two tubes are sufficient. However, in the case of an unknown or unusual material, three tubes should be used, one to serve as a guide to establish the 75–175 meq range and the other two for making duplicate determinations of the final end point. It is usually advantageous to have the starting time for two or more tubes spaced 1 or 2 hrs apart.
4. With some experience, the approach of the end point can be judged by the odor of the effluent air from the sample tube.
5. It is desirable to heat and aerate samples continuously until the end point is reached. Where this is not practical, remove the tube from the heater, chill immediately and hold below 10°C until ready to start again. The regular procedure should be followed starting at paragraph 3 in Procedure.
6. In 1990, the Uniform Methods Committee adopted as a Recommended Practice Method Cd 8b-90 for determining peroxide value, using isooctane in place of chloroform as specified in AOCS Official Method Cd 8-53. It has been reported that the isooctane method gives erratic results at peroxide values ≥70, so Method Cd 8b-90 should not be used in the AOM test in which peroxide values ≥70 may be encountered.

PRECISION

1. The coefficient of variation, that is, the standard deviation expressed as a percent of the AOM value, is approximately 13.4%. This signifies that a maximum variation between laboratories of ± 25 might be expected on a 100-hour sample, or ± 2.5 on a 10-hour sample.

312

AOCS Official Method Ti 1a-64

Replaces Ka 13-56 • Reapproved 1997

Spectrophotometric Determination of Conjugated Dienoic Acid

DEFINITION

This method determines the diene conjugation of unsaturated linkages present, expressing this value as a percentage of conjugated dienoic acid.

SCOPE

Applicable to dehydrated castor oil, dehydrated castor fatty acids and their methyl or ethyl esters.

APPARATUS

1. Ultra-violet spectrophotometer, covering a spectral range of 220–350 nm with wavelength scale readable to 0.1 nm, equipped with absorption cuvette compartment for holding 1.000, 2.500 and 5.000 cm cuvettes (see Notes, 1).
2. Absorption cuvettes—quartz, matched pairs of length 1.000 ± 0.005 cm, 2.500 ± .005 cm and 5.000 ± 0.005 cm. The cuvettes in a pair when filled with water or isooctane must match within 0.01 absorbency unit. Use 1.000 cm non-demountable type consisting of Pyrex™ glass cuvette body of 22 mm o.d. with centered ground glass stopper, threaded metal caps, polished crystalline quartz windows and cork gaskets.
3. Filter tube—32 in. by 1¾ in., with glass stopcock sealed at one end.
4. Cork stopper—covered with aluminum foil to fit loosely in the top of the filter tube.
5. Dropping bottle—stoppered, for weighing the samples.
6. Volumetric flasks—glass stoppered, having capacities of 100, 50 and 25 mL.
7. Volumetric pipets—precision grade, assorted sizes, for making dilutions when required.

REAGENTS

1. Silica gel—40–60 mesh, Code 11-08, Davison Chemical Co., Baltimore, MD, USA, or equivalent.
2. Glass wool.
3. Isooctane (2,2,4 trimethyl pentane)—Pure or Spetro grades of isooctane are satisfactory and should be used. Check the absorbency of a 1 cm layer of the isooctane against distilled water at 233 nm. The absorbency compared with distilled water set at zero absorbency shall not be more than 0.070 at 233 nm. If the isooctane does not meet these requirements, purify it as follows:

   Place about 3½ in. of glass wool above the lower end of the filter tube. Add about 20 in. of silica gel and about 2 in. of glass wool. Fasten the tube vertically to a ring stand and place a funnel and 2-L amber bottle under the tube. Pour the isooctane slowly into the tube, filling about three-fourths full, and allow the isooctane to filter through the silica gel. Renew the silica gel in the tube as often as necessary to yield isooctane conforming to the transmission limit given above. A constant flow can be maintained by feeding the isooctane from a 500-mL glass-stoppered separation funnel, the tip of which is below the surface of the isooctane in the filter tube. The 2-L amber bottle used for storing the purified isooctane should be rinsed with a little of the first eluate from the column, and the rinse should be discarded. An aluminum-lined screw cap should be used to close the bottle for storage.

PROCEDURE

1. Weigh by difference into a 100-mL volumetric flask, to the nearest 0.1 mg, 90–130 mg of the sample. Add about 75 mL of purified isooctane.
2. Rotate the flask and warm the contents, if necessary, to completely dissolve the sample. Cool to room temperature, and allow to stand at least 15 min to attain temperature equilibrium. Dilute to volume with purified solvent and mix thoroughly.
3. Make the necessary dilutions, in no more than tenfold steps, to give a final concentration of about 0.01 g of the sample per L (see Notes, 2).
4. Measure the absorbency of the solution at 233 nm. Use a matching cuvette containing purified solvent only for the blank cuvette. Take several readings to obtain an average value.
5. The observed absorbency reading should lie between 0.2 and 0.8; otherwise change the weight of sample or the dilution to give the required reading.

CALCULATIONS

Report to three significant figures, see Precision statement below.

$$\text{Conjugated Dienoic Acid, \%} = 0.84\left(\frac{A_s}{bc} - K_o\right)$$

Where—

K_o = absorptivity by acid or ester groups,
= 0.07 for esters
= 0.03 for acids

A_s = observed absorbency at 233 nm

b = cuvette length in cm

c = concentration of sample, g/L of the final dilution used for the absorption measurement

313

PRECISION

1. Repeatability results determined by the same operator and the same apparatus should not differ from the mean by more than ±0.39%.
2. Reproducibility results in different laboratories by different operators should not differ from the mean by more than ±1.26%.

NOTES

1. Suitable apparatus may be obtained from Beckman Division, Beckman Instruments, Inc., 2500 Fullerton Road, Fullerton, CA, USA.
2. When making dilutions, allow sufficient time for solutions to reach room temperature.

AOCS Official Method Cd 22-91
Reapproved 1997

Determination of Polymerized Triglycerides by Gel-Permeation HPLC

DEFINITION

This method is for the determination of polymerized triglyceride content in oils and fats. The separation is based on the relative retention of solubilized polymer molecules in terms of their molecular size by gel-permeation chromatography.

SCOPE

This method is applicable to vegetable and animal fats and oils, heated or not. It has been applied to the determination of polymers in animal feed fats and used frying fats or oils.

APPARATUS

Note—HPLC equipment suitable for gel-permeation chromatography is required, including the following:

1. Solvent reservoir—with a mobile-phase line filter (pore size 1 µm).
2. HPLC pump—pulseless, with a flow of 0.7–1.5 mL/min.
3. Injection port—with low dead volume, applicable with high pressure, provided with a 20-µL sample loop.
4. Syringe—with a volume of 50–100 µL, compatible with injector.
5. Stainless steel column—length 300 mm, 7.8-mm i.d.
6. Stationary phase—consisting of a high-performance spherical gel made of polystyrene–divinylbenzene microporous particles with 5-µm particle size and 10-nm pore size (see Notes, 1)

   Note—The column must be stored in toluene (solvent B) (Reagents, 2).
7. Detector—refractive index detector with minimum sensitivity at full-scale of at least 1.10×10^{-4} refraction index units, capable of being maintained at a temperature a few degrees above ambient temperature. If the detector is not provided with built-in heating, a recirculating water bath with constant temperature may be used.
8. Recorder—suitable to display, and to quantify with accuracy, the peak areas. Suggested specifications:
   - —response time 1.5 sec (time to give a response of 90% with a signal at 100%)
   - —paper at least 20-cm wide
   - —chart speed 0.1–10 mm/min
   - —sensitivity 1–100 mV, adjustable to signal output from the refractive index detector
9. Calculator and/or integrator.
10. Round-bottom flask—250 mL, with suitable heating mantle capable of heating the contents of the flask to 180 ± 2°C.
11. Thermometer—to measure a temperature of 180 ± 1°C.
12. Conical flask—25–50-mL capacity, for sample preparation.

REAGENTS

1. Tetrahydrofuran (solvent A)—analytical reagent grade (see Notes, *Caution*).
2. Toluene (solvent B)—analytical reagent grade (see Notes, *Caution*).
3. Soybean oil—refined.
4. Sodium sulfate—anhydrous.

PROCEDURE

1. Starting up HPLC equipment—
   - (a) It is advisable to read and to follow carefully the manufacturer's recommendations. In choosing the optimal working conditions, take into account the following variables:
     - —choice of column for optimal separation
     - —concentration of the sample
     - —sensitivity of the refractive index detector
     - —constant temperature of the refractive index detector
     - —pump speed
     - —optimal pump performance
     - —straight baseline
     - —time of analysis
   - (b) To condition and stabilize the column before sample analyses are carried out, the mobile phase must be changed stepwise from the storage solvent, toluene (solvent B), to tetrahydrofuran (solvent A). Prepare mixtures of solvent B with increasing amounts of solvent A (e.g., step 1, 25% A in B; step 2, 50% A in B; step 3, 75% A in B; step 4, 100% A). To stabilize the column, pump solvent A for at least 12 hr. The mobile phase must also be changed stepwise when changing back to solvent B for storage of the column.
   - (c) Pump tetrahydrofuran at a rate of 1 mL/min to purge the whole system up to the injection valve.
   - (d) Connect the column to the injection valve and wash it with about 30 mL of tetrahydrofuran.
   - (e) Connect the column to the sample cell of the detector.
   - (f) Fill the reference cell with tetrahydrofuran.
   - (g) Adjust the mobile-phase flow to 0.5–1.0 mL/min.
   - (h) Wait until the system becomes stabilized (no appreciable deviation of the baseline).
2. Preparation of standard oil—With the aid of a heating mantle, heat a 250-mL round-bottom flask containing approximately 185 mL of refined soybean oil to 180 ± 2°C until the amount of polymerized triglycerides is between 10 and 15%, analyzed and calculated as noted in Procedure, step 5, below.
3. Preparation of sample—If the sample is not completely melted at ambient temperature, heat the sample to a temperature of not more than 10°C above the temperature at which it is completely melted. Mix the sample thoroughly.

4. Determination of theoretical plates—Analyze the standard oil (Procedure, 2) according to step 5 in Procedure, in such a way that the peak of the triglycerides is more than half the chart width (refer to Fig. 1). Calculate the theoretical plate number with the formula:

$$n = 16\left(\frac{dR}{w}\right)^2$$

Calculate the resolution with the formula:

$$R = \frac{\Delta}{w}$$

Where—

n = theoretical plate number
R = resolution
dR = retention distance from the start of the chromatogram to the peak maximum for triglycerides, in mm
w = width of the triglycerides peak at the baseline, measured between points of intersection between tangents and baseline, in mm
Δ = the distance between peak maxima of the triglycerides peak and the neighbor peak of the polymerized triglycerides (peaks numbered 3 and 2, respectively, in Fig. 1), in mm

Conditions for the analysis shall be chosen in such a way that the theoretical plate number (n) for the triglycerides is at least 6000 and the resolution (R) as calculated above is at least 1.

5. Analysis—
   (a) Accurately weigh 0.2 ± 0.01 g prepared sample into a conical flask. Add 15 mL tetrahydrofuran (see Notes, 2), swirl and leave until the fat is completely dissolved. The samples must be free from water. Add about 50 mg anhydrous sodium sulfate, shake and wait 2 min. Filter through medium-porosity filter paper.
   (b) Using the syringe, take 50–100 µL of the sample solution. Fill the injection loop with 20 µL of filtered (see Notes, 3) sample solution.
   (c) Inject the sample onto the HPLC column and simultaneously switch on the integrator, or note injection point on chart recorder.
   (d) Elute the sample with a mobile-phase flow rate of 0.5–1 mL/min. At a flow rate of 1 mL/min, the analysis takes about 10 min.

RESULTS

1. The basic chromatographic pattern (e.g., Fig. 1) resulting from this determination shows a main peak, representing monomeric triglycerides (MW about 900), and one or several smaller peaks with a shorter retention time than the triglycerides peak, representing polymerized triglycerides (dimers and oligomers).
2. Three typical chromatograms are presented (Fig. 2). Under suitable conditions, triglycerides and polymerized triglycerides can be separated with good resolution (Fig. 2, A and B), even at low levels of polymerized triglycerides (Fig. 2, A). However, with those fats and oils in which complex degradation phenomena (possibly hydrolysis) may have occurred, the peak pattern preceding the triglyceride peak is less well defined (Fig. 2, C), with corresponding difficulties in calculation.

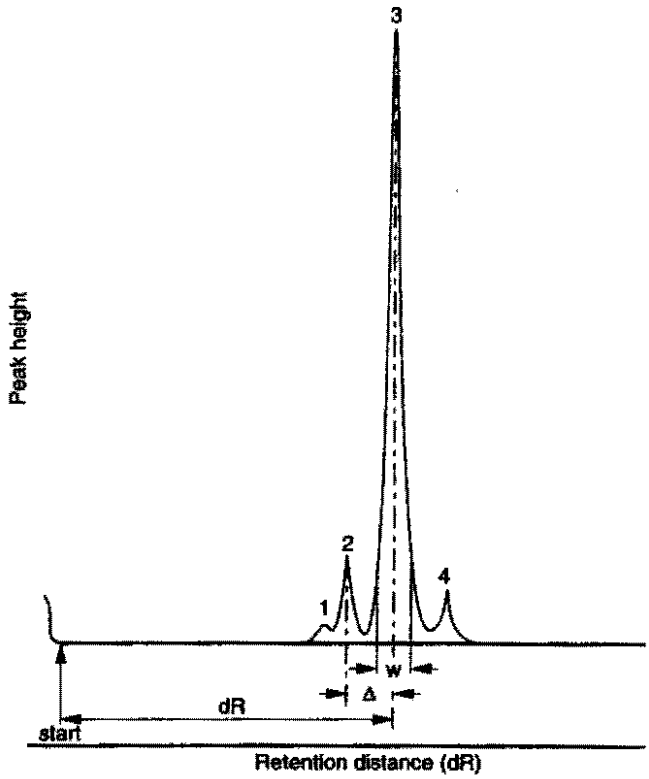


Figure 1. Determination of resolution and theoretical plate number for standard oil (heated soybean oil). 1 and 2, polymerized triglycerides; 3, triglycerides; 4, free fatty acids.

3. Figure 1 is the chromatograph of the standard oil sample (heated soybean oil, prepared as noted in Procedure, 2), used to check the efficiency and the resolution power of the column, which should be at least as good as shown in the reference chromatogram.

CALCULATIONS

1. The area normalization method is used to calculate the percentage of polymerized triglycerides, under the assumption that all components of the sample are eluted.
2. In case there is a valley between the triglycerides peak and the highest peak of the polymerized triglycerides, and the lowest point of that valley is lower than 75% of the height of that highest peak of the polymerized triglycerides, then the amount of polymerized triglycerides has to be calculated using the formula:

$$PT = \frac{A_{PT}}{\Sigma A} \times 100$$

Where—

PT = content of polymerized triglycerides, in % (A/A)
A_{PT} = sum areas of the polymerized triglycerides peaks (these are the peaks with a shorter retention distance than the triglycerides peak)
ΣA = sum of areas of all peaks

Give the result to one decimal place.

316

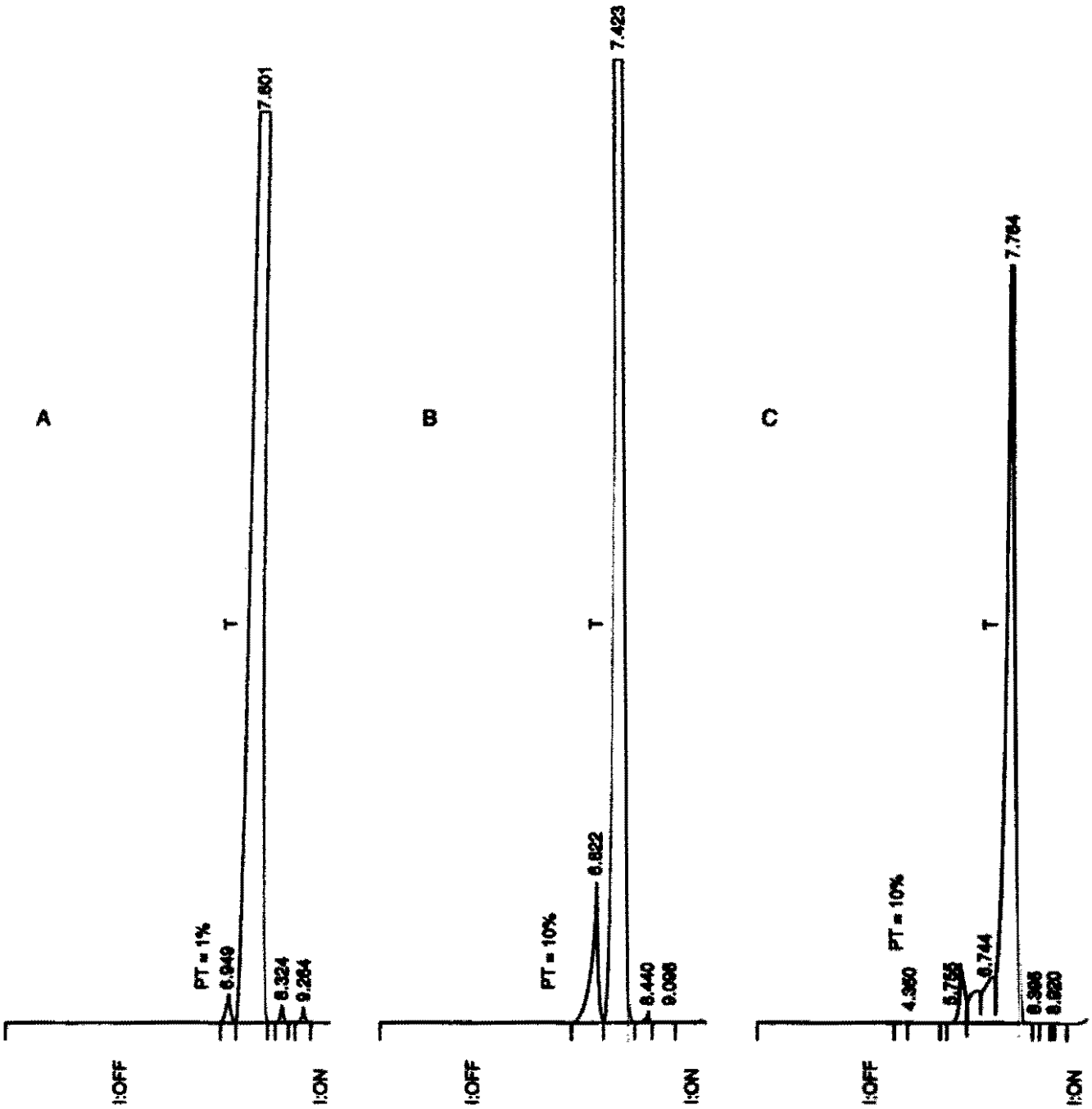


Figure 2. Typical chromatograms. T, triglycerides; PT, polymerized triglyceride; I, injector. Conditions: chart speed, 0.5 cm/min; attenuation, 16; zero, 10%; 5 min/tick.

3. In case there is no valley between the triglycerides peak and the highest peak of the polymerized triglycerides, or the lowest point of that valley is higher than 75% of the height of that highest peak of the polymerized triglycerides, then the amount of the polymerized triglycerides has to be calculated using the formula (see Notes, 4):

$$PT = \frac{A'_{PT}}{\Sigma A} \times 100$$

Where—

PT = content of polymerized triglycerides, in % (A/A)

317

A_{PT} = area above the baseline measured from the start until the retention distance (dR – w/2) (see Procedure, 4)

ΣA = sum of areas of all peaks

Give the result to one decimal place.

4. For calculating A_{PT} two cases are possible:
   (a) Good resolution between peaks (R > 1) (Fig. 2, A and B). The general methods of integration (manual and electronic) can be used to calculate individual and total areas.
   (b) Poor resolution between peaks (R < 1) (Fig. 2, C). It is assumed that all components before (dR – w/2) are polymerized triglycerides (see Fig. 1), where w is the width of the triglycerides peak at the baseline, measured between points of intersection between tangents and baseline, and dR is the retention distance from the start of the chromatogram to peak maximum for triglycerides.

   With a manual technique, it is necessary to determine the triglyceride peak area by triangulation.

   With electronic integration, the integrator has to be carefully adjusted (backward horizontal integration) to integrate all the surfaces included between the curve and the baseline.

PRECISION

1. The statistical analysis of collaborative study results is shown in Table 1.

Table 1
Statistical analysis of collaborative study results.

Sample no.	1	2	3	4
No. of labs	17	17	17	17
No. of assays	34	34	34	34
No. of labs retained[a]	16	15	16	16
No. of assays retained[a]	32	30	32	32
Average value, PT, %	9.7	1.8	5.2	9.7
Repeatability standard deviation (S_r)	0.3	0.1	0.1	0.2
RSD_r, %	3.2	6.2	2.3	2.3
Repeatability value, r ($2.83 \times S_r$)	0.9	0.3	0.3	0.6
Reproducibility standard deviation (S_R)	0.4	0.4	0.3	1.8
RSD_R, %	4.6	23.7	6.71	8.6
Reproducibility value, R ($2.83 \times S_R$)	1.3	1.2	1.0	5.1

[a]Outliers detected and eliminated by the Dixon and Cochran test methods.

NOTES

Caution

Tetrahydrofuran is flammable and a dangerous fire risk. Flammable limits in air are 2–11%. It is toxic by ingestion and inhalation. The TLV in air is 200 ppm. A fume hood should be used at all times when using tetrahydrofuran.

Toluene is flammable and a dangerous fire risk. Explosive limits in air are 1.27–7%. It is toxic by ingestion, inhalation and skin absorption. The TLV is 100 ppm in air. A fume hood should be used at all times when using toluene.

NUMBERED NOTES

1. Suppliers: Phenomenex, Torrance, CA, USA, or Polymer Laboratories, Stretton, Shropshire, United Kingdom.
2. To avoid possible interferences from tetrahydrofuran breakdown products, collect tetrahydrofuran at the end of a chromatographic run, after the tetrahydrofuran breakdown products have eluted from the column, and use this tetrahydrofuran to dissolve the sample.
3. To avoid blockage of the porous fritted filter at the top of the column, when the sample contains suspended particles, filter the sample through 1-µm pore size polytetrafluoroethylene (Teflon™ TFE) or cellulose esters filter (commercial LC disposable syringe filter units are suitable).
4. With an increasing amount of products of hydrolysis in a feed fat or in used frying oils, the peak pattern preceding the triglycerides peak becomes less clear or even disappears. It is, however, important that for these fats (especially feed fats) the amount of polymerized triglycerides can be determined. In Calculations, 3, it is assumed that all components eluting before (dR – w/2) are polymerized triglycerides.

REFERENCES

1. *Nederlandse Norm*, Draft NEN 6348, November, 1986.
2. *Pure Appl. Chem. 63:*1163 (1991).
3. *Standard Methods for the Analysis of Oils, Fats and Derivatives*, International Union of Pure and Applied Chemistry, 7th edn., Blackwell Scientific Publications, 1987, IUPAC Method 2.508.

AOCS Official Method Cc 13d-55
Reapproved 1997

Chlorophyll Pigments

DEFINITION

This method is used to determine mg/kg (ppm) of chlorophyll-related pigments (predominantly pheophytin a) in oils from spectrophotometric absorption measurements at 630, 670 and 710 nm (see Notes, 1).

SCOPE

Applicable to expelled, refined and bleached oils. This method is not applicable to hydrogenated oils, deodorized oils and finished products, because in these processed oils, the absorption maximum does not occur at 670 nm (see Notes, 2 and 3).

APPARATUS

1. Spectrophotometer—Beckman Model B (Beckman Instruments, Fullerton, CA, USA), with red phototube and modified to provide for continuous sensitivity control (See Notes, 4, 5 and 6).
2. Cuvettes—50 mm long, 20 mm wide and 40 mm deep. These cuvettes must be matched and differ from each other by not more than 0.5% transmittance when checked with distilled water at 400 nm. Any suitable matched cuvettes with 50-mm light path may be used. Cuvettes with shorter light path, carefully matched, should be available for oils with absorbencies >0.8 at the wavelengths used.

REAGENTS

1. Methylene chloride (CH_2Cl_2) or methylisobutylketone (MIBK)—reagent grade (see Notes, *Caution*). The transmittance should not differ from that of distilled water by more than 0.5% at 400 nm.

ADJUSTMENT OF SPECTROPHOTOMETER

1. Before using, the spectrophotometer should be checked carefully following the instructions given in the operating manual obtained with the instrument. The wavelength setting of the instrument should be checked with the blue sensitive phototube in the instrument. With the instrument turned on and with no cuvettes in the cuvette compartment, the maximum sensitivity obtainable with the instrument should occur at approximately 540 nm. It is advisable to check the instrument occasionally against a mercury vapor lamp using 579.1, 546.1, 435.8 and 404.7 nm as check points. Any mercury vapor lamp can be focused to pass through the instrument in place of the regular incandescent lamp normally used. Transmission peaks should occur at the specified wavelengths.

PROCEDURE

1. Turn on the spectrophotometer and allow at least a suitable warm-up period before adjusting or making measurements.
2. Place a 50-mm cuvette containing the CH_2Cl_2 or MIBK in the cuvette compartment. Set the instrument to the desired wavelength. With cuvette aperture closed, "zero" the instrument and adjust the dark current to give a reading of zero. With the slit width adjusted to 0.1 mm, open the cuvette aperture and adjust the instrument to 100% transmittance. Repeat this process until the instrument is properly adjusted to a zero reading. Close the cuvette aperture.
3. Adjust the temperature of the oil to 25 ± 2°C. The oil sample must be clear and free of moisture, haze and particulate matter. Fill a second 50-mm cuvette with the oil sample and place in the cuvette compartment of the spectrophotometer.
4. Open the aperture and measure the absorption of the oil sample. Close the cuvette aperture and change the wavelength setting to the next desired wavelength with either CH_2Cl_2 or MIBK in the light path. Adjust the instrument to give a zero reading with the cuvette aperture closed and 100% transmittance with the cuvette aperture open. Place the sample in the light path and measure the absorbance. Make all desired readings following this procedure.
5. All absorbance values should preferably fall between 0.3 and 0.8. Use the maximum cuvette size to give the desired values. On bleached oils, absorbencies of less than 0.3 will be obtained.

CALCULATIONS

1. For Beckman Model B—

$$\text{mg/kg (ppm) } C = \frac{A_{670} - (A_{630} + A_{710})/2}{0.0964 \times L}$$

Where—
C = chlorophyll pigments
A = absorbance
L = cuvette length, cm

NOTES

Caution

Methylene chloride is toxic and a carcinogen. The TLC is 100 ppm in air. A fume hood should be used at all times when using methylene chloride.

NUMBERED NOTES

1. A new method, based on the complete conversion of chlorophyll to pheophytin, is currently under study by the NMR and the Seed and Meal Analysis technical committees of the AOCS.
2. This method is not directly applicable to processed and heated oils, because in such oils part or all of the chlorophyll-related pigments may be converted to products that do not exhibit an absorbance maximum at 670 nm.

3. Several variations of this method have been proposed; they include the following:
   (a) Treatment with acetic acid (to convert any remaining chlorophyll to pheophytin), and determining the total pheophytin content, to yield more accurate results. (The pheophytin content is related to the original chlorophyll content.)
   (b) Individually determine chlorophyll and pheophytin by HPLC, using appropriate chlorophyll and pheophytin standards.
   (c) Determine the actual absorbance maximum of the sample and substitute this value in the equation used in the calculation for mg/kg (ppm) chlorophyll.
   (d) Determine the chlorophyll pigments spectrophotometrically as pheophytin a as follows (References, 1 and 2):

$$C = \frac{345.3 \times (A_{670} - 0.5 \times A_{630} - 0.5 \times A_{710})}{L}$$

Where—
C = concentration of pigments in mg/kg (ppm) oil
A = absorbance at respective wavelengths
L = thickness of spectrophotometer cuvette

4. While the method was written for use with the Beckman Model B spectrophotometer, the Beckman DU, or Cary, any other spectrophotometer allowing measurements in the range of 630–710 nm may be used, following the manufacturer's instructions for balancing the instrument and making absorbance measurements.
   (a) Beckman DU—

$$\text{mg/kg (ppm) } C = \frac{A_{670} - (A_{630} + A_{710})/2}{0.1016 \times L}$$

   (b) Cary—

$$\text{mg/kg (ppm) } C = \frac{A_{670} - (A_{630} + A_{710})/2}{0.1086 \times L}$$

5. The Coleman Jr. spectrophotometer can be used for determining chlorophyll pigments in amounts of approximately 0.1 mg/kg (ppm). Measurements are made in a 25-mm cuvette vs. the solvent at 630 and 670 nm.

$$\text{mg/kg (ppm) } C = \frac{A_{670} - A_{630}}{0.0668}$$

6. Factors may be determined for spectrophotometer models currently available, using chlorophyll standards. However, due to the instability of chlorophyll, if this approach is taken, the analyst is advised to check the composition of the chlorophyll standards by an appropriate method, e.g., HPLC. The true composition of the standards as so determined may then be used to calibrate the spectrophotometer.

REFERENCES

1. *La Revista Italiana delle Sostanze Grasse 67*:521 (1990).
2. IUPAC Commission on Oils and Fats, Working Report, International Union of Pure and Applied Chemistry, July 9, 1990, p. M-10.

OTHER REFERENCES

Fette, Seifen, Anstrichm. 86 (8):304 (1984).
J. Am. Oil Chem. Soc. 62 (1):113 (1985).
Rev. Fr. Corps Gras 32 (6–7):257 (1985).

AOCS Official Method Cc 13b-45

Reapproved 1997

Color

Wesson Method Using Color Glasses Calibrated in Accordance with the AOCS–Tintometer Color Scale

DEFINITION

This method determines color by comparison with glasses of known color characteristics.

SCOPE

Applicable to all normal fats and oils, providing no turbidity is present in the sample. In international trade, the British Standard Lovibond color, AOCS Official Method Cc 13e-92, may be preferred or required.

APPARATUS

1. Colorimeter specifications—The instrument consists of a light-proof box with a dull black interior, illuminated by a 100-watt blue frosted electric lightbulb.

   A block of magnesia is placed in the instrument at the proper angle to reflect the light from the electric bulb vertically upward through the color tube and color glasses. The block must be free from oil and dirt and positioned to give equal illumination to both sides of the viewing field.

   An eye piece finished with a dull black interior is fitted over (outside) the rectangular top of the tube holder so that the light passes through the color tube and color glasses. Eye pieces with split fields are *not* permitted.

   The tube holder (1 in. or 2.54 cm i.d.) is fitted with $1\frac{3}{16}$ in. (30.2 mm) i.d. rings at the bottom. One ring is to retain the color tube containing the oil sample, and the other is to permit an equal amount of light to reach the color glasses.
2. Colorimeters—The following colorimeters have been found to be acceptable for use in this method:
   (a) The Wesson colorimeter, constructed as shown in Figure 1.
   (b) The Stevenson colorimeter conforms to the specifications noted in Apparatus, 1 for the Wesson colorimeter and may be used in this method (see References, 1).
   (c) The Lovibond Tintometer model AF 710 (Notes, 1) conforms to the specifications noted in Apparatus, 1 and may be used in this method when operated according to the manufacturer's instructions. Any subsequent improved model(s), using the same optical principles as the AF 710, may also be used in this method.
3. Color cabinet—The colorimeter is maintained in a booth or cabinet, not less than 40 in. wide × 30 in. deep (102 cm wide × 76 cm deep). The booth or cabinet is closed so that no external light can enter. The inside of the booth is painted a dull, neutral gray of Munsell value 4/.

   The booth or cabinet is indirectly illuminated by means of a bulb and fixture mounted 48 in. (122 cm) above the colorimeter in such a way that no direct light rays strike the colorimeter or the eye of the observer. The level of illumination of the booth (at the top of the box of the colorimeter) should be not less than 1 nor more than 5 foot candles.
4. Color glasses—Glasses must conform to the AOCS–Tintometer Color Scale, within tolerances as follows:

Value of glasses	Tolerance, ±
0.1 to 0.9 red, inclusive	0.05
1.0 to 5.0 red, inclusive	0.10
6.0 to 10.0 red, inclusive	0.20
Above 10.0 red	4% of declared value

   Glasses with valid calibrations to the N° Priest–Gibson Scale conform to the AOCS–Tintometer Color Scale. Glasses are supplied and guaranteed by the Tintometer Company (USA) and Tintometer, Ltd. (UK and Europe), to conform to the AOCS–Tintometer Color Scale. Such glasses are identified by the letter "T" engraved on the glass with the color value. Red glasses of the Lovibond Color Scale Series 200 do not conform.

   Standard-type glasses of the AOCS–Tintometer Color Scale are deposited with Tintometer, Ltd., Salisbury, England and the National Institute of Testing, Washington, D.C., USA. In the case of disputes, arrangements for checking glasses may be made with the manufacturer or the National Institute of Testing.

   The minimum standard set shall consist of the following numbers of red and yellow glasses:

   Red*: 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 7.0, 7.6, 8.0, 9.0, 10.0, 11.0, 12.0, 16.0, 20.0

   Yellow: 1.0, 2.0, 3.0, 5.0, 10.0, 20.0, 35.0, 50.0, 70.0

   *Glasses above 1.0 red need not have the exact value shown, but should cover the same range.

   Note—Keep the color glasses clean and free from any oil film. Handle carefully and protect against scratches. It is especially important that every color glass used be clean at the time of use.
5. Color tubes—
   (a) Description—Color tubes should be made of clear, colorless glass with a smooth, flat, polished bottom and have dimensions as follows: length, 154 mm; overall inside diameter, 19 mm; outside diameter, 22 mm.
   (b) Markings—The color tubes should have two etched marks: one to indicate an oil column of 133.35 mm (5.25 in.), and another to indicate an oil column of 25.4 mm (1 in.).

321

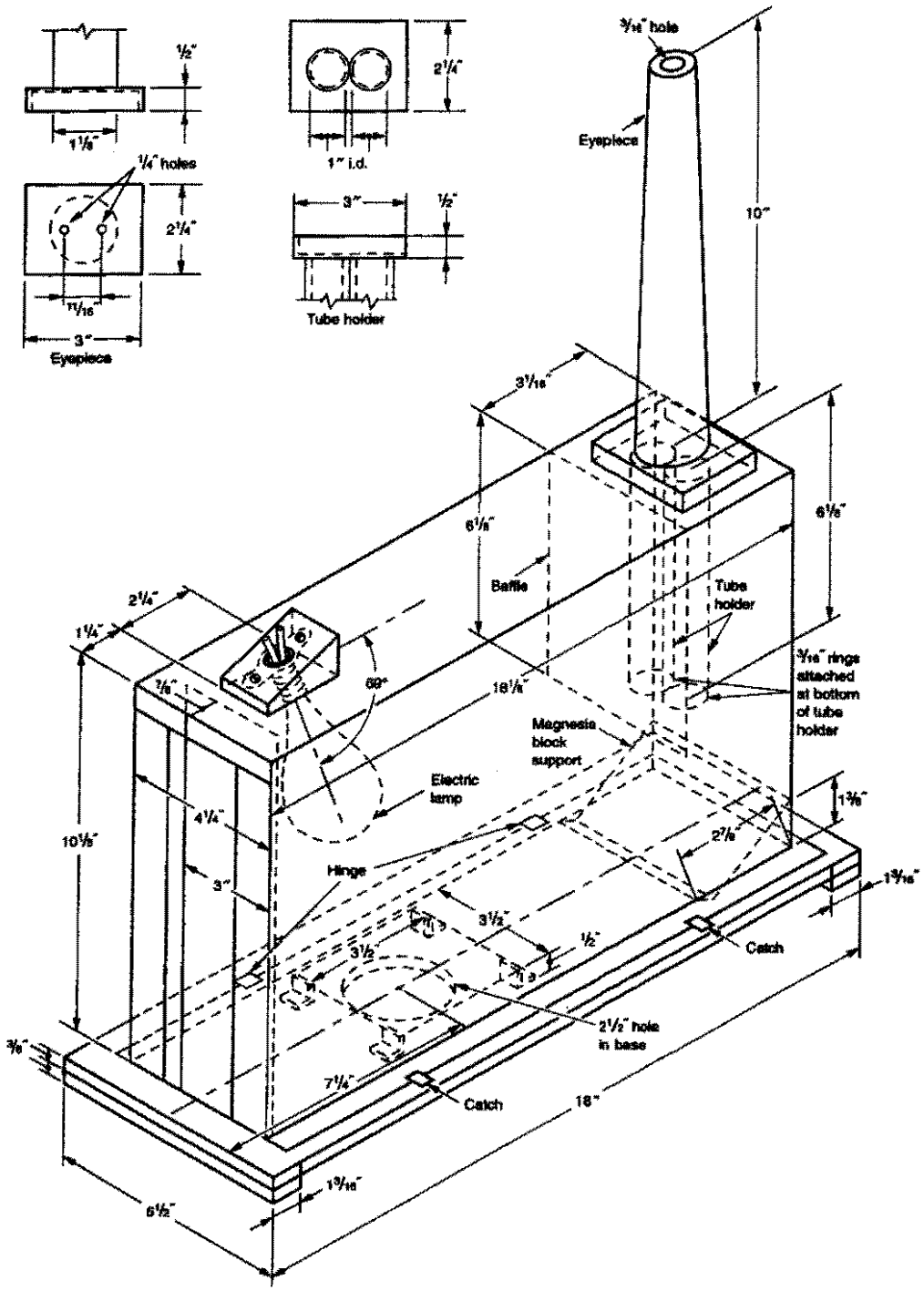


Figure 1. Color measurement apparatus.

(c) Suppliers—In the USA, suppliers of these color tubes include Fisher Scientific and Wilkens–Anderson Co., Chicago, IL.

6. Filter paper—qualitative, medium porosity, to provide medium rate of filtration, 250 mm.

PROCEDURE

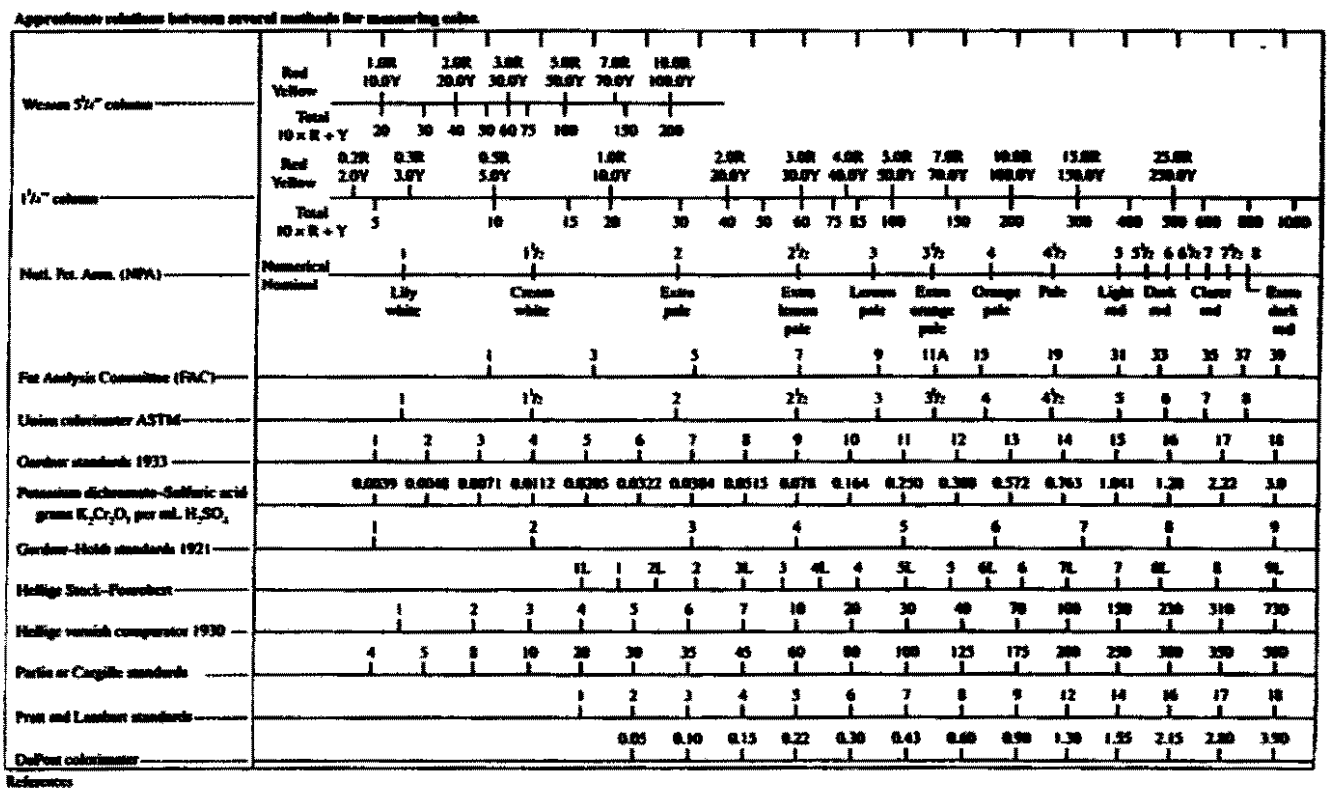
1. Crude, raw and refined oil samples must be treated with 0.5 g of diatomaceous earth (filter aid) per 300 g of oil. Add the diatomaceous earth to the oil and agitate for 2.5 min at 250 rpm at room temperature (or at no more than 10°C above the melting point of the sample, if it is necessary to maintain the sample in the liquid state), and filter the sample through an appropriate filter paper (Apparatus, 6). Oils that have been bleached in the laboratory in accordance with AOCS Official Methods Cc 8a-52, Cc 8b-52 or CC 8d-55 normally are sufficiently clear for the color determination. Suspended material, even if of colloidal size, will cause light scattering. If the sample is not absolutely clear, treat with diatoma-

322

ceous earth (filter aid) as noted, and filter before proceeding with the color determination.

2. Adjust the temperature of the sample to 25–35°C, and fill the color tube to the desired mark. If the sample is not completely liquid at 25–35°C, heat to a temperature of not more than 10°C above the melting point of the sample.
3. Place the tube containing the sample in the colorimeter and place along side of it such red and yellow glasses [see paragraphs (a–e) immediately following and Notes, 2] as are necessary to match the color of the oil, observing the sample of the oil and the glasses through the eyepiece.
   (a) Crude and raw oil color—For crude oils of the coconut type, read the color using proper ratio of yellow to red listed below:
   Up to 3.9 red use 6 yellow to 1 red
   4.0 to 4.9 red use 25 yellow to 1 red
   5.0 to 5.9 red use 30 yellow to 1 red
   6.0 to 6.9 red use 35 yellow to 1 red
   7.0 to 7.9 red use 40 yellow to 1 red
   8.0 to 10.9 red use 50 yellow to 1 red
   11.0 to 14.9 red use 70 yellow to 1 red
   15.0 to 19.9 red use 100 yellow to 1 red
   Above 20.0 red use 150 yellow to 1 red
   If the above ratios fail to give a satisfactory match, this fact should be noted and a second reading made using the amount of yellow color required for a good match. *Report both readings.*
   (b) Raw inedible oils—For raw inedible oils (such as tallows, greases, fatty acids, etc.), use the ratios of yellow to red listed below:
   Up to 3.5 red use 10 yellow to 1 red
   3.5 to 5.0 red use 35 yellow to 1 red
   Above 5.0 red use 70 yellow to 1 red
   (c) Dark oils—If the color of the oil or fat sample exceeds 40.0 red when using the regular 133.35-mm column, fill another tube to the 25.4-mm mark and read the color under the same conditions as described for the longer column. Report the height of the column used, because it is assumed that any color result in which the column height is not designated has been determined on a 133.35-mm column.
   (d) Refined oils—For determining refined oil color, use only one yellow glass; 35 yellow for refined cottonseed and refined peanut oils, 70 yellow for refined soybean oil. Use not more than two red glasses up to and including 13.0 red, and not more than three red glasses above 13.0 red.
   (e) Refined and bleached oils—For refined and bleached oils, the ratios of yellow to red to be used in determining color are as follows, except where trading rules specify the yellow and/or red to be used in classifying particular grades:
   Cottonseed, peanut and corn oils—
   Up to 3.5 red use 10 yellow to 1 red
   Above 3.5 red use 35 yellow to 1 red
   Coconut and palm kernel oils—
   Up to 3.9 red use 6 yellow to 1 red

Approximate relations between several methods for measuring color.



References
1. Mehlenbacher, V. C., *The Analysis of Fat and Oils*, 1960, p. 443.
2. *J. Am. Oil Chem. Soc.* 30:259 (1953).

Above 3.9 red use 10 yellow to 1 red

Soybean oil—

Up to 3.5 red use 10 yellow to 1 red

Above 3.5 red use 70 yellow to 1 red

Tallows, greases, fatty acids, etc.—

Up to 3.5 red use 10 yellow to 1 red

3.5 to 5.0 red use 35 yellow to 1 red

Above 5.0 red use 70 yellow to 1 red

REPORTING

1. When any color result is reported, it is usually assumed that the column height of the oil on which the color of the sample is determined is 133.35 mm (5.25 in.). Therefore, if the color of a sample is determined on a column of oil other than 133.35 mm (5.25 in.), the height of the column *must* be reported with the color value.

NOTES

1. The US supplier of the AOCS–Tintometer apparatus is HF Scientific, Inc., Ft. Myers, FL, 33916-7597.
2. Some oils are at times subject to abnormalities in the composition of their pigment content, leading to off colors (or "hues") in the oils. The hues that occur usually cannot be matched, even approximately, using the fixed yellow or yellow-to-red ratios noted in this method. In such cases, if a standard combination is specified, report whether the oil is lighter or darker than the standard. If a standard combination is not specified, report the red and yellow glasses whose color most closely resembles the color of the sample.

REFERENCES

1. *Oil and Soap 13:*18 (1936).

AOCS Recommended Practice Cc 17-95

Formerly Cc 17-79 • Reapproved 1997

Soap in Oil Titrimetric Method

DEFINITION

The titrimetric method determines the alkalinity of the sample as sodium oleate.

SCOPE

Applicable only to refined vegetable oils (see References, 1).

APPARATUS

1. Test tubes—approximately 150 × 40 mm of borosilicate glass fitted with ground glass stoppers and flattened at the base.
2. Microburet—5 mL.
3. Steam bath—hot water bath may also be used.

REAGENTS

1. Acetone—containing 2% water, prepared by adding 20 mL distilled water to 980 mL of reagent-grade acetone (see Notes, *Caution*).
2. Hydrochloric acid (HCl)—approximately 0.01 N, accurately standardized. See AOCS Specification H 14-52.
3. Bromophenol blue indicator solution—1.0 % in water.
4. Sodium hydroxide (NaOH)—approximately 0.01 N.

PROCEDURE

1. Just prior to the analysis, prepare the test solution by adding 0.5 mL of the bromophenol blue indicator solution (Reagents, 3) to each 100 mL of the aqueous acetone solution (Reagents, 1) and titrating with 0.01 N HCl (Reagents, 2) or 0.01 N NaOH (Reagents, 4) until the test solution is just yellow in color.
2. Weigh 40 g (Notes, 1) of the oil or fat to be tested into a test tube (Apparatus, 1) which has been well rinsed with the test solution (Procedure, 1).
3. Add 1 mL of water to the test sample, warm on a steam bath (or in water bath) and shake vigorously. Add 50 mL of the test solution (Procedure, 1), and after warming, shake the tube well and allow the contents to separate until two distinct layers are formed. *Note*—If soap is present in the oil or fat, the upper layer will be colored green to blue.
4. Slowly add 0.01 N HCl (Reagents, 2) from the microburet until the color just changes from green/blue to yellow. Repeat the warming, shaking and addition of 0.01 N HCl until the yellow color of the upper layer remains permanent. Record the total volume of acid required as mL_s.
5. A blank correction should be determined on soap-free oil, using Procedure, steps 1–4. Record the volume of acid required for the blank as mL_b.

CALCULATIONS

1. ppm soap as sodium oleate =

$$\frac{(mL_s - mL_b) \times N \times 304{,}400}{\text{sample mass, g}}$$

Where—

mL_s = volume, mL HCl obtained in Procedure, 4
mL_b = volume, mL HCl obtained in Procedure, 5
N = normality of HCl

NOTES

Caution

Acetone is highly flammable. Forms explosive peroxides with oxidizing agents. Use effective fume-removal device. Do not mix with chloroform.

Hydrochloric acid is a strong acid and will cause severe burns. Protective clothing should be worn when working with this acid. It is toxic by ingestion and inhalation and a strong irritant to eyes and skin. The use of a properly operating fume hood is recommended. When diluting the acid, always add the acid to the water, never the reverse.

NUMBERED NOTES

1. The method as written is suitable for the determination of soap at concentrations of up to 0.05% in oils and fats. At higher concentrations it is better to analyze 4 g of sample and use 0.01 N HCl.

REFERENCES

1. A study among seven industrial organizations indicated that this method is suitable only for refined oils. *Yukagaku (Japan)* 39:1056 (1990).
2. This method is identical with Codex Alimentarius method CAC/RM 13-1969 and similar to British Standard 648:1958.
3. Wolff, J.P. *Oléagineux*, p. 197 (April, 1948).

AOCS Recommended Practice Cd 1c-85

Reapproved 1997

Calculated Iodine Value

DEFINITION

This method determines the iodine value for edible oils directly from fatty acid compositions (see Notes, 1 and 2).

SCOPE

Applicable to triglycerides and free fatty acids and their hydrogenated products (see Notes, 3).

APPARATUS

1. Gas chromatograph—to provide fatty acid composition by accepted validated methodology, e.g., AOCS Official Method Ce 1-62, AOCS Official Method Ce 1c-89, etc.

PROCEDURE

1. Proceed as directed in the accepted validated method of choice.
2. Determine the fatty acid composition of the oil or fatty acid mixture.

CALCULATIONS (see Notes, 4)

1. Triglycerides, iodine value = (% hexadecenoic acid × 0.950) + (% octadecenoic acid × 0.860) + (% octadecadienoic acid × 1.732) + (% octadecatrienoic acid × 2.616) + (% eicosenoic acid × 0.785) + (% docosenoic acid × 0.723).
2. Free fatty acids, iodine value = (% hexadecenoic acid × 0.9976) + (% octadecenoic acid × 0.8986) + (% octadecadienoic acid × 1.810) + (% octadecatrienoic acid × 2.735) + (% eicosenoic acid × 0.8175) + (% docosenoic acid × 0.7497).

NOTES

1. While this procedure provides an iodine value, it is not intended to be a rapid method, but rather gives two results from one analysis.
2. In 1994, the AOCS Uniform Methods Committee reviewed the factors used in the calculations and concluded that no changes were necessary at that time.
3. For oils with an unsaponifiable content greater than 0.5% (e.g., fish oils), the calculation tends to be low.
4. The calculation tends to be low for materials with low iodine value.
5. Calculated IV based on GC fatty acid composition of non-triglyceride lipid materials such as partial esters of glycerol, partial esters of sorbitol/sorbitan/isosorbide esters, partial esters of polyoxyethylene sorbitol/sorbitan/isosorbide or glycerol, etc. will provide the calculated IV of only the fatty acids used to prepare the partial esters. To obtain the actual IV of partial esters with non-fatty acid polyol diluents, the chlorinated Wijs Reagent IV method should be used. IV values of partial esters via the Wijs method are lower than those obtained by GC because of the dilution effect of the polyol material.

326

AOCS Official Method Cd 1-25
Withdrawn (Historical Interest Only)

Iodine Value of Fats and Oils Wijs Method

DEFINITION
The iodine value is a measure of the unsaturation of fats and oils and is expressed in terms of the number of centigrams of iodine absorbed per gram of sample (% iodine absorbed).

SCOPE
Applicable to all normal fats and oils that do not contain conjugated double bonds (see Notes, 1).
Applicable to all normal fats and oils with samples having iodine values between 0–15.

APPARATUS

1. Glass-stoppered iodine flasks—500 mL.
2. Glass-stoppered volumetric flasks—1000 mL, for preparing standard solutions.
3. Pipet—25 mL, for accurately dispensing 25.0 mL of Wijs solution.
4. Volumetric dispenser—20 mL, 1-mL adjustability, for 10% potassium iodide (KI) solution.
5. Volumetric dispenser—2 mL, 1-mL adjustability, for starch solution.
6. Volumetric dispenser—50 mL, 1-mL adjustability, for distilled water.
7. Repeater pipet—with filling flask, 20 mL, for cyclohexane.
8. Analytical balance—accurate to ±0.0001 g.
9. Magnetic stirrer.
10. Filter paper—Whatman no. 41H, or equivalent.
11. Beakers—50 mL.
12. Hot air oven.
13. Timer.

REAGENTS

1. Wijs solution—see Notes, 2 and *Caution*.
2. Potassium iodide (KI)—reagent grade.
3. Carbon tetrachloride—reagent grade (see Notes, *Caution* and Recommendations). The absence of oxidizable matter in this reagent is verified by shaking 10 mL of the reagent with 1 mL of saturated aqueous potassium dichromate solution and 2 mL of concentrated sulfuric acid; no green coloration should appear.
4. Soluble starch solution—recently prepared, tested for sensitivity (see Notes, 3). Make a paste with 1 g of natural, soluble starch (see Notes, 4) and a small amount of cold distilled water. Add, while stirring, to 100 mL of boiling water.

   Test for sensitivity—Place 5 mL of starch solution in 100 mL of water and add 0.05 mL of *freshly prepared* 0.1 N KI solution and one drop of a 50 ppm chlorine solution made by diluting 1 mL of a commercial 5% sodium hypochlorite (NaOCl) solution to 1000 mL. The deep blue color produced must be discharged by 0.05 mL of 0.1 N sodium thiosulfate.
5. Potassium dichromate—primary standard grade. The potassium dichromate is finely ground and dried to constant weight at about 110°C before using.

   Note—A standard sample of potassium dichromate with a certificate of analysis may be obtained from the National Bureau of Standards in Washington, D.C., USA. This sample, or equivalent, is strongly recommended for the primary standard for this method. Treat as directed in the certificate of analysis accompanying the sample.
6. Sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$)—reagent grade.

SOLUTIONS

1. Potassium iodide (KI) solution—100 g/L (10% solution), prepared by dissolving 100 g of reagent grade KI in 1000 mL of deionized water.
2. Starch indicator solution—prepared and tested as noted in Reagents, 4. Salicylic acid (1.25 g/L) may be added to preserve the indicator. If long storage is required, the solution must be kept in a refrigerator at 4–10°C (40–50°F). Fresh indicator must be prepared when the end point of the titration from blue to colorless fails to be sharp. If stored under refrigeration, the starch solution should be stable for about 2–3 weeks.
3. Sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$) solution—0.1 N (see Notes, 5), accurately standardized vs. potassium dichromate primary standard as follows:
   (a) Sodium thiosulfate solution 0.1 N, prepared by dissolving 24.9 g of sodium thiosulfate in distilled water and diluting to 1 L.
   (b) Weigh 0.16–0.22 g of finely ground and dried potassium dichromate into a 500-mL flask or bottle by difference from a weighing bottle. Dissolve in 25 mL of water, add 5 mL of concentrated hydrochloric acid, 20 mL of potassium iodide solution and rotate to mix. Allow to stand for 5 min, and then add 100 mL of distilled water. Titrate with sodium thiosulfate solution, shaking continuously until the yellow color has almost disappeared. Add 1–2 mL of starch indicator and continue the titration, adding the thiosulfate solution slowly until the blue color just disappears. The strength of the sodium thiosulfate solution is expressed in terms of its normality.

   Normality of $Na_2S_2O_3$ solution =
   20.394 × mass of $K_2Cr_2O_7$ in g/mL of sodium thiosulfate
4. Wijs solution—see Notes, 2 and *Caution*.

Table 1
Sample weights.

Iodine value	Mass of sample 100% excess	Mass of sample 150% excess	Weighing accuracy
	g	g	g
<3	10	10	±0.001
3	10.576	8.4613	0.005
5	6.346	5.0770	0.0005
10	3.1730	2.5384	0.0002
20	1.5865	1.2720	0.0002
40	.7935	.6346	0.0002
60	.5288	.4231	0.0002
80	.3966	.3173	0.0001
100	.3173	.2538	0.0001
120	.2644	.2115	0.0001
140	.2266	.1813	0.0001
160	.1983	.1587	0.0001
180	.1762	.1410	0.0001
200	.1586	.1269	0.0001

PROCEDURE

1. Melt the sample, if it is not already liquid (the temperature during melting should not exceed the melting point of the sample by more than 10°C), and filter through two pieces of filter paper to remove any solid impurities and the last traces of moisture. The filtration may be performed in an air oven at 80–85°C, but should be completed within 5 min ± 30 sec. The sample must be absolutely dry.

   Note—All glassware must be absolutely clean and completely dry.
2. After filtration, allow the filtered sample to achieve a temperature of 68–71 ± 1°C before weighing the sample.
3. Once the sample has achieved a temperature of 68–71 ± 1°C, immediately weigh the sample into a 500-mL iodine flask, using the weights and weighing accuracy noted in Table 1 (see Notes, 6).
4. Add 15 mL of carbon tetrachloride on top of the sample and swirl to ensure that the sample is completely dissolved.
5. Dispense 25 mL of Wijs solution using the pipet (Apparatus, 3) into flask containing the sample, stopper the flask and swirl to ensure an intimate mixture. Immediately set the timer for 30 min.
6. Immediately store the flasks in the dark for the required reaction time at a temperature of 25 ± 5°C.
7. Remove the flasks from storage and add 20 mL of KI solution, followed by 100 mL of distilled water (see Notes, 8 and 9).
8. Titrate with 0.1 N $Na_2S_2O_3$ solution, adding it gradually and with constant and vigorous shaking (see Notes, 10). Continue the titration until the yellow color has almost disappeared. Add 1–2 mL of starch indicator solution and continue the titration until the blue color just disappears.
9. Prepare and conduct at least one blank determination with each group of samples simultaneously and similar in all respects to the samples.

CALCULATIONS

1. The iodine value =

$$\frac{(B - S) \times N \times 12.69}{\text{mass, g of sample}}$$

Where—

B = volume of titrant, mL of blank
S = volume of titrant, mL of sample
N = normality of $Na_2S_2O_3$ solution

NOTES

Caution

Wijs solution causes severe burns, and the vapors can cause lung and eye damage. Use of a fume hood is recommended. Wijs solution without carbon tetrachloride is available commercially.

Carbon tetrachloride is a known carcinogen. It is toxic by ingestion, inhalation and skin absorption. It is a narcotic. It should not be used to extinguish fires. It decomposes to phosgene gas at high temperature. The TLV is 5 ppm in air. A fume hood should be used at all times when using carbon tetrachloride.

Hydrochloric acid is a strong acid and will cause severe burns. Protective clothing should be worn when working with this acid. It is toxic by ingestion and inhalation and is a strong irritant to eyes and skin. The use of a properly operating fume hood is recommended. When diluting the acid, always add the acid to the water, never the reverse.

Chlorine is a poisonous gas. The TLV is 1 ppm in air. It is a strong oxidizing agent and should not be allowed to come in contact with organic materials, hydrogen, powdered metals and reducing agents. A fume hood should be used at all times when using chlorine.

Sulfuric acid is a strong acid and will cause severe burns. Protective clothing should be worn when working with this acid. It is an oxidizing agent and should not be stored in the vicinity of organic materials. Use great caution in mixing with water due to heat evolution that can cause explosive spattering. Always add the acid to water, never the reverse.

Acetic acid in the pure state is moderately toxic by ingestion and inhalation. It is a strong irritant to skin and tissue. The TLV in air is 10 ppm.

Potassium dichromate is toxic by ingestion and inhalation. There is sufficient evidence in humans for the carcinogenicity of chromium [+6], in particular lung cancer. It is a strong oxidizing agent and a dangerous fire risk in contact with organic chemicals.

RECOMMENDATIONS

The most satisfactory replacement found to date for carbon tetrachloride has been cyclohexane + acetic acid, 1:1, v/v (see AOCS Recommended Practice Cd 1b-87). Because of environmental concerns, 1,1,2-trichloro-1,2,2-trifluoroethane (Freon 113) is not recommended. Acetic acid alone and a mixture of cyclohexane + acetic acid (4:1, v/v), respectively, have been shown to be unsatisfactory replacements for carbon tetrachloride.

328

NUMBERED NOTES

1. When the iodine value is determined on materials having conjugated systems, the result is not a measure of total unsaturation, but rather is an empirical value indicative of the amount of unsaturation present. Reproducible results are obtained which afford a comparison of total unsaturation. When the iodine value is required on fatty acids, the preparation and separation are performed as directed in AOCS Official Method Cd 6-38.
2. Because the preparation of the Wijs solution is time-consuming and involves the use of both hazardous and toxic chemicals, this solution may be purchased from a chemical supplier. Solutions are available which contain no carbon tetrachloride, and such solutions should be used. All Wijs solutions are sensitive to temperature, moisture and light. Store in a cool and dark place, and never allow to come to a temperature above 25–30°C. The laboratory preparation of Wijs solution is noted for reference purposes:

   Reagents

   (a) Glacial acetic acid—reagent grade (see Notes, *Caution*). The permanganate test should be applied to ensure that this specification is met.

   Permanganate test—Dilute 2 mL of the acid with 10 mL of distilled water and add 0.1 mL of 0.1 N $KMnO_4$. The pink color must not be entirely discharged within 2 hr.

   Alternate test—The absence of oxidizable matter in the reagent may be verified by shaking 10 mL of the reagent with 1 mL of saturated aqueous potassium dichromate solution and 2 mL of concentrated sulfuric acid; no green coloration should appear.

   (b) Chlorine—99.8% (see Notes, *Caution*). Satisfactory commercial grades are available in cylinders, but the gas must be dried by passing through sulfuric acid (sp. gr. 1.84) before introducing into the iodine solution.

   (c) Iodine—reagent grade.

   (d) Hydrochloric acid (HCl)—reagent grade, concentrated, sp. gr. 1.19 (see Notes, *Caution*).

   (e) Sulfuric acid (H_2SO_4)—reagent grade, concentrated, sp. gr. 1.84 (see Notes, *Caution*).

   (f) Iodine monochloride—reagent grade.

LABORATORY PREPARATION

(a) Dissolve 13.0 g of iodine in 1 L of glacial acetic acid. Gentle heat may be necessary to promote solution. Cool, remove a small quantity (100–200 mL) and set aside in a cool place for future use. Pass dry chlorine gas into the iodine solution until the original titration is not quite double. A characteristic color change takes place in the Wijs solution when the desired amount of chlorine has been added. This may be used to assist in judging the end point. A convenient procedure is to add a small excess of chlorine and immediately bring back to the desired titration by addition of some of the original iodine solution which was taken out at the beginning. The original solution and finished Wijs solution are both titrated with $Na_2S_2O_3$ solution as directed in Procedure, 6 and 7.

(b) The Wijs solution may be prepared from commercial iodine monochloride as follows:

Stock solution—Add 317 ± 0.1 g of iodine monochloride to 1 L of glacial acetic acid and filter through Whatman no. 41H filter paper, or equivalent, into a clean and dry actinic glass bottle. Filter rapidly to prevent contamination with moisture and store in a cool place. Discard the solution if a precipitate forms on standing.

Wijs solution—Using a graduate, pour 117.0 ± 0.1 mL of the stock solution into a standard 5-lb bottle of glacial acetic acid and mix well by shaking.

(c) The I/Cl ratio of the Wijs solution should be within the limits of 1.10 ± 0.1. The procedure for determining the ratio is as follows:

Iodine content—Pour 150 mL of saturated chlorine water into a 500-mL Erlenmeyer flask and add a few glass beads. Pipet 5 mL of the Wijs solution into the flask containing the saturated chlorine water. Shake, and heat to boiling. Boil briskly for 10 min, cool and add 30 mL of 2% sulfuric acid and 15 mL of 15% potassium iodide solution. Mix well and titrate immediately with 0.1 N sodium thiosulfate solution to a starch end point.

Total halogen content—Pour 150 mL of recently boiled distilled water into a clean, dry 500-mL Erlenmeyer flask. Add 15 mL of 15% potassium iodide solution. Pipet 20 mL of Wijs solution into the flask and mix well. Titrate with 0.1 N sodium thiosulfate solution to a starch end point.

Calculation of halogen ratio—

$$\text{Halogen ratio } R = \frac{2A}{3B - 2A}$$

Where—

A = volume of titrant, mL of iodine content as mL sodium thiosulfate

B = volume of titrant, mL of total halogen content as mL sodium thiosulfate

3. 1% starch solution may be purchased from a chemical supplier.
4. "Potato Starch for Iodometry" is recommended, because this starch produces a deep blue color in the presence of the iodonium ion. "Soluble Starch" is not recommended because a consistent deep blue color may not be developed when some soluble starches interact with the iodonium ion. The following are suitable starches: Soluble Starch for Iodometry, Fisher S516-100; Soluble Potato Starch, Sigma S-2630; Soluble Potato Starch for Iodometry, J.T. Baker 4006-04.
5. The sodium thiosulfate solution may be purchased from a chemical supplier. However, it still must be accurately standardized in the laboratory.
6. The weight of the sample must be such that there will be an excess of Wijs solution of 50–60% of the amount added; i.e., 100–150% of the amount absorbed. In the case of tung oil, weigh 0.1–0.2 g of sample, using an excess of 55 ± 3% Wijs solution. In the case of dehy-

drated castor oils and dehydrated castor oil fatty acids, weigh 0.11–0.13 g.

7. The indicated reaction times are those specified in the International Union of Pure and Applied Chemistry (IUPAC) Iodine Value Method 2.205 (References, 1) and were the reaction times used in the IUPAC/ISO validation study of the cyclohexane + acetic acid method (AOCS Official Method Cd 1d-92). Previous AOCS versions of iodine value methods specified a reaction time of 0.5 hr, regardless of the iodine value, but noted that "a longer reaction time may be necessary for oils with high iodine value." The longer reaction times appear to be particularly critical when cyclohexane is used as a replacement for carbon tetrachloride.
8. If the reaction is not terminated within 3 min after the reaction time, the sample must be discarded.
9. The sample must be titrated within 30 min of reaction termination, after which the analysis is invalid.
10. Mechanical stirring is recommended for agitation during the addition of thiosulfate.

REFERENCES

1. *Standard Methods for the Analysis of Oils, Fats and Derivatives*, International Union of Pure and Applied Chemistry, 7th edn., Blackwell Scientific Publications, 1987, IUPAC Method 2.205.

AOCS Official Method Cd 16-81
Reapproved 1997

Solid Fat Content (SFC) by Nuclear Magnetic Resonance Spectrometry (NMR)

DEFINITION

This method determines the quantity of solid triglycerides in an oil or fat, at the temperatures at which measurements are made (in the US: 10.0°C, 21.1°C, 26.7°C, 33.3°C, 37.8°C and 40°C; in Europe: 10°C, 20°C, 25°C, 30°C, 35°C and 40°C). Only the pulsed NMR signal of the liquid fat is measured. The total liquid content is obtained by melting and measuring the sample at 60°C. The pulsed NMR signal at lower temperatures of the partially crystallized oil or fat is proportional to the total liquid measured at 60°C. An olive oil liquid reference sample is measured at all temperatures to compensate for instrument and sample differences.

SCOPE

Applicable to margarine oils, plasticized shortenings and non-cocoa butter-type confectionery fats with a solid fat content of 95% or less at 10.0°C. This method is dependent upon the tempering history of the sample before measurement and departures from the procedure will cause variations in results. Poor reproducibility can be expected when applied to unhydrogenated palm oils due to different tempering conditions for these types of samples (see Notes, 1). Furthermore, the values obtained by NMR (SFC) are not directly comparable to those by dilatometry (AOCS Official Method Cd 10-57). AOCS Official Method Cd 16b-93 is the more recent version of the NMR method for determining solid fat content (see Notes, 1).

APPARATUS

1. Praxis Pulsed NMR SFC 900 Solid Fat Analyzer—available from The Praxis Corporation, San Antonio, Texas, 78251, USA, or equivalent.
2. Sample tubes—test tubes, culture, disposable, Pyrex™ or Kimax glass, 10 mm o.d. × 75 mm length without rims, with i.d. of 0.380 ± 0.005 inches (9.65 ± 0.13 mm).
3. Oven—maintained at 70 ± 2°C.
4. Corks—size 0.
5. Tissues—for wiping sample tubes.

REAGENTS

1. Olive oil reference liquids having the following compositional analysis (see Notes, 2)—

	Spanish	Italian
Iodine value:	84–87	85–88
Saponification value:	189–195	192–195
Fatty acid composition		
C16	9.5 ± 1.0	14.0 ± 1.5
C18	3.5 ± 0.5	2.5 ± 0.5
C18:1	76 ± 2.0	65.0 ± 3.0
C18:2	7.0 ± 1.5	14.0 ± 1.5
C18:3	1.0 ± 0.2	1.0 ± 0.2

PROCEDURE

1. Filling the sample tube—
   (a) Heat the sample in the 70°C oven until liquid and mix well.
   (b) Fill the sample tube with the melted sample to approximately 15 mm from the top.
   (c) Place cork in top of sample tube.
   (d) Wipe sample tube with tissue making sure outside of tube is clean.
2. Tempering of the sample and pulsed NMR measurements—
   (a) Insert sample tubes containing all samples to be measured and the reference olive oil sample into the sample tempering ports of the 60°C probe.
   (b) Equilibrate all samples and reference oil for 30 min.
   (c) Set the instrument conditions as follows—

Auto/manual switch	Auto
Probe selector	6
FID/temp switch	FID
Response	Fast
Variable delay	100 × 1
Clock	2 × 1.0
Function	90°
Program counter	8
Gain and instrument	Refer to operator's manual
Background	Refer to operator's manual
Probe temperatures	Refer to operator's manual

   (d) Insert the olive oil reference sample into the analysis port and measure the NMR reading (see Notes, 3).
   (e) Insert each sample into the analysis port and measure the NMR reading of each sample.
   (f) Transfer the reference oil and samples to the 26.7°C probe and equilibrate for exactly 15 min.
   (g) Transfer the reference oil and samples to the 0°C probe and equilibrate for exactly 15 min.
   (h) Transfer the reference oil and samples to the 26.7°C probe and temper for exactly 30 min.
   (i) Transfer the reference oil and samples to the 0°C probe and chill for exactly 15 min.
   (j) Transfer the reference oil and samples to the 10°C probe and equilibrate for exactly 30 min.
   (k) Set clock to 1 × 1.0 and Probe Selector to 1.

(l) Measure the NMR reading of the reference oil and samples.
(m) Transfer the reference oil and samples to the 21.1°C probe and equilibrate for exactly 30 min.
(n) Set Probe Selector to 2.
(o) Measure the NMR reading of the reference oil and the samples.
(p) Transfer the reference oil and samples to the 26.7°C probe and equilibrate for exactly 30 min.
(q) Set Probe Selector to 3.
(r) Measure the NMR reading of the reference oil and the samples.
(s) Transfer the reference oil and samples to the 33.3°C (or to 40.6°C) probe and equilibrate for exactly 30 min.
(t) Set Probe Selector to 4.
(u) Measure the NMR reading of the reference oil and samples.
(v) Transfer the reference oil and samples to the 37.8°C (or to 40.6°C) probe and equilibrate for exactly 30 min.
(w) Set Probe Selector to 5.
(x) Measure the NMR reading of the reference oil and samples.

CALCULATIONS

1. Solid fat content (SFC) at temperature T°C =

$$100 - \frac{\text{reference oil at 60°C}}{\text{sample at 60°C}} \times \frac{\text{sample at T°C}}{\text{reference oil at T°C}} \times 100$$

Example—

NMR Readings:

Reference oil at 60°C	105.6
Sample at 60°C	105.4
Reference oil at T°C (10.0)	98.8
Sample at T°C (10.0)	80.2

$$\% \text{ Solid fat} = 100 - \frac{105.6}{105.4} \times \frac{80.2}{98.8} \times 100 = 18.67\%$$

PRECISION

Data from the AOCS collaborative study which validated this method show that the following reproducibility can be expected—

1. Within and between laboratories, separate determinations of margarine-type oils should be plus or minus one standard deviation for temperatures listed:

Temp.	10.0°C	21.1°C	26.7°C	33.3°C	37.8°C
SD	0.6	0.5	0.6	0.6	0.4

2. Within and between laboratories, separate determinations of plasticized shortening-type oils should be plus or minus one standard deviation for temperatures listed:

Temp.	10.0°C	21.1°C	26.7°C	33.3°C	37.8°C
SD	0.7	0.7	0.6	0.5	0.4

3. Within and between laboratories, separate determination of non-cocoa butter-type confectionery fats should be plus or minus one standard deviation for temperatures listed:

Temp.	10.0°C	21.1°C	26.7°C	33.3°C	37.8°C
SD	0.6	1.0	1.1	0.5	0.4

4. Within and between laboratories, separate determinations for unhydrogenated palm-type oils should be plus or minus one standard deviation for temperatures listed (see Notes, 4):

Temp.	10.0°C	21.1°C	26.7°C	33.3°C	37.8°C
SD	1.0	0.8	0.6	0.5	0.4

5. Within and between laboratories, separate determinations of tristearin in olive oil standards should be plus or minus one standard deviation at 10°C for the percent tristearin in olive oils listed (see Notes, 5):

Temp.	10%	25%	50%	75%	90%
SD	0.2	0.2	0.4	0.2	0.1

NOTES

1. The basic procedure is applicable at temperatures and times other than those specified and the Committee recognizes that sometimes such deviations are necessary. In 1989, an international collaborative study effort by the AOCS NMR Technical Committee was begun to establish harmonized tempering conditions for existing NMR instruments and for other types of oils and fats. The AOCS NMR Technical Committee completed the study in 1993 and published the results as AOCS Official Method Cd 16b-93. The second phase of the study will include the application of the method to cocoa butter and cocoa butter substitutes.
2. The basic procedure described is dependent upon the chemical stability of the olive oil reference sample. Excessive heating or abuse can cause oxidation resulting in the formation of solids and lower pulsed NMR readings. The Committee recommends replacement of the reference sample oil every 3 mon with fresh olive oil which has been kept under refrigeration. While it is not the Committee's place or intent to recommend a specific oil, it is noted that the collaborative study was conducted using a high grade Lucca Olive Oil from Italy.
3. Push retest button one time before making NMR reading on first sample tested in each probe, as recommended by the instrument manufacturer.
4. The basic procedure described is applicable to unhydrogenated palm oils and blends containing unhydrogenated palm oils. The Committee recognized that other palm oil-type samples and/or blends do produce poor reproducibility and further work is planned in this direction to establish tempering conditions prior to measurement.
5. These values of reproducibility are an indication of the precision obtainable by this method of measurement when polymorphic stability of the sample is not a factor. The tristearin in olive oil mixtures are also used as reference samples for the calibration of the instrument. These mixtures are melted and liquid readings taken at 70°C before being tempered and measured by this method. The SFC values measured agree well with the percentage of solids by weight. A single fifth order polynomial regression equation is recommended for solids contents of 95% or less. Polynomial coefficients, correlation coefficients and interpolation tables for conversion of calculated solids to calibration-corrected solids can be obtained from the manufacturer for each instrument. The calibration procedure is also provided by the manufacturer.

AOCS Official Method Cd 10-57
Reapproved 1997

Solid Fat Index Dilatometric Method

DEFINITION

The solid fat index (SFI) is an empirical measure of the solid fat content of the sample. It is calculated from the specific volumes associated with combined liquid and solid phases at specified temperatures, utilizing a dilatometric scale graduated in units of mL × 1000. Results are therefore expressed as melting dilation in mL/kg of sample.

SCOPE

Applicable to margarine oils, shortenings and other fats with an SFI of 50 or less at 10°C. The method is empirical, and departures from the procedure as written may cause variations in results. It is essential that the temperatures utilized in the test procedure be reported with the results of the test.

APPARATUS

1. Pyrex™ dilatometers—constructed according to the specifications given in Figure 1. The stem should be made from precision bore capillary tubing graduated in 0.005-mL increments from 0 to 1.400 mL with an overall accuracy of at least ± 0.005 mL. The scale should be marked 0 to 1400 in intervals of 50. The dilatometers should have identification numbers on the stems and stoppers (see Notes, 1).
2. Polyacetal clamp—to hold the dilatometer stopper in place.
3. Thermometer clamps—for holding the dilatometers in the constant temperature baths.
4. Constant-temperature water baths—accurate to ± 0.05°C, equipped with a means for adequate circulation. Solid fat indices are normally determined at 10, 21.1, 26.7, 33.3 and 37.8°C for the characterization of margarine oils and shortenings. For special cases, higher temperatures may also be used. Therefore, the water baths required should be maintained at 10, 21.1, 26.7, 33.3, 37.8 and 60°C, and at higher temperatures if necessary (see Notes, 2 and 3).
5. Vacuum pump—capable of reducing the pressure to 2 mm Hg or less.
6. Capillary 2-way stopcock—2 mm i.d., with buret tip, made of Pyrex™.
7. Pyseal cement—Fisher Scientific, or equivalent.

REAGENTS

1. Potassium dichromate indicator solution—1% in distilled water (see Notes, 4 and Caution).
2. Grease—suitable for use at high vacuum, silicone type.
3. Petroleum ether—AOCS Specification H 2-41 (see Notes, Caution).
4. Distilled mercury—see Notes, 5.

CALIBRATION OF DILATOMETER

Note—All new dilatometers should be checked for accuracy (see Notes, 5).

1. Thoroughly clean and dry the dilatometer.
2. Securely clamp the dilatometer in an inverted position.

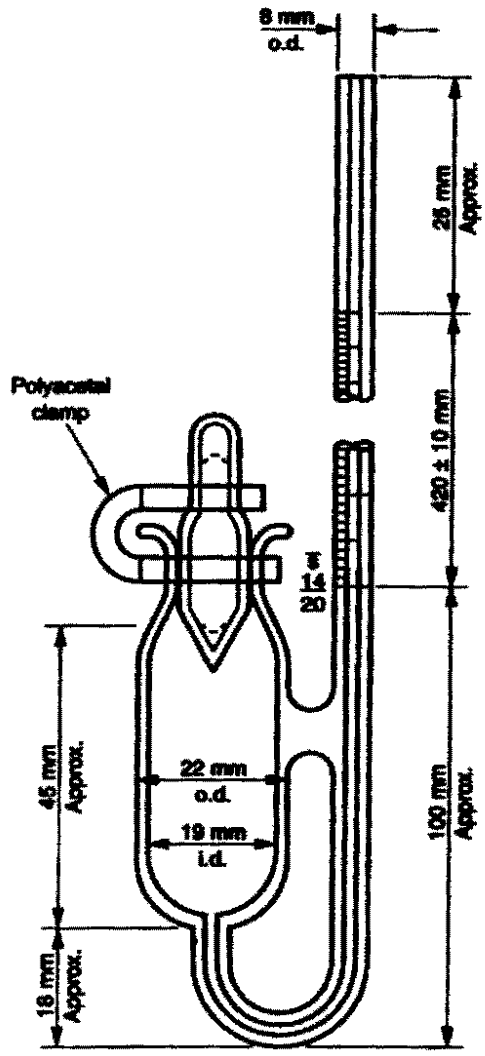


Figure 1. Sketch of dilatometer tube, showing dimensions (see Notes, 1).

333

3. Clamp the capillary stopcock (Apparatus, 6) in place at the end of the dilatometer stem, and make a seal with the Pyseal cement (Apparatus, 7).
4. After the cement has hardened, immerse the tip of the stopcock into a reservoir of clean, distilled mercury which is at room temperature.
5. Using a vacuum, draw the mercury into the dilatometer stem until the calibrated portion is full.
6. Successively withdraw 0.200-mL portions of the mercury into a tared 50-mL beaker and record the weights.
7. Calculate the true volume (in mL) contained in each measured scale interval as follows (see Notes, 4):

$$\frac{\text{mass of mercury, g}}{\text{Final} - \text{initial scale reading}} \times (\text{specific volume of mercury at } T_R) \times 1000$$

Where—

T_R = measured ambient temperature at time of calibration

1 mL = 1000 on dilatometer scale

PROCEDURE

1. Filling the dilatometer—
   (a) Deaerate about 50 mL of the indicator solution for 3 min in a 250-mL filter flask, or other suitable strong-walled container, at a pressure slightly above the vapor pressure of the solution at the temperature of deaeration (the vapor pressure of water at 25°C is 24 mm of Hg). The indicator may also be deaerated by vigorously boiling for 15 min at atmospheric pressure, but should be cooled to room temperature before use.
   (b) Heat the sample to 80°C and deaerate in a 250-mL filter flask, or other suitable strong-walled container, at a pressure of 2 mm of Hg until no more gas bubbles are seen (for at least 2 min). The sample must be maintained in a liquid state and agitated vigorously during deaeration.
   Note—The indicator and sample should be used as soon as possible after they have been deaerated. The sample must be completely melted. Even slight crystallization occludes air.
   (c) Pipet 2 mL of the indicator solution into the dilatometer bulb. Lubricate the stopper lightly with silicone grease and weigh the assembled dilatometer to the nearest 0.01 g on a suitable balance.
   (d) Carefully overlay the indicator with the sample and fill until the sample overflows. Insert the stopper so that the indicator solution rises to approximately the 1200 mark of the stem when the stopper is securely sealed. The reading should be 1200 ± 100 at 60°C; if not, the determination must be started from the beginning.
   (e) Wash any sample from the outer surface of the dilatometer with petroleum ether. Attach the retaining clamp to the stopper and reweigh the dilatometer when the solvent has been completely removed.
2. Measurement of thermal expansion—
   (a) Immerse the dilatometer to the 300 mark in the 60°C bath and record the reading after 15 min and then at 5-min intervals until the change is less than 2 units in 5 min. Rechecks of the 60°C reading at the end of the determination should agree with the 60°C reference reading. Significant variations indicate faulty technique.
   (b) Transfer the dilatometer to the 37.8°C bath and immerse to the 300 mark. Read the level of the indicator solution at intervals of 5 min until the change is less than 2 units in 5 min. Record the readings.
   Note—It is necessary for the sample to be completely melted at the lower temperature. If any seeding or clouding occurs, remelt the sample in a 60°C bath and raise the temperature of the other bath. If the reference bath temperatures are changed, appropriate changes must be made in the calculations.
3. Conditioning of the sample—
   (a) Transfer the dilatometer to the 0°C bath, immerse to the 300 mark and hold for 15 min.
   (b) Transfer to a 26.7°C bath and hold for 30 min.
   (c) Transfer back to the 0°C bath and hold for 15 min.
   Note—If an ice bath is used at 0°C, provisions should be made for adequate water circulation.
4. Measurement of dilation—
   (a) Transfer the dilatometer from the 0°C bath to a bath at the lowest desired temperature. Immerse to the 300 mark, and record the reading at the end of exactly 30 min.
   (b) At the end of the 30-min period, transfer the dilatometer to the next-highest temperature bath for 30 min and repeat this process until readings have been obtained at all of the desired temperatures (see Notes, 3).

CALCULATIONS

1. Solid fat index (SFI) at temperature T =
   (total dilation) − [(thermal expansion) × (60 − T)]

Where—

Total dilation between T and 60°C, mL/kg =

$$\frac{R(60) - R(T) - V_c(T)}{W}$$

Thermal expansion of sample per °C, mL/kg =

$$\frac{R(60) - R(37.8) - V_c(37.8)}{W \times (60 - 37.8)}$$

T = observed temperature

$V_c(T)$ = volume correction for expansion of Pyrex™ and water at temperature T (see Table 1 and Notes, 6 and 7).

R(T) = dilatometer reading at T

W = mass, g of sample

PRECISION

Data from the Edible Fat Series in the Smalley Check Sample Program have shown that the following reproducibility can be expected—

1. Within or between laboratories, separate determinations of margarine-type oils should be ± the standard deviations for the temperatures listed:

% Solids variation				
50°F	70°F	80°F	92°F	100°F
± 0.64	± 0.49	± 0.38	± 0.32	± 0.25

2. Within or between laboratories, separate determinations of shortening-type oils should be ± the standard deviations for the temperatures listed:

% Solids variation				
50°F	70°F	80°F	92°F	100°F
± 0.87	± 0.57	± 0.69	± 0.46	± 0.69

NOTES

Caution

Petroleum ether is extremely flammable. Avoid static electricity. Use effective fume-removal device.

Potassium dichromate is toxic by ingestion and inhalation. There is sufficient evidence in humans for the carcinogenicity of chromium [+6], in particular lung cancer. It is a strong oxidizing agent and a dangerous fire risk in contact with organic chemicals (see Notes, 4).

NUMBERED NOTES

1. The supplier of the dilatometers is Kimble Kontes, Vineland, NJ, USA.
2. The basic procedure is applicable at temperatures other than those specified, and the AOCS Technical Committee recognizes that sometimes deviations from the procedure as noted are necessary. The necessary deviations depend on the composition and character of the sample. It is hoped, however, that within limits, a uniform temperature range will become established in the fats and oils industry for the particular type of sample being tested.

As noted in Apparatus 4, specifications suggest bath temperatures be set and SFI values calculated from the following temperatures:

°C	°F
10	50
21.1	70
26.7	80
33.3	92
37.8	100
40	104

When using the equation (Calculations, 1) for calculating SFI $V_C(T)$ values, volume correction factors are to be taken from Table 1. These values are given in five degree C increments from 0°C to 60°C. Unless these values are extrapolated to fit the actual bath temperature, it is conceivable that small errors will exist, making it harder to compare data.

Table 1
Volume corrections for Pyrex™ brand glass and water.

Bath temp, °C	60°C Reading 1000	1100	1200	1300	1400
0	22.0	20.3	18.6	16.9	15.2
5	22.2	20.5	18.7	17.0	15.3
10	21.8	20.1	18.4	16.7	15.1
15	21.0	19.5	17.8	16.2	14.6
20	19.8	18.4	16.8	15.3	13.8
25	18.4	17.0	15.6	14.1	12.7
30	16.6	15.3	14.0	12.7	11.4
35	14.4	13.3	12.2	11.1	10.0
40	12.0	11.0	10.2	9.2	8.3
45	9.4	8.7	8.0	7.2	6.5
50	6.6	6.1	5.6	5.1	4.5
55	3.2	3.0	2.8	2.5	2.3
60	0	0	0	0	0

It is recommended that the bath temperatures for SFI (and SFC) should be as follows:

°C	°F
10	50
20	68
25	77
30	86
40	104

3. Several laboratories have reported difficulty in determining the SFI value at 10.0°C for fats having a SFI greater than 50 at 10.0°C. No official action has been taken on these observations and recommendations. One finding reported to reduce the magnitude of the problem consists of removing the dilatometer from the 50.0°C reference bath and placing it, sequentially, in the 26.7, 21.1 and 10.0°C baths for 15 min at each temperature, transferring to the 0°C bath and then proceeding with the tempering as usual. Another finding consists of taking the reading at 10.0°C at the official time of 30 min, but then every 5 or 10 min thereafter until a reading is the same as, or maximum only one unit higher than, the previous reading.
4. Due to the carcinogenicity of chromium[VI] compounds, the following indicator solution has been proposed as a replacement: Using water-soluble food dyes, mix FD & C Blue #1 with FD & C Yellow #5 in equal proportions to form a green dye. To 200 mL of distilled water, add 0.56 g (about 15 drops) of FD & C Blue #1 and 0.35 g (about 11 drops) of the green dye. The proportions are not critical, but enough food dye should be used to create an opaque solution. The dye mixture described should produce a solution having an absorbance of 0.854 (14% transmittance) at a wavelength of 505 nm.

When the SFIs of emulsified shortenings are determined, the addition of 1–2% sodium chloride (NaCl) to the indicator solution is recommended to reduce foaming at the interface between the sample and the indicator solution.

5. Mercury is used for the calibration of the dilatometers. Kimble Kontes will calibrate new dilatometers, providing a certificate of calibration with the dilatometer, thereby avoiding the concerns associated with the use of mercury. There is an additional charge for this service. Kimble Kontes should be contacted for further information.
6. V_c from Table 1 represents the combined correction for the expansion of glass and water, and applies to Pyrex™ brand glass only. If the dilatometer is constructed of glass other than Pyrex™ brand, appropriate corrections must be made for the type of the type of glass used.
7. The liquid thermal expansion is basic for calculating the solid fat index. It must, therefore, be accurate. Repeated analyses have shown values of 0.83–0.85 mL/kg to be normal for cottonseed oil, soybean oil, lard and tallow. Lauric acid oils, such as coconut, have values of 0.85–0.87 mL/kg. If determined values are abnormal, the analyses should be repeated. Standard thermal expansions may be applied in routine determinations where results are used within an organization, provided they are checked by actual measurement.

REFERENCES

1. *Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 49, World Health Organization/International Agency for Research on Cancer (IARC), 1990, p. 213.

336

AOCS Official Method Cd 14-95

Replaces Cd 14-61 • Reapproved 1997

Isolated *trans* Isomers Infrared Spectrometric Method

DEFINITION

Isolated *trans* bonds in long-chain fatty acids, esters and triglycerides may be measured by infrared spectrometry. An absorption band with a maximum at about 966 cm^{-1} (10.3 m), arising from a C-H deformation about a *trans* double bond, is exhibited in the spectra of all compounds containing an isolated *trans* group, and not observed in the spectra of the corresponding *cis* and saturated compounds. Measurement of the intensity of this absorption band under analytically controlled conditions is the basis for a quantitative method for the determination of isolated *trans* content. For high accuracy, common interfering absorptions associated with the glycerol backbone of triglycerides and the carboxyl group of fatty acids must be eliminated by conversion of these samples to their methyl esters prior to analysis.

SCOPE

This method is applicable to the accurate determination of isolated *trans* bonds in natural or processed long-chain acids, esters and triglycerides with *trans* levels ≥0.5%. It is not applicable or is applicable only with specific precautions to fats and oils containing large quantities (over 5%) of conjugated unsaturation, such as tung oil; to materials containing functional groups which modify the intensity of the C-H deformation about the *trans* double bond, such as castor oil containing ricinoleic acid or its geometrical isomer, ricinelaidic acid (12-hydroxy-9-octadecenoic acid); to mixed glycerides having long- and short-chain moieties, such as diacetostearin; or in general, to any materials containing constituents which have functional groups which give rise to specific absorption bands at or sufficiently close to interfere with the 966 cm^{-1} band of the C-H deformation of the isolated *trans* double bond. For accurate determinations on materials with *trans* levels below 0.5%, AOCS method Ce 1c-89 or Ce 1f-96 is recommended. For the direct analysis of triglycerides, Association of Official Analytical Chemists (AOAC) method 965.35 is recommended.

APPARATUS

1. Infrared spectrometer (FT–IR or dispersive) capable of making measurements at 8 cm^{-1} resolution in the spectral range covering 1050–900 cm^{-1}. The instrument's data handling capabilities should allow conversion of spectra to absorbance, scale expansion of the x and y axes, and readout of wavenumbers to the nearest 1 cm^{-1} and absorbance to the nearest 0.001 A.U. FT–IR spectrometers should employ TGS or DTGS detectors to ensure linearity.
2. Infrared liquid sampling cells with NaCl or KBr windows and a fixed path-length of 1 mm. For use in null-type instruments, pairs of cells matched to within 0.01 absorbance units are required. In split-beam type instruments with both cells filled with the solvent, electronic balance of the two beams to within these limits should be attained.
3. Volumetric flasks—Class A, 10 mL, 25 mL, and 50 mL.
4. Pipets—Class A, 1 mL, 3 mL, 4 mL, 5 mL, 7 mL, 9 mL.
5. Disposable Pasteur pipets for filling of infrared cells.
6. Analytical balance with a 60-g capacity, capable of weighing 0.2 g to an accuracy of ± 0.0001 g.

REAGENTS

1. Carbon disulfide (CS_2)—dry, reagent grade (see Notes, *Caution*).
2. Primary standards—Methyl elaidate and methyl oleate with purity of 99+% (see Notes, 1).

PREPARATION OF STANDARDS

1. *Stock solutions.* Weigh accurately to the nearest 0.0001 g, 0.5 g ± 0.01 g of methyl elaidate into a 25-mL volumetric flask. Dilute to volume with CS_2, stopper, and mix well. Prepare a second solution of methyl oleate as above. Prepare a 0.0020 g/mL stock solution of methyl elaidate by pipetting 5.00 mL of the 0.020 g/mL methyl elaidate stock solution into a 50-mL volumetric flask. Dilute to volume with CS_2, stopper, and mix well.
2. *1, 4 and 7%* trans *standards.* Into 10-mL volumetric flasks, weigh to the nearest 0.0001 g, the weight of methyl oleate specified below to ± 0.005 g. Pipet the specified volume of the 0.0020 g/mL methyl elaidate stock solution into each, then dilute each to volume with CS_2, stopper, and mix well.

Nominal % *trans*	mL Elaidate Soln.	g Methyl Oleate
1	1.00	0.198
4	4.00	0.192
7	7.00	0.186

3. *10, 30, 50 and 70%* trans *standards.* From the 0.020 g/mL stock solutions prepared above, prepare a set of calibration solutions by pipetting the following specified volumes of solutions into 10-mL volumetric flasks. Bring to volume, stopper and mix well.

Nominal % *Trans*	mL Elaidate Soln.	mL Oleate Soln.
10	1.00	9.00
30	3.00	7.00
50	5.00	5.00
70	7.00	3.00

CALIBRATION (See Notes, 2)

1. For each standard solution, calculate the exact weight of methyl elaidate diluted in 10 mL of solvent. Analyze each solution and determine the baseline corrected infrared absorbance at 966 cm^{-1} as described in the Procedure and Calculations sections below.
2. Trans ≤*10%*. Using a first-order regression analysis, determine the slope and intercept of the line which best fits the plot of the baseline corrected absorbance at 966 cm^{-1} for the 1, 4, 7 and 10% *trans* standards (y-axis) as a function of the grams of methyl elaidate/10 mL of solution (x-axis).
3. Trans >*10%*. Repeat Step 2 for the 10, 30, 50 and 70% *trans* standards.

PREPARATION OF SAMPLES

1. Solid fats must be melted on a steam bath and mixed before sampling. Samples that appear cloudy due to presence of water should be treated with anhydrous sodium sulfate and filtered before removing the sample for analysis.
2. Convert 0.5–1 g of the sample to methyl esters using AOCS method Ce 2-66, AOAC method 969.33, or other procedure which is appropriate for the sample type.
3. Weigh accurately to the nearest 0.0001 g, 0.20 g ± 0.01 g of the prepared undiluted methyl esters into a 10-mL volumetric flask. Dilute to volume with CS_2, stopper, and mix well.

PROCEDURE

1. Set up the infrared spectrometer operating parameters according to the manufacturer's recommendations to collect infrared spectra at 4 cm^{-1} resolution covering the range from 1050–900 cm^{-1}. Conditions employed should be identical for samples and standards. (See Notes, 3)
2. *For single-beam instruments.* Fill a clean infrared liquid cell with CS_2, remove any air bubbles, stopper, and place in the spectrometer's cell holder. Collect the single-beam spectrum to be used as a reference (background). Refill the clean cell with the sample solution and collect the single-beam spectrum of the sample. Ratio the sample spectrum against the background and convert to absorbance.
   For split or dual-beam instruments. Fill a clean infrared liquid cell with CS_2, remove any air bubbles, stopper, and place in the reference beam cell holder. Fill a second matched cell with the sample solution, remove any air bubbles, stopper, and place in the sample beam cell holder. Collect the spectrum and convert to absorbance.

CALCULATIONS

With the spectrum of the sample scale expanded in the region from 1050–900 cm^{-1}, draw a baseline (XY) tangent to the "feet" of the infrared absorbance band at 966 cm^{-1} as demonstrated in Figure 1. The points in a spectrum between which line XY is drawn will vary with peak absorbance. For accurate results, baselines must be drawn the same for both samples and standards. For high *trans* containing samples, the baseline should be drawn to the minimum located at 985 cm^{-1}. Occasionally, this minimum may be absent and the point to which the baseline is drawn will need to be estimated.

Determine the baseline corrected absorbance of the absorbance band (AC) by subtracting the absorbance of the baseline at the peak maximum (AB) from the absorbance of the peak at 966 cm-1 (AP). (The measured position of the peak maximum will vary with instrument and trans level of the sample analyzed. The position of the maximum should be established separately for each instrument with the 70% trans standard. This same position should then be used at all concentrations.)

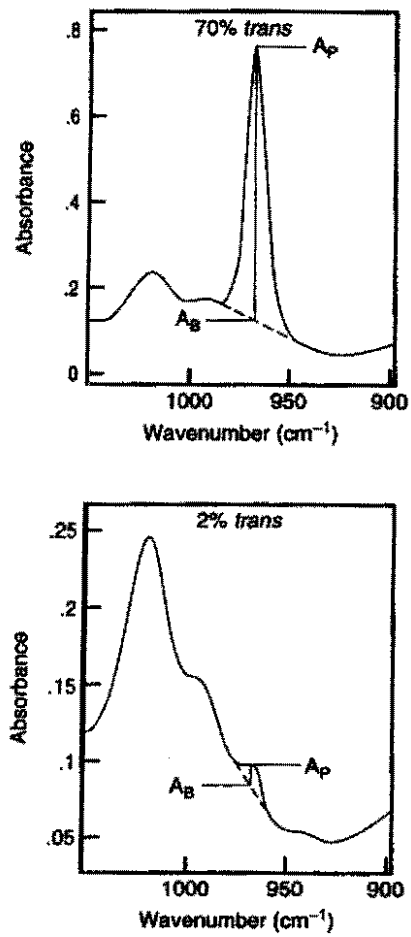


Figure 1. Infrared spectra of methyl esters containing 70 and 2% *trans*.

338

From Figure 1,

$$A_C = (A_P - A_B)$$

Using the calibration data for the appropriate trans range (≤10% or >10%), calculate the weight equivalent of methyl elaidate in the sample from the following equation:

$$\text{Methyl elaidate mass equivalent, g} = (A_C - \text{intercept}) / \text{slope}$$

Table 1

An international interlaboratory study completed by the AOCS Chromatography Committee in 1995, coordinated by David Henry, in which 16 laboratories participated, each obtaining two test results for each sample analyzed by IR, gave statistical results determined by ISO 5725-1986, which are summarized in the following table

	Me-1	Tg-1	Tg-2	Tg-3	Tg-4	Tg-5	Tg-6
Labs after outliers	15	16	16	15	16	16	14
Labs removed	1	0	0	1	0	0	2
Values, *n*	30	32	32	30	32	32	27
Mean, x, %	20.23	6.09	1.33	16.14	16.02	30.91	47.25
Known value, %	21.15	6.14	—	—	—	—	—
S_r	0.49	0.22	0.18	0.20	0.38	0.46	0.81
RSD_r	2.40	3.65	13.86	1.21	2.38	1.49	1.71
$r = 2.8 \times S_r$	1.37	0.62	0.50	0.56	1.09	1.29	2.27
S_R	1.30	0.60	0.44	1.19	1.07	1.86	1.22
RSD_R	6.42	9.82	32.78	7.38	6.69	6.03	2.59
$R = 2.8 \times S_R$	3.64	1.68	1.23	3.33	3.00	5.21	3.42

Sample key for Table 1:

Me-1 = Methyl elaidate/Methyl stearate with 21.15% *trans*
Tg-1 = Trielaidin in zero-*trans* peanut oil with 6.14% *trans*
Tg-2 = Canola oil
Tg-3 = }Shortening—
Tg-4 = }Blind duplicates
Tg-5 = Margarine
Tg-6 = Par fry oil

$$\%\ \textit{trans}\ \text{as methyl elaidate} = \frac{\text{Methyl elaidate mass equivalents, grams}}{\text{Sample mass, grams/10 mL of } CS_2} \times 100$$

Report results to the nearest 0.1%.

REPRODUCIBILITY

The absolute difference between two single test results, obtained with the same method on identical test material in different laboratories with different operators using different equipment, should not be greater than the reproducibility limit R as shown in Table 1.

REPEATABILITY

Absolute difference between two independent single test results, obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within short intervals of time, should not be greater than repeatability limit *r* as shown in Table 1.

NOTES

Caution

Prolonged breathing of carbon disulfide (CS2) vapors is dangerous. This solvent should be handled only under conditions which provide adequate ventilation, preferably under a chemical hood.

NUMBERED NOTES

1. Primary standards are available from Nu-Check-Prep, Inc., P.O. Box 295, Elysian, MN, 56028, USA. Telephone (507) 267-4689.
2. Once calibration data have been established, they should be checked periodically to ensure their validity. For highest quantitative accuracy and precision, a primary or secondary reference should be methylated and analyzed with each group of samples, then checked to ensure that its measured *trans* level is in agreement with the known or established mean value.
3. Infrared spectrometers should be checked to ensure that they are operating within the manufacturer's established specifications. Checks should include signal-to-noise as well as photometric and wavelength accuracy.

AOCS Official Method Cd 14-95

Replaces Cd 14-61 • Reapproved 1997 • Revised 1999

Isolated *trans* Isomers Infrared Spectrometric Method

DEFINITION

Isolated *trans* bonds in long-chain fatty acids, esters and triglycerides may be measured by infrared spectrometry. An absorption band with a maximum at about 966 cm^{-1} (10.3 µm), arising from a C-H deformation about a *trans* double bond, is exhibited in the spectra of all compounds containing an isolated *trans* group, and not observed in the spectra of the corresponding *cis* and saturated compounds. Measurement of the intensity of this absorption band under analytically controlled conditions is the basis for a quantitative method for the determination of isolated *trans* content. For high accuracy, common interfering absorptions associated with the glycerol backbone of triglycerides and the carboxyl group of fatty acids must be eliminated by conversion of these samples to their methyl esters prior to analysis.

SCOPE

This method is applicable to the accurate determination of isolated *trans* bonds in natural or processed long-chain acids, esters and triglycerides with *trans* levels ≥1%. It is not applicable or is applicable only with specific precautions to fats and oils containing large quantities (over 5%) of conjugated unsaturation, such as tung oil; to materials containing functional groups which modify the intensity of the C-H deformation about the *trans* double bond, such as castor oil containing ricinoleic acid or its geometrical isomer, ricinelaidic acid (12-hydroxy-9-octadecenoic acid); to mixed glycerides having long- and short-chain moieties, such as diacetostearin; or in general, to any materials containing constituents which have functional groups which give rise to specific absorption bands at or sufficiently close to interfere with the 966 cm^{-1} band of the C-H deformation of the isolated *trans* double bond. For accurate determinations on materials with *trans* levels below 0.5%, AOCS method Ce 1c-89 or Ce 1f-96 is recommended. For the direct analysis of triglycerides, Association of Official Analytical Chemists (AOAC) method 965.35 is recommended.

APPARATUS

1. Infrared spectrometer (FT–IR or dispersive) capable of making measurements at 8 cm^{-1} resolution in the spectral range covering 1050–900 cm^{-1}. The instrument's data handling capabilities should allow conversion of spectra to absorbance, scale expansion of the x and y axes, and readout of wavenumbers to the nearest 1 cm^{-1} and absorbance to the nearest 0.001 A.U. FT–IR spectrometers should employ TGS or DTGS detectors to ensure linearity.
2. Infrared liquid sampling cells with NaCl or KBr windows and a fixed path-length of 1 mm. For use in null-type instruments, pairs of cells matched to within 0.01 absorbance units are required. In split-beam type instruments with both cells filled with the solvent, electronic balance of the two beams to within these limits should be attained.
3. Volumetric flasks—Class A, 10 mL, 25 mL, and 50 mL.
4. Pipets—Class A, 1 mL, 3 mL, 4 mL, 5 mL, 7 mL, 9 mL.
5. Disposable Pasteur pipets for filling of infrared cells.
6. Analytical balance with a 60-g capacity, capable of weighing 0.2 g to an accuracy of ± 0.0001 g.

REAGENTS

1. Carbon disulfide (CS_2)—dry, reagent grade (see Notes, *Caution*).
2. Primary standards—Methyl elaidate and methyl oleate with purity of 99+% (see Notes, 1).

PREPARATION OF STANDARDS

1. *Stock solutions.* Weigh accurately to the nearest 0.0001 g, 0.5 g ± 0.01 g of methyl elaidate into a 25-mL volumetric flask. Dilute to volume with CS_2, stopper, and mix well. Prepare a second solution of methyl oleate as above. Prepare a 0.0020 g/mL stock solution of methyl elaidate by pipetting 5.00 mL of the 0.020 g/mL methyl elaidate stock solution into a 50-mL volumetric flask. Dilute to volume with CS_2, stopper, and mix well.
2. *1, 4 and 7%* trans *standards.* Into 10-mL volumetric flasks, weigh to the nearest 0.0001 g, the weight of methyl oleate specified below to ± 0.005 g. Pipet the specified volume of the 0.0020 g/mL methyl elaidate stock solution into each, then dilute each to volume with CS_2, stopper, and mix well.

Nominal % *trans*	mL Elaidate Soln.	g Methyl Oleate
1	1.00	0.198
4	4.00	0.192
7	7.00	0.186

3. *10, 30, 50 and 70%* trans *standards.* From the 0.020 g/mL stock solutions prepared above, prepare a set of calibration solutions by pipetting the following specified volumes of solutions into 10-mL volumetric flasks. Bring to volume, stopper and mix well.

Nominal % *Trans*	mL Elaidate Soln.	mL Oleate Soln.
10	1.00	9.00
30	3.00	7.00
50	5.00	5.00
70	7.00	3.00

CALIBRATION (See Notes, 2)

1. For each standard solution, calculate the exact weight of methyl elaidate diluted in 10 mL of solvent. Analyze each solution and determine the baseline corrected infrared absorbance at 966 cm^{-1} as described in the Procedure and Calculations sections below.
2. Trans $\leq$*10%*. Using a first-order regression analysis, determine the slope and intercept of the line which best fits the plot of the baseline corrected absorbance at 966 cm^{-1} for the 1, 4, 7 and 10% *trans* standards (y-axis) as a function of the grams of methyl elaidate/10 mL of solution (x-axis).
3. Trans >*10%*. Repeat Step 2 for the 10, 30, 50 and 70% *trans* standards.

PREPARATION OF SAMPLES

1. Solid fats must be melted on a steam bath and mixed before sampling. Samples that appear cloudy due to presence of water should be treated with anhydrous sodium sulfate and filtered before removing the sample for analysis.
2. Convert 0.5–1 g of the sample to methyl esters using AOCS method Ce 2-66, AOAC method 969.33, or other procedure which is appropriate for the sample type.
3. Weigh accurately to the nearest 0.0001 g, 0.20 g ± 0.01 g of the prepared undiluted methyl esters into a 10-mL volumetric flask. Dilute to volume with CS_2, stopper, and mix well.

PROCEDURE

1. Set up the infrared spectrometer operating parameters according to the manufacturer's recommendations to collect infrared spectra at 4 cm^{-1} resolution covering the range from 1050–900 cm^{-1}. Conditions employed should be identical for samples and standards. (See Notes, 3)
2. *For single-beam instruments.* Fill a clean infrared liquid cell with CS_2, remove any air bubbles, stopper, and place in the spectrometer's cell holder. Collect the single-beam spectrum to be used as a reference (background). Refill the clean cell with the sample solution and collect the single-beam spectrum of the sample. Ratio the sample spectrum against the background and convert to absorbance.

   For split or dual-beam instruments. Fill a clean infrared liquid cell with CS_2, remove any air bubbles, stopper, and place in the reference beam cell holder. Fill a second matched cell with the sample solution, remove any air bubbles, stopper, and place in the sample beam cell holder. Collect the spectrum and convert to absorbance.

CALCULATIONS

With the spectrum of the sample scale expanded in the region from 1050–900 cm^{-1}, draw a baseline (XY) tangent to the "feet" of the infrared absorbance band at 966 cm^{-1} as demonstrated in Figure 1. The points in a spectrum between which line XY is drawn will vary with peak absorbance. For accurate results, baselines must be drawn the same for both samples and standards. For high *trans* containing samples, the baseline should be drawn to the minimum located at 985 cm^{-1}. Occasionally, this minimum may be absent and the point to which the baseline is drawn will need to be estimated.

Determine the baseline corrected absorbance of the absorbance band (AC) by subtracting the absorbance of the baseline at the peak maximum (AB) from the absorbance of the peak at 966 cm-1 (AP). (The measured position of the peak maximum will vary with instrument and trans level of the sample analyzed. The position of the maximum should be established separately for each instrument with the 70% trans standard. This same position should then be used at all concentrations.)

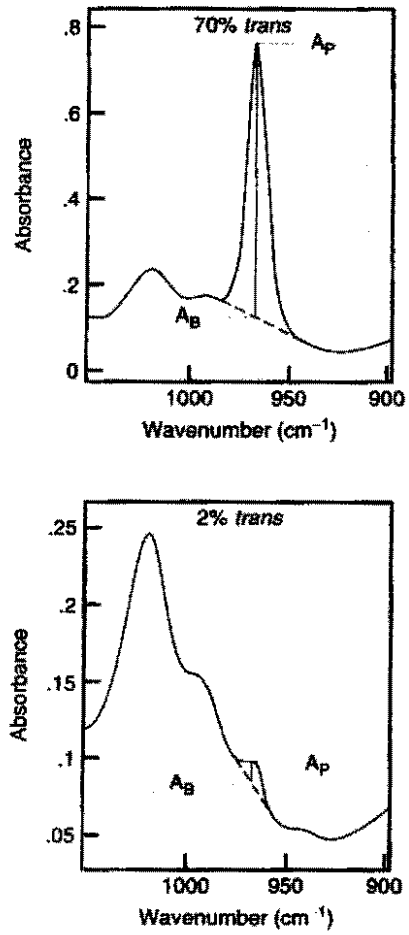


Figure 1. Infrared spectra of methyl esters containing 70 and 2% *trans*.

341

From Figure 1,

$$A_C = (A_P - A_B)$$

Using the calibration data for the appropriate trans range (≤10% or >10%), calculate the weight equivalent of methyl elaidate in the sample from the following equation:

$$\text{Methyl elaidate mass equivalent, g} = (A_C - \text{intercept}) / \text{slope}$$

Table 1
An international interlaboratory study completed by the AOCS Chromatography Committee in 1995, coordinated by David Henry, in which 16 laboratories participated, each obtaining two test results for each sample analyzed by IR, gave statistical results determined by ISO 5725-1986, which are summarized in the following table

	Me-1	Tg-1	Tg-2	Tg-3	Tg-4	Tg-5	Tg-6
Labs after outliers	15	16	16	15	16	16	14
Labs removed	1	0	0	1	0	0	2
Values, *n*	30	32	32	30	32	32	27
Mean, %	20.23	6.09	1.33	16.14	16.02	30.91	47.25
Known value, %	21.15	6.14	—	—	—	—	—
S_r	0.49	0.22	0.18	0.20	0.38	0.46	0.81
RSD_r	2.40	3.65	13.86	1.21	2.38	1.49	1.71
$r = 2.8 \times S_r$	1.37	0.62	0.50	0.56	1.09	1.29	2.27
S_R	1.30	0.60	0.44	1.19	1.07	1.86	1.22
RSD_R	6.42	9.82	32.78	7.38	6.69	6.03	2.59
$R = 2.8 \times S_R$	3.64	1.68	1.23	3.33	3.00	5.21	3.42

Sample key for Table 1:
Me-1 = Methyl elaidate/Methyl stearate with 21.15% *trans*
Tg-1 = Trielaidin in zero-*trans* peanut oil with 6.14% *trans*
Tg-2 = Canola oil
Tg-3 = } Shortening—
Tg-4 = } Blind duplicates
Tg-5 = Margarine
Tg-6 = Par fry oil

$$\%\ \textit{trans}\ \text{as methyl elaidate} = \frac{\text{Methyl elaidate mass equivalents, grams}}{\text{Sample mass, grams/10 mL of } CS_2} \times 100$$

Report results to the nearest 0.1%.

REPRODUCIBILITY

The absolute difference between two single test results, obtained with the same method on identical test material in different laboratories with different operators using different equipment, should not be greater than the reproducibility limit R as shown in Table 1.

REPEATABILITY

Absolute difference between two independent single test results, obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within short intervals of time, should not be greater than repeatability limit r as shown in Table 1.

NOTES

Caution

Prolonged breathing of carbon disulfide (CS_2) vapors is dangerous. This solvent should be handled only under conditions which provide adequate ventilation, preferably under a chemical hood.

NUMBERED NOTES

1. Primary standards are available from Nu-Check-Prep, Inc., P.O. Box 295, Elysian, MN, 56028, USA. Telephone (507) 267-4689.
2. Once calibration data have been established, they should be checked periodically to ensure their validity. For highest quantitative accuracy and precision, a primary or secondary reference should be methylated and analyzed with each group of samples, then checked to ensure that its measured *trans* level is in agreement with the known or established mean value.
3. Infrared spectrometers should be checked to ensure that they are operating within the manufacturer's established specifications. Checks should include signal-to-noise as well as photometric and wavelength accuracy.

Official Method Cd 14d-99
Revised 1999

Rapid Determination of Isolated *trans* Geometric Isomers in Fats and Oils by Attenuated Total Reflection Infrared Spectroscopy

DEFINITION

In most naturally occurring vegetable fats and oils, unsaturated constituents contain only isolated, i.e., non-conjugated, double bonds in the *cis* configuration. These *cis* bonds may be isomerized to the *trans* configuration during extraction and processing procedures, due to oxidation, conversion during heating, and/or partial hydrogenation. Animal and marine fats may contain measurable amounts of naturally occurring *trans* isomers. Isolated *trans* bonds in long-chain fatty acids, fatty acid methyl esters (FAME), soaps, and triacylglycerols (TAG) may be measured by infrared spectroscopy. An absorption band with a maximum of about 966 cm^{-1} (10.3 μ), arising from a C-H deformation about a *trans* double bond, is exhibited in the spectra of all compounds containing an isolated *trans* group. This band is not observed in the spectra of the corresponding *cis* and saturated compounds. Measurement of the intensity of this absorption band under analytically controlled conditions is the basis for a quantitative method for the determination of isolated *trans* content.

A Fourier transform infrared spectrometer capable of operating at 4 cm^{-1} resolution, equipped with a TGS or DTGS detector, and an attenuated total reflection (ATR) infrared cell are required.

SCOPE

This method is applicable to the rapid (about 5 min) and accurate determination of isolated *trans* double bonds in natural or processed oils and fats with *trans* levels equal to or greater than about 1.0%.

It is not applicable to fats and oils containing over about 1% of conjugated unsaturation, such as tung oil; to materials containing functional groups which modify the intensity of the C-H deformation about the *trans* double bond, such as castor oil, which contains hydroxy groups; or in general, to any materials containing constituents with functional groups that give rise to specific absorption bands at or sufficiently close to interfere with the 966 cm^{-1} band of the C-H deformation of the isolated *trans* double bond. For accurate determinations of materials with *trans* levels below about 1.0%, gas chromatography of FAME (eg., AOCS Method Ce 1f-96, JAOCS 73:275-282, 1996) is recommended; GC has also been the industry standard for the routine determination of fatty acid composition.

This method requires no weighing and no quantitative dilution of TAG test samples in any solvent.

APPARATUS

1. Fourier transform infrared (FTIR) spectrometer—capable of making measurements at 4 cm^{-1} resolution in the spectral range covering 1050–900 cm^{-1}. The instrument data handling capabilities should allow conversion of the spectra to absorbance, scale expansion of the *x* and *y* axes, readout of wavenumbers to the nearest 1 cm^{-1} and absorbance to the nearest 0.001 A.U., and integration of the area under the absorption band at 966 cm^{-1}. FTIR spectrometer should be equipped with TGS and DTGS detectors for greatest linearity. With no test sample on the attenuated total reflection (ATR) crystal (see number 2 below), a 1 min data collection at 4 cm^{-1} resolution should yield an absorption spectrum between 1050 and 900 cm^{-1}, with a peak-to-peak noise level of 0.0005 A.U. or less.
2. ATR infrared cell—with a zinc selenide (or equivalent) crystal. The capacity of the ATR cell should be about 50 μL. For the 966 cm^{-1} band, the signal-to-noise ratio (SNR) in the 1050–900 cm^{-1} range must equal or exceed 10:1 for a 1% trielaidin test sample.
3. Analytical balance—capable of weighing 0.3 g to an accuracy of ±0.0001 g.
4. ATR accessory—capable of maintaining a constant temperature of about 65 (±2) °C.

REAGENTS

1. Primary standards: trielaidin (TE) and triolein (TO) with purity of 99% (see Numbered Notes, 1).

PREPARATION OF STANDARDS

1. *Trans* calibration standards: weigh accurately to the nearest 0.0001 g, (0.3-x) g of TO, and x g of TE, into a 10-mL beaker, where x equals 0.0030, 0.0150, 0.0300, 0.0600, 0.0900, 0.1200 and 0.1500 g in order to prepare 1, 5, 10, 20, 30, 40, and 50% *trans* calibration standards, respectively.

CALIBRATION (See Numbered Notes, 2)

1. For each neat TE/TO standard mixture, calculate the exact percent *trans* as TE per total TAG. Analyze each mixture and determine the integrated area under the absorption band at 966 cm^{-1} as described in the Procedures and Calculations sections below.
2. Using a first-order regression analysis, determine the slope and intercept of the line which best fits the plot of the area of the *trans* band for all the *trans* standard mixtures (y axis) as a function of % *trans* expressed as percent TE in TO (x axis).

PREPARATION OF SAMPLES

Solid fats must be gently melted and mixed before sampling. Samples that appear cloudy due to the presence of water should be treated with anhydrous sodium sulfate and filtered before removing the test sample for analysis.

PROCEDURES

1. Set up the FTIR spectrometer operating parameters according to the manufacturer's recommendations for using an ATR cell with the following parameters: 1050–900 cm^{-1} range, 64- scan (or appropriate number of scans needed to give a peak-to-peak noise level of of 0.0005 A.U. or less in this range), 4 cm^{-1} resolution, and triangular apodization (if available). Conditions should be identical for test samples and calibration standards (see Numbered Notes, 3). For solid TAG test samples maintain ATR cell at 65°C (±2°C).
2. Using a disposable pipet, transfer about 50 µL without weighing (use enough material to cover the entire surface of the ATR crystal) of the neat reference background material; for TE/TO calibration standard mixtures, use TO as reference background material. For spiked (accuracy standards) test samples, use the unspiked material for measuring the reference background single-beam spectrum. For unknown test samples, use an appropriate reference as background, for example, the refined bleached source oil for the material to be analyzed (see Numbered Notes, 4). Place the reference background material on the horizontal (face-up) ZnSe sampling surface of the ATR cell (see Numbered Notes, 5). Collect and save the single-beam spectrum to be used as background. Clean the ZnSe crystal by wiping off the TAG analyte with a disposable soft lint-free or low-lint tissue paper (see Numbered Notes, 6). Place the neat test sample (without weighing) on the horizontal ZnSe crystal. Collect and save the single-beam spectrum of the test sample. Ratio the sample single-beam spectrum against that of the background, and convert to absorbance. Save the absorption spectrum.

CALCULATIONS

With the absorbance spectrum wavenumber scale expanded in the region from 1050 to 900 cm^{-1}, integrate (electronically) the area under the 966 cm^{-1} band between the limits 990 cm^{-1} and 945 cm^{-1}. Calculate the linear regression equation for area vs. % *trans* plot of the *trans* calibration standard mixtures.

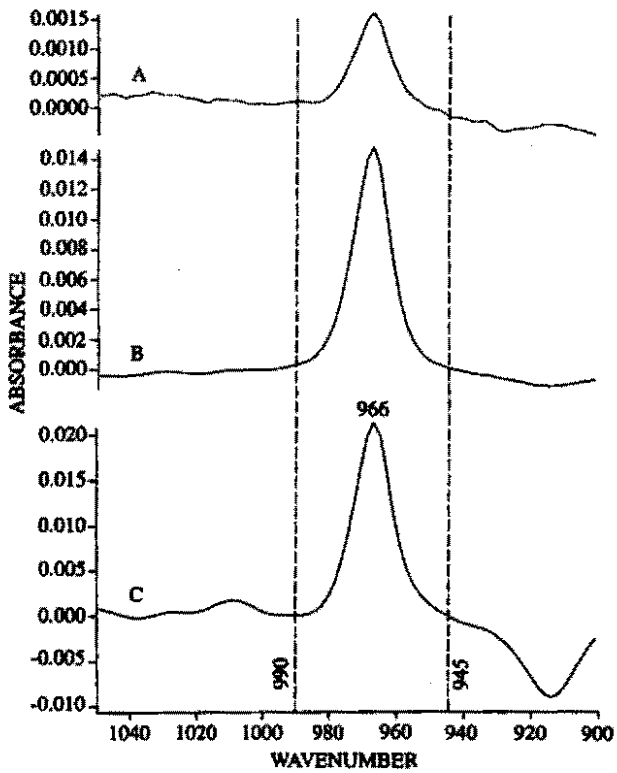


Figure 1. ATR-FTIR spectra in the range 1050–900 cm^{-1} showing the 966 cm^{-1} band attributed to isolated *trans* double bonds for: (A) a 1% trielaidin standard. The signal-to-noise ratio in this range was equal to 16:1 under our experimental conditions of 64 scans and 4 cm^{-1} resolution; (B) a 10% trielaidin standard; and (C) a test sample consisting of a blend of commercial vegetable oils and partially hydrogenated soybean oil found to contain about 14% *trans* fat, as percent of total fat.

Using the slope and intercept generated for *trans* standard mixtures, calculate the % *trans* for test samples by plugging the value of the integrated area of the *trans* band in the following equation:

$$\%\ trans\ \text{as ME} = \frac{(\text{area} - \text{intercept})}{\text{slope}}$$

Report results to the nearest 0.1%.

NUMBERED NOTES

1. Primary standards are available from Nu-Check-Prep, Inc., P.O. Box 295, Elysian, MN 56028.
2. Once a calibration curve has been established, it should be checked periodically to ensure that it has not shifted.
3. The performance of FTIR spectrometers should be evaluated for wavenumber accuracy and noise level to ensure that they are operating within the manufacturer's established specifications.
4. TAG reference material ultra degummed bleached expeller soybean oil is recommended (available commercially from Owensboro Grain Co, Owensboro, KY).

344

5. The analyte must *completely* cover the horizontal surface of the ZnSe crystal.
6. In order to minimize contamination, wipe the ZnSe crystal with the next test sample to be analyzed, and then clean the crystal.

REFERENCES

1. Mossoba, M.M., M.P. Yurawecz, and R.E. McDonald, Rapid Determination of the Total *trans* Content of Neat Hydrogenated Oils by Attenuated Total Reflection Spectroscopy, *J. Am. Oil Chem. Soc. 73*:1003 (1996).
2. Ali, L.H., G. Angyal, C.M. Weaver, J.I. Rader, and M.M. Mossoba, Determination of Total *trans* Fatty Acids in Foods: Comparison of Capillary Column GC and SB-HATR Infrared Spectroscopy, *J. Am. Oil Chem. Soc. 73*:1699 (1996).
3. Adam, M., M. Chew, S. Wasserman, A. McCollum, R.E. McDonald, and M.M. Mossoba, Determination of *trans* Fatty Acids in Hydrogenated Vegetable Oils by Attenuated Total Reflection Infrared Spectroscopy: Two Limited Collaborative Studies, *J. Am. Oil Chem. Soc. 75*:353 (1998).
4. Adam, M., M.M. Mossoba, M. Chew, and S. Wasserman, Comparison of Attenuated Total Reflection Infrared Spectroscopy to Capillary Gas Chromatography for *trans* Fatty Acid Determination, *J. Am. Oil Chem. Soc. 76*:375 (1999).

APPENDIX 1

Summary of statistical results, determined by the AOAC AOACBUBR computer program, for an international collaborative ATR FTIR study among 12 laboratories that was coordinated by Michael Adam (Lipton, Baltimore, MD) and Magdi M. Mossoba (FDA, Washington, DC).

The study reports data for seven pairs of blind duplicate accuracy standards that were spiked with known amounts of *trans* fat, and 10 pairs of blind duplicate test samples.

Table 1
Pairs of blind duplicate accuracy standards

Lab	Acc1/a	Acc2/b	Acc3/c	Acc4/d	Acc5/e	Acc6/f	Acc7/g
one	0.93	1.08	5.12	10.13	15.40	20.16	40.36
	0.80	1.14	5.27	10.17	15.42	19.97	43.30
two	0.61	0.89	5.10	9.95	15.20	20.40	40.44
	0.64	0.85	5.14	10.24	15.07	20.50	40.37
three	0.81	0.81	4.90	10.13	15.37	20.61	40.41
	0.81	0.81	4.90	10.13	15.37	20.03	40.41
four	1.13	1.40	5.10	10.86	16.53	15.69[b]	38.07
	1.24	1.33	4.87	11.46	15.99	17.87[b]	43.63
five	0.54	0.78	5.37	10.44	16.11	20.99	39.38
	0.52	0.69	5.40	10.83	15.86	21.01	39.37
six	0.74	0.85	5.15	9.53	15.61	20.59	40.31
	0.95	1.23	5.29	10.21	15.18	20.45	40.29
seven	0.93	1.15	5.03	9.75	16.06	20.82	37.48
	0.97	1.15	5.11	10.01	17.14	23.70	38.02
eight	0.84	0.99	5.02	10.12	15.43	20.22	39.96
	0.80	1.26	5.34	10.16	15.33	20.06	40.53
nine	0.81	1.08	5.01	9.88	15.51	19.79	38.87
	0.75	0.99	5.00	10.54	15.03	19.56	39.22
ten	0.07[a]	0.31	5.17	10.73	16.12	20.36	41.34
	0.10[a]	0.28	5.09	9.50	15.27	20.63	39.11
eleven	0.84	0.90	5.03	11.33	15.44	23.54	40.69
	0.78	0.91	5.38	11.15	15.71	20.32	40.35
twelve	0.93	1.15	5.26	10.04	15.30	20.09	40.63
	0.94	1.14	5.26	10.37	15.23	20.09	39.47
TRUE[c]	0.81	1.00	5.04	10.00	15.03	20.00	39.98
XBAR	0.83	0.97	5.14	10.32	15.61	20.63	40.08
s_r	0.06	0.10	0.12	0.37	0.34	0.93	1.40
s_R	0.18	0.28	0.16	0.52	0.51	1.04	1.41
RSD_r	7.46	10.46	2.30	3.55	2.17	4.53	3.48
RSD_R	21.07	29.27	3.06	5.06	3.26	5.03	3.52
r	0.17	0.28	0.33	1.03	0.95	2.62	3.91
R	0.49	0.79	0.44	1.46	1.42	2.91	3.95

[a]Single Grubbs' outlier
[b]Cochran's outlier
[c]Determined gravimetrically

Table 2
Pairs of blind duplicate test samples

Lab	TS1/j	TS2/i	TS3/h	TS4/g	TS5/f	TS6/e	TS7/d	TS8/c	TS9/b	TS10/a
one	0.75	0.06	39.93	0.32	4.99	14.79	1.39	28.72	19.77	1.73
	0.71	0.21	40.12	0.35	4.83	14.94	1.49	28.64	20.19	1.84
two	0.12	0.11	39.95	0.00	4.30	14.58	1.20	28.99	20.22	1.05
	0.12	0.13	39.92	0.00	4.27	14.66	1.23	28.86	20.20	0.96
three	0.23	0.23	39.83	0.23	4.30	14.79	1.39	28.76	20.61	1.39
	0.23	0.23	40.41	0.23	4.30	14.79	1.39	28.76	20.61	1.39
four	0.81	0.00	40.32	0.32	4.94	15.00	1.82	29.86	21.67	2.12
	0.80	0.00	40.46	0.37	4.47	15.16	1.76	29.13	21.83	2.13
five	0.07	0.00	40.23	0.00	4.83	15.78	1.26	30.24	21.50	1.75
	0.00	0.00	40.87	0.00	4.68	15.74	1.30	30.37	21.25	1.77
six	0.28	0.00	39.30	0.00	4.64	14.59	1.36	28.94	20.26	2.21
	0.41	0.00	39.24	0.00	4.69	14.45	1.61	28.85	20.47	1.81
seven	0.64	0.66[a]	37.34	0.44	4.45	13.97	1.56	27.10	19.23	2.03
	0.64	0.66[a]	37.37	0.37	4.49	13.90	1.56	27.06	19.30	2.05
eight	0.53	0.00	39.85	0.00	4.66	14.49	1.38	28.74	20.00	1.89
	0.45	0.00	39.75	0.00	4.50	14.40	1.41	28.97	20.09	1.85
nine	0.44	0.00	39.00	0.00	4.43	14.19	1.38	28.13	19.84	1.88
	0.40	0.00	39.82	0.00	4.37	14.33	1.37	28.15	19.83	1.85
ten	0.00	0.00	41.42	0.00	4.32	15.38	0.66[a]	30.41	21.60	1.00
	0.00	0.00	41.66	0.00	4.22	15.50	0.66[a]	30.35	21.43	0.95
eleven	0.48	0.37	39.14	0.00	4.17	13.75	1.48	28.50	19.81	1.84
	0.59	0.27	39.35	0.00	4.35	13.72	1.61	27.83	19.64	2.19
twelve	0.91	0.13	39.40	1.00[b]	4.65	14.25	1.57	28.97	18.92	1.93
	0.80	0.29	35.48	0.63[b]	4.78	14.52	1.83	25.15	18.05	2.23
XBAR	0.43	0.09	39.59	0.12	4.53	14.65	1.47	28.73	20.26	1.74
s_r	0.05	0.05	0.84	0.02	0.12	0.09	0.09	0.81	0.22	0.13
s_R	0.30	0.12	1.34	0.17	0.24	0.59	0.18	1.19	0.96	0.41
RSD_r	11.11	55.88	2.12	16.25	2.72	0.62	5.87	2.81	1.07	7.39
RSD_R	68.35	133.46	3.38	143.15	5.32	4.00	12.35	4.15	4.74	23.69
r	0.13	0.14	2.35	0.05	0.34	0.25	0.24	2.26	0.61	0.36
R	0.83	0.34	3.75	0.48	0.67	1.64	0.51	3.34	2.69	1.16

[a]Single Grubbs' outlier
[b]Cochran's outlier

For test samples, the reference background material was ultra-degummed bleached expeller soybean oil (Owensboro Grain Co.). Test samples consisted of a commercial vegetable oil (namely, soybean oil, rapeseed oil, palm kernel oil, cottonseed oil, or sunflower oil), partially hydrogenated soybean oil, or the same oil (or a blend of two oils) mixed with partially hydrogenated soybean oil (Lipton, Baltimore, MD).

Accuracy standards consisted of cold-pressed bleached degummed soybean oil spiked with trielaidin; the reference gackground material was cold-pressed bleached degummed soybean oil (Lipton).

The statistical parameters:

XBAR, the overall mean of the laboratory values
s(r), repeatability standard deviation
s(R), reproducibility standard deviation
RSD(r), repeatability relative standard deviation
RSD(R), reproducibility relative standard deviation
r, repeatability value = 2.8 s(r)
R, reproducibility value = 2.8 s(R)

346

AOCS Official Method Ak 2-92

Reapproved 1997

Determination of Chlorophyll Content in Rapeseed/Canola (Colza) by Spectrometry

DEFINITION

This method, adopted from ISO 10519 (References, 1), defines the chlorophyll content as the total of the substances contributing to the absorption band near 665 nm as determined under the operating conditions specified in this method, measured as chlorophyll a and expressed in milligrams per kilogram (mg/kg) of the product as received. The chlorophyll content of the rapeseed/canola (colza) is determined by extraction of a test portion in a suitable apparatus with a solution of ethanol and isooctane, or 2-propanol, methanol and isooctane, followed by the spectrometric determination of the chlorophyll contained in the extraction solution.

SCOPE

This method specifies a spectrometric method for the determination of the chlorophyll content of rapeseed (colza) used as an industrial raw material.

APPARATUS

Usual laboratory apparatus and, in particular, the following:

1. Analytical balance.
2. Mechanical grinder—blade type, or coffee mill, or equivalent.
3. Mechanical microgrinder (see Fig. 1)—stainless steel tubes of approximately 50-mL volume and which can be securely stoppered, stainless steel ball bearings (16 mm in diameter) and an apparatus to shake the securely stoppered tubes horizontally at a frequency of 240 min^{-1}, with a horizontal displacement of 3.5 cm, or, for example, a Dangoumau ball mill (see Notes, 1).

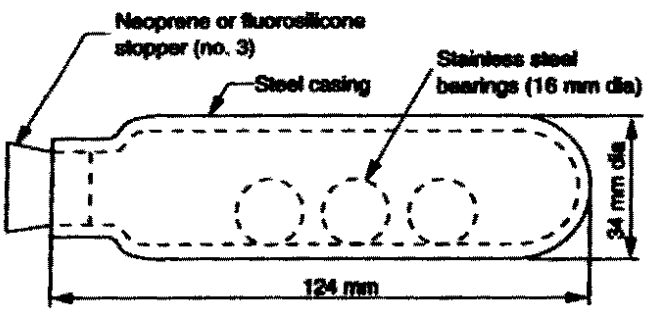


Figure 1. Mechanical microgrinder.

4. Filter paper—medium speed, V-folded.
5. Spectrometer—suitable for carrying out absorbance measurements between 600 and 700 nm, with a spectral band path of 2 nm. If the spectrometer is not able to achieve a spectral band width of 2 nm, it is necessary to carry out a calibration with pure chlorophyll a as described in Notes, 2.
6. Optical cuvettes—having a path length of at least 1.0 cm.
7. Pipets—30-mL capacity, class A, or a repetitive dispenser with a capability of dispensing 30 mL with an accuracy of <1%.
8. Culture tubes—20-mL capacity, with stoppers.

REAGENTS

The reagents used for the preparation of the extraction solvent shall be of recognized analytical grade unless otherwise stated.

1. Extraction solvent—prepared as follows:
   Transfer to a 500-mL beaker either
   (a) 100 mL of anhydrous ethanol, or
   (b) 50 mL of anhydrous 2-propanol and 50 mL of anhydrous methanol.
2. Add to the contents of the beaker 300 mL of anhydrous isooctane (2,2,5-trimethylpentane), or technical n-heptane, or petroleum ether (essentially composed of C7 hydrocarbons, with a boiling range between 90 and 100°C).

PROCEDURE

1. Sampling to obtain the laboratory sample and the reduction of the laboratory sample (as received or after separation of impurities) to the test sample shall be carried out in accordance with accepted standardized techniques and with any contractual agreement.
2. Seeds with a moisture content of >10% (w/w) should be dried for 12 hr at 45°C to reduce the moisture level to 10% (w/w) or less without breaking down chlorophyll pigments.
3. Transfer 50 g of the test sample to the mechanical grinder (Apparatus, 2) and grind to produce a uniform ground seed. If a small grinder such as a coffee mill is used, grind several portions of 10 g and then combine and mix thoroughly the ground portions.
4. For the test portion, weigh, to the nearest 1 mg, 2 g of the test sample (Procedure, 3) into the stainless steel tube (Apparatus, 3), or the extraction vessel of a Dangoumau ball mill (Apparatus, 3).
5. For the extraction of the test portion add, using a pipet (Apparatus, 7), 30 mL of the extraction solvent (Reagents, 1 and 2) to the tube or vessel. If using a tube, add three stainless steel balls to the tube (Fig. 1) and shake for 1 hr. For Dangoumau ball mills, add steel balls (at least four medium-sized balls) to the vessel and extract for 20 min.

347

6. Allow the extract to settle for 10 min and then decant a sufficient volume of the extract through the filter paper (Apparatus, 4) into a culture tube (Apparatus, 8) to fill the optical cuvette (Apparatus, 6). Stopper the tube as soon as possible to minimize evaporation.

   Note—The presence of more than one phase in the extraction solvent indicates the presence of excessive moisture, either in the sample [which should contain < 10% (w/w) moisture] or in the solvents (which should be anhydrous).
7. Transfer the filtered extract to the cuvette (Apparatus, 6) and determine the absorbance at 665, 705 and 625 nm (the readings at 705 and 625 nm are used to calculate a baseline correction).

CALCULATIONS

1. The chlorophyll content, in milligrams per kilogram (mg/kg) of the product as received, is given by the formula

$$\text{chlorophyll content, mg/kg} = \frac{k \times A_{corr} \times V}{m \times l}$$

Where—

A_{corr} (the corrected absorbance) = A665 − [(A705 + A625)/2]
A665 = absorbance at 665 nm
A705 = absorbance at 705 nm
A625 = absorbance at 625 nm
k = 13
l = path length of the optical cuvette, in cm
m = mass of the test portion, in g
V = volume of solvent added to the tube (Apparatus, 8), in mL. If it is desired to express the chlorophyll content relative to the dry product, take into account in the calculation the water content of the sample, determined in accordance with ISO 665.

PRECISION

1. Repeatability—The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, should not be greater than the following values:
   (a) chlorophyll content between 10 mg/kg and 20 mg/kg, mg/kg = 2
   (b) chlorophyll content between 20 mg/kg and 30 mg/kg, mg/kg = 3

   Note—Values are not fixed for chlorophyll contents below 10 mg/kg because the industry accepts a content up to 25 mg/kg.
2. Reproducibility—The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment should not be greater than the following values:
   (a) chlorophyll content between 10 mg/kg and 20 mg/kg, mg/kg = 3
   (b) chlorophyll content between 20 mg/kg and 30 mg/kg, mg/kg = 6

   Note—Values are not fixed for chlorophyll contents below 10 mg/kg because the industry accepts a content up to 25 mg/kg.

TEST REPORT

1. The test report shall specify the method used and the results obtained. It shall also mention all operating conditions not specified in the method, or regarded as optional, together with details of any incidents which may have influenced the results.
2. The test report shall include all information necessary for the complete identification of the sample.

NOTES

Caution

The solvents used in this method are flammable liquids with low flash points. These solvents may defat (dry out) or irritate the skin, eyes and mucous membranes. Consult the appropriate manufacturer's material safety data sheets.

When handling solvents, use protective eye wear, gloves and a static-free (not polyester) laboratory coat. Handle the solvents in a well-ventilated area (preferably a fume hood) away from heat, sparks and open flame. Conduct bulk transfers of the solvents in a fume hood. Take care during transfer, as the liquids are nonconducting and may generate a static charge. Store large volumes of solvents in a cool, ventilated location. Separate from oxidizing material. Isolate from other combustible material.

Diethyl ether may form organic peroxides during storage. All organic peroxides are extremely flammable and unstable (explosive). They are hazardous because of their extreme sensitivity to heat, friction, shock and light, as well as to strong oxidizing and reducing agents. Store diethyl ether away from heat and light in closed vessels, preferably in the container furnished by the supplier. Containers of ether should be labelled with the dates received, opened and of expiration. The contents should be used within 1 month of opening. If a bottle is several years old, or if there is any visible solid around the cap, then no attempt should be made to open it.

Used solvents shall be stored and disposed of in accordance with local regulations.

NUMBERED NOTES

1. Dangoumau ball mill is an example of a suitable apparatus available commercially. This information is given for the convenience of users of this method and does not constitute an endorsement by AOCS of this apparatus.
2. Calibration for routine operation on spectrometers unable to achieve a spectral band width of 2 nm requires the preparation of solutions of pure chlorophyll a, followed by the determination, using these solutions, of the slope of the calibration curve for the spectrometer.

   Reagents
   (a) Diethyl ether—of recognized analytical grade.
   (b) Chlorophyll a—standard solution (Sigma Chemical, St. Louis, MO, USA). Place about 1 mg of crystalline chlorophyll a of at least 95% purity in a 10-mL volumetric flask, dissolve in the diethyl ether and make up to the mark with diethyl ether.

Apparatus

(a) Spectrometer—other than the unit to be calibrated, suitable for carrying out absorbance measurements between 600 and 700 nm and with a spectral band width of < 2 nm. This instrument is required to determine the concentration of chlorophyll a in the standard solution.
(b) Micropipets—capable of delivering 0.200 mL, 0.400 mL, 0.600 mL, 0.800 mL and 1.000 mL.
(c) Volumetric flasks—10-mL capacity.
(d) Working spectrometer—i.e., a spectrometer other than that specified in Notes, 2, Apparatus (a), for which a calibration is desired.

Calibration

(a) To determine the chlorophyll a content in the standard solution, transfer 1.0 mL of the chlorophyll a standard solution [Notes, 2, Reagents, (b)] to a 10-mL volumetric flask [Notes, 2, Apparatus, (c)] and dilute to the mark with the diethyl ether [Notes, 2, Reagents, (a)]. Measure the absorbance of this solution using the spectrometer [Notes, 2, Apparatus, (a)] at 660 nm (peak wavelength) and 642.5 nm, using a 1.0-cm cuvette and a spectral band width of 2 nm.
(b) Calculate the concentration of the chlorophyll a, mg/L, in the standard solution using the formula

$$\text{Chlorophyll a (mg/L)} = (99.3 \times A660) - (7.8 \times A642.5)$$

Where—

A660 = absorbance of the standard chlorophyll a solution at 660 nm

A642.5 = absorbance of the standard chlorophyll a solution at 642.5 nm

Note—The factors used for the determination of chlorophyll a (99.3 and 7.8) are derived from the factors published in References, 2. The factors 9.93 and 0.777 from this source are adjusted for a 10-fold dilution.

Preparation of the Calibration Curve

(a) To a series of five 10.0-mL volumetric flasks, add 0.200, 0.400, 0.600, 0.800 and 1.000 mL of the standard chlorophyll a solution [Notes, 2, Reagents (b)], the concentration of which was calculated in Notes, 2, Calibration, (b). Fill the flasks to the mark with the extraction solvent (Reagents, 1 and 2).

Note—The small amounts of diethyl ether in these solutions do not interfere significantly with the determination.

(b) Measure the corrected absorbance A_{corr} (Calculations, 1) of these calibration solutions and plot this against the concentration of chlorophyll a in the solutions. The slope of the curve thus plotted is the constant k, in mg/L, per corrected absorbance.

REFERENCES

1. *Rapeseed—Determination of chlorophyll content—Spectrometric method*, International Organization for Standardization, Geneva, 1992, ISO 10519:1992(E).
2. *Official Methods of Analysis*, AOAC International, 16th ed., Vol. I, Gaithersburg, MD, 1995, Chapt. 3, p. 27, Method 942.04.

AOCS Recommended Practice Cc 13i-96
Reapproved 1997

Determination of Chlorophyll Pigments in Crude Vegetable Oils

DEFINITION

The content of chlorophyll pigments in vegetable oils is expressed as mg of pheophytin a in 1 kg of oil.

SCOPE

This method determines total chlorophyll pigments, expressed as pheophytin a, in crude vegetable oils. The method is suitable for the determination of quantities of chlorophyll pigments higher than 1 mg/kg (1 ppm). The chlorophyll pigments are determined by measuring the absorbance at 670 nm, correcting the result for the background absorption, and calculating the content with use of the absorptivity of pheophytin a, which is the main chlorophyll pigment in crude vegetable oils.

APPARATUS

1. Spectrophotometer allowing the measurement in the range of 630–710 nm.
2. Glass spectrophotometer cells, with light path of 5 mm and 10 mm.
3. Glass funnel—diameter 40–60 mm.

PROCEDURE

1. The sample is heated to a temperature no more than 5°C higher than the melting point of the sample, if the sample is solid at room temperature. The liquid sample is mixed thoroughly, and if it is turbid, it is filtered immediately prior to the analysis, using a filter paper of medium porosity.
2. The sample is measured at 630 nm, 670 nm and 710 nm in a 5 mm or 10 mm spectrophotometer cell (Apparatus, 2) against air rather than a reference cell.

CALCULATION OF RESULTS

1. The content of chlorophyll pigments is expressed in mg of pheophytin a, which is calculated as follows:

$$C = 345.3 \times \frac{(A_{670} - 0.5 \times A_{630} - 0.5 \times A_{710})}{L}$$

Where—

C = content of chlorophyll pigments as mg of pheophytin a in 1 kg of oil;
A = absorbance at the respective wavelength (nm);
L = light path of the spectrophotometer cell (mm).

PRECISION

1. Repeatability, expressed by the coefficient of variation, RSD_r is—0.4 at high concentration of chlorophyll pigments; 1.0 at moderate concentration of chlorophyll pigments; and 1.4 at low concentration of chlorophyll pigments.
2. Reproducibility, expressed by the coefficient of variation, RSD_R, is—1.3 at high concentration of chlorophyll pigments; 3.5 at moderate concentration of chlorophyll pigments; and 6.0 at low concentration of chlorophyll pigments.

REFERENCE

1. Results of the IUPAC Commission on Oils, Fats and Derivatives collaborative study for the determination of chlorophyll pigments in crude vegetable oils, *Pure & Applied Chemistry* 67(10):1781 (1995).

AOCS Official Method Cc 1-25

Reapproved 1997

Melting Point Capillary Tube Method

DEFINITION

The natural fats and oils, that is, those of animal and vegetable origin, are mixtures of glycerides and a number of other minor components (pigments, sterols, tocopherols, etc.). They do not exhibit either a definite or sharp melting point. Therefore, the term "melting point" does not imply the same characteristics that it does with pure substances of a definite crystalline nature. Fats pass through a stage of gradual softening before they become totally liquid. The melting point then must be defined by the specific conditions of the method by which it is determined and, in this case, it is the temperature at which the sample becomes completely clear and liquid.

SCOPE

Applicable to all normal animal and vegetable fats.

APPARATUS

1. Melting-point tubes—capillary glass tubing, i.d. 1 mm, o.d. 2 mm maximum, convenient length 50–80 mm. Available from Kontes, Vineland, NJ, USA.
2. Thermometer—range −2–68°C or −2–80°C (AOCS Specification H 6-40 or H 7-45, respectively), which should be calibrated against a National Institute of Testing traceable thermometer.
3. Glass beaker—600 mL.
4. Heat source—electric hot plate with rheostat control (see Notes, 1).

PROCEDURE

1. Melt the sample, and filter through filter paper to remove any impurities and the last traces of moisture. It is essential that the sample be absolutely dry.
2. Dip at least three clean capillary tubes in the completely liquid sample so that the sample rises about 10 mm high in the tubes. Fuse the end of the tube (where the sample is located) in a small flame, taking care not to burn the fat.
3. Place the tube in a beaker and hold in a refrigerator (see Notes, 2) at 4–10°C for 16 hr (may be done overnight).
4. Remove the tubes from the refrigerator, and attach with a rubber band or by any suitable means to the thermometer so that the lower ends of the melting point tubes are even with the bottom of the mercury bulb of the thermometer.
5. Suspend the thermometer in a 600-mL beaker that is about half-full of clear distilled water. The bottom of the thermometer is immersed in the water to the immersion mark.
6. Adjust the starting bath temperature to 8–10°C below the melting point of the sample at the beginning of the test. Agitate the water bath with a small stream of air or other suitable means, and apply gentle heat to increase the bath temperature at the rate of 0.5°C/min.
7. Fats usually pass through an opalescent stage before melting completely. The heating is continued until the tubes are completely clear throughout. Observe the temperature at which each tube becomes clear and calculate the average of all tubes. Results should agree within 0.5°C. Report this average as the melting point.

NOTES

1. A water bath with thermostat may be used for closer temperature control.
2. The sample must be completely liquid when the tubes are placed in the refrigerator. It is good practice to pass the ends of the tubes containing the sample momentarily through the flame just before they are taken to the refrigerator.

AOCS Official Method Cc 18-80
Reapproved 1997

Dropping Point

DEFINITION

The dropping point of a fat or oil is the temperature at which the sample will become fluid to flow under the conditions of the test.

SCOPE

Applicable to hydrogenated and nonhydrogenated fats and oils that solidify sufficiently when held at −5°C or lower for the allotted time.

APPARATUS

1. Mettler FP90 control unit and FP83HT dropping point furnace or their equivalent. Mettler Instrument Corp., Hightstown, NJ, USA (see Notes, 1).
2. Sample cups—drip hole diameter 0.11 in., Mettler no. 18712, or equivalent.
3. Sample cup holder—Mettler no. 18711, or equivalent.
4. Sample collecting sleeve—Mettler no. 18713, or equivalent.
5. Freezer—capable of maintaining samples at a temperature of −5°C or lower (see Notes, 2).

SAMPLE CONDITIONING

1. Place a sample cup in the freezer on a clean smooth surface at least 10 min prior to filling. The sample cup must be absolutely clean and free from any residual fat or oil.
2. Melt the sample and mix thoroughly.
3. Fill the sample cup in the freezer to the top with the melted sample.
4. Allow the cup to stand in the freezer for 15 min after filling.

PROCEDURE

1. Preheat the dropping point furnace to a temperature of at least 5°C below the dropping point of the sample (see Notes, 3).
2. Set the heating rate of the dropping point apparatus at 1°C.
3. Insert the sample cup into the cartridge and remove from the freezer, avoiding repeated handling of the filled sample cup.
4. Place the sample holder immediately into the dropping point furnace, making certain that it is seated properly.
5. Activate the heater.
6. Read and record the dropping point temperature from the display board.

PRECISION

1. Table 1 lists the expected precision at a confidence level of 95% for Mettler dropping point values above and below 33°C.

Table 1
Expected precision of Mettler dropping point determinations.

At a confidence level of 95% in the range of values of:	Below 33°C	Above 33°C
Two single determinations on the same sample performed in one laboratory should not differ by more than	1.5°C	0.7°C
The average of duplicate determinations on the same sample performed by two or more laboratories should not differ for the mean value by more than	±1.6°C	±1.0°C

NOTES

1. Based on a collaborative study carried out by the AOCS Commercial Oils and Fats Analysis Committee in 1993, the following recommendations are made regarding the operation and standarization of the apparatus:
   (a) Diode voltage should be between 8 and 12 volts. Low diode voltage (<8) may result in the failure of the photo detector to detect the initial drop of clear materials, in particular, standard reference materials such as lauric acid.
   (b) When using clear standard reference materials, such as lauric acid, finely powdered carbon should be added to the reference material to improve detector response. The powdered carbon will not affect the melting point. When powdered carbon is used, the sample cup should be cleaned thoroughly.
   (c) A test should be considered valid only if the following two criteria are met: it is verified that some sample has indeed fallen into the collection sleeve, and, at least half of the original sample still remains in the sample cup. Any test not meeting these two requirements should be considered invalid and the test repeated. Continuous failure of the tests could indicate a malfunctioning instrument, and the manufacturer should be contacted.
2. Optimum precision is obtained for samples with dropping points below 33°C when a freezer temperature of −20°C or lower is used.
3. In cases where the dropping point of the sample requires the furnace temperature to be set below room temperature, the furnace may be placed in a styrofoam box with dry ice, a refrigerator or other suitable cooling device.

AOCS Official Method Ca 15-75
Reapproved 1997

Analyses for Chromium, Copper, Iron and Nickel in Vegetable Oils by Atomic Absorption Spectrophotometry

DEFINITION

Vegetable oils dissolved in methyl isobutyl ketone (MIBK) are analyzed for metals by direct aspiration. Calibration is based on MIBK solutions of organometallic reference compounds solubilized in purified vegetable oil.

SCOPE

This method is intended for the analysis of crude or partially refined vegetable oils that contain at least 2.0 ppm of each measured metal in the form of oil-soluble derivatives. Hardened oils and animal fats have limited solubilities in MIBK and, even at reduced solute concentrations, they readily foul the burner. For these reasons this method is not recommended for such samples (see Notes, 1).

AOCS Method Ca 18-79 has a lower detection limit (0.1 mg/kg, or 0.1 ppm, for an individual metal) as opposed to 2 mg/kg, or 2 ppm, for AOCS Method Ca 15-75. Therefore, when lower detection limits are required, as in the case of contractual trade methods, AOCS Method Ca 18-79 should be used.

APPARATUS

1. Atomic absorption spectrophotometer—air–acetylene laminar-flow burner head and appropriate hollow cathode lamps.
2. Desiccator—containing fresh phosphorus pentoxide.
3. Weighing bottles—10–15 mL.
4. Hot plate—with rheostat temperature control.
5. Oven.
6. Beakers—25 and 100 mL.
7. Volumetric flasks—25, 50 and 250 mL.
8. Polyethylene bottles—60 and 120 g.
9. Pipets—graduated, to deliver 5 and 50 mL.
10. Balance—top loading, 500-g capacity with ±0.01 g sensitivity.
11. Balance—analytical, 200-g capacity with ±0.0001 g sensitivity.

REAGENTS

1. Organometallic standards—National Institute of Testing (NIST); Conoco, (Ponca City, OK) or equivalent.
   (a) tris(1-phenyl-1,3-butanediono)chromium III.
   (b) bis(1-phenyl-1,3-butanediono)copper II.
   (c) tris(1-phenyl-1,3-butanediono)iron III.
   (d) Nickel cyclohexanebutyrate.
2. 4-Methyl-2-pentanone (methyl isobutyl ketone, MIBK)—reagent grade.
3. Xylene—reagent grade.
4. 2-Ethylhexanoic acid—reagent grade.
5. 2-Ethylhexylamine—reagent grade.
6. Purified vegetable oil (base oil)—Corn or soybean oil containing not more than 0.02 ppm Cr, Cu, Ni or Fe can be prepared by distilling a low-ash refined oil on a rotating-disk molecular still, rejecting the first and last 15% fractions.
7. Fresh desiccant—Calcium chloride is not recommended. See AOCS Specification H 9-87.

PREPARATION OF STANDARDS

1. Chromium stock standard, 100 µg/g—Dry about 0.1 g tris(1-phenyl-1,3-butanediono)chromium III 1 hr at 110°C. Weigh 51.6 mg into a 25-mL beaker, add 3 mL xylene, add 3 mL 2-ethylhexanoic acid and warm on hot plate without refluxing, swirling occasionally until salt dissolves. Let cool to ambient. Transfer solution to a tared 60-g polyethylene bottle, rinse beaker with five 5-mL portions of base oil, bring the contents to 50 mL with base oil, cap with nitrogen, mix and store in a cool location. Standard solution should be stable for at least 6 months.
2. Copper stock standard, 100 µg/g—Dry about 0.05 g bis(1-phenyl-1,3-butanediono)copper II 30 min at 110°C. Weigh 30.3 mg into a 25-mL beaker, add 2 mL xylene, add 2 mL 2-ethylhexylamine and warm on hot plate without refluxing, swirling occasionally until salt dissolves. Let cool to ambient. Transfer solution to a tared 60-g polyethylene bottle, rinse beaker with five 5-mL portions of base oil, bring the contents to 50 mL with base oil, cap with nitrogen, mix and store in a cool location. Standard solution should be stable for at least 6 months.
3. Iron stock standard, 100 µg/g—Dry about 0.1 g tris(1-phenyl-1,3-butanediono)iron III for 1 hr at 100°C. Weigh 48.6 mg into a 25-mL beaker, add 3 mL xylene, add 3 mL 2-ethylhexanoic acid and warm on hot plate without refluxing, swirling occasionally until salt dissolves. Let cool to ambient. Transfer solution to a tared 60-g polyethylene bottle, rinse beaker with five 5-mL portions of base oil, bring the contents to 50 mL with base oil, cap with nitrogen, mix and store in a cool location. Standard solution should be stable for at least 6 months.
4. Nickel stock standard, 100 µg/g—Desiccate about 0.05 nickel cyclohexane butyrate (13.89% Ni) for 48 hr over phosphorus pentoxide. Quickly weigh 36.0 mg into a 25-mL beaker, add 3 mL xylene, add 3 mL 2-ethylhexanoic acid and warm on hot plate without refluxing,

swirling occasionally until salt dissolves. Let cool to ambient. Transfer solution to a tared 60-g polyethylene bottle, rinse beaker with five 5-mL portions of base oil, bring the contents to 50 mL with base oil, cap with nitrogen, mix and store in a cool location. Standard solution should be stable for at least 6 months.

5. Mixed stock standard, 20 µg/g Cr, Cu, Fe and Ni—Weigh carefully by additions into a tared 120-g bottle 20 ± 0.02 g each of the 100 µg/g Cr, Cu, Fe and Ni stock standards, and 20 ± 0.02 g base oil. Purge with nitrogen, cap and mix.
6. Mixed working standards—Weigh 50 ± 0.1 g base oil into a 100-mL beaker, transfer to 250-mL volumetric flask with MIBK, dilute to volume, and mix (20% wt/vol base oil in MIBK). Weigh accurately (± 5 mg) 5.00, 3.75, 2.50, 1.25 and 0.625 g mixed stock standard into 50-mL volumetric flasks. In order, add by pipet 25.0, 31.3, 37.5, 43.8 and 46.9 mL of the 20% base oil in MIBK solution. Dilute to volume with MIBK and mix. These solutions contain 2.0, 1.5, 1.0, 0.5 and 0.25 µg/mL Cr, Cu, Ni and Fe. Retain the remaining 20% base oil solution for zeroing the spectrophotometer and, if necessary, diluting the sample oil.

PROCEDURE

1. Weigh accurately 5.0 ± 0.0005 g of sample oil into a 25-mL volumetric flask, dilute with MIBK and mix.
2. Set up the instrument for operation with an air–acetylene–organic solvent flame according to the manufacturer's recommendations (see Notes, 2).
3. Install the appropriate hollow cathode lamp, turn on the instrument, adjust to the recommended operating lamp current, set the monochromator to the recommended wavelength (see Table 1) and allow the instrument to stabilize.

Table 1
Recommended wavelengths.

Element	Wavelength, nm
Cr	357.9
Cu	324.8
Fe	248.3
Ni	232.0

4. Light the burner and allow to warm to equilibrium (approx. 3 min).
5. While aspirating the 20% base oil solution, reduce the acetylene flow until the yellow incandescence of the flame just disappears. Next, optimize the fuel–air ratio for the determination (Notes, 3) by varying the acetylene flow while aspirating one of the mixed standards until a maximum absorption is obtained. Flush the burner by aspirating MIBK for 15 sec (Notes, 4) and then zero the instrument while aspirating the 20% base oil solution.
6. Record absorptions for the five mixed working standards in order of increasing concentration. Record the absorption of the sample solution and then aspirate the 20% base oil solution to check the zero point (Notes, 5). If the sample absorption exceeds the highest standard, prepare a new sample solution by weighing a suitable ratio of sample oil to base oil to produce a 20% oil solution that absorbs within the calibration range and repeat the absorption measurements.
7. From the standard absorptions, construct a calibration curve to be used to determine the concentration (µg/mL) of metal in the sample solution.
8. Repeat Procedure, 3–7 for each analysis, using the appropriate source lamp and conditions.

CALCULATIONS

1. Metal, µg/g (ppm) $= C \times \frac{25}{W}$

Where—
C = metal in µg/mL from calibration graph
W = mass of sample, g in 25 mL of sample solution

PRECISION

1. Table 2 gives the standard deviations for each metal analysis. Eight collaborators analyzed two samples of soybean oil. Test solutions were retained and absorption measurements were repeated on the following day.

Table 2
Precision.

		Standard deviations		
Element	Level, µg/g	Day 1	Day 2	Average
Cr	2.5	0.0536	0.1155	0.084
	8.5	0.2654	0.3007	0.283
Cu	3.5	0.1137	0.1344	0.124
	9.5	0.1574	0.1940	0.176
Fe	2.0	0.0627	0.1640	0.113
	8.0	0.4990	0.2875	0.393
Ni	3.0	0.3599	0.3239	0.342
	9.0	0.3159	0.2797	0.298

NOTES

Caution—Xylene is flammable and a dangerous fire risk. The TLV is 100 ppm in air.

Methyl isobutyl ketone (MIBK) is flammable and a dangerous fire risk. Explosive limits in air are 1.4–7.5%. Avoid inhalation and ingestion. It is absorbed by the skin. The TLV is 50 ppm in air.

Chloroform is a known carcinogen. It is toxic by inhalation and has anesthetic properties. Avoid contact with the skin. Prolonged inhalation or ingestion can lead to liver and kidney damage and may be fatal. It is nonflammable, but will burn on prolonged exposure to flame or high temperature. The TLV is 10 ppm in air. A fume hood should be used at all times when using chloroform.

NUMBERED NOTES

1. Mixed solvent systems, i.e., chloroform–MIBK, may reduce fat solubility problem and lessen burner fouling, but it will be necessary to prepare the standards in the same solvent system. However, the use of chloroform is

354

not recommended due to the potential of forming phosgene gas under the test conditions.

2. If a Boling burner head is used, be sure that it is designed to withstand the highly oxidizing conditions required for the nickel determination. Boling burners of an early design could be permanently warped under these conditions.
3. Chromium requires a rich (yellow) flame, copper and iron a lean (blue) flame and nickel a very lean flame.
4. The MIBK flush removes contaminated oil and minimizes fouling of the burner head by oil buildup.
5. With correct fuel adjustment, a linear calibration curve that intercepts the origin can usually be obtained.

AOCS Recommended Practice Ca 15b-87
Reapproved 1997

Sodium and Calcium by Atomic Absorption Spectrophotometry

DEFINITION
Sodium (sodium soap) and calcium (calcium soap) present in a fat or oil sample are acidified and then extracted into a known volume. The sodium and calcium concentrations are measured using flame AAS and can be directly measured after calibration of the instrument.

SCOPE
The method is applicable to unemulsified fats and oils.

APPARATUS

1. Atomic absorption spectrometer—Perkin–Elmer 306, or equivalent, equipped for flame analysis.
2. Sodium hollow cathode lamp—Perkin–Elmer 303-6065, or equivalent.
3. Calcium hollow cathode lamp—Perkin–Elmer 303-6017, or equivalent.
4. Screw-cap culture tubes—16 × 125 mm (Corning #9825, or equivalent).
5. Disposable glass pipets.
6. Volumetric pipets—1, 4, 5, 10, 20 and 25 mL, class A.
7. Volumetric flasks—50 and 1000 mL, class A.

REAGENTS

1. Sodium standard solution—aqueous, 1000 ppm (Fisher SO-S-139, or equivalent). See Notes, 1.
2. Calcium standard solution—aqueous, 1000 ppm (Fisher SC-S-191, or equivalent). See Notes, 1.
3. Deionized water.
4. Potassium chloride (KCl)—reagent grade.
5. Methylene chloride—reagent grade (see Notes, *Caution*).
6. 0.2 N Hydrochloric acid (HCl) standard volumetric solution—prepared by diluting the contents of two 0.1 N HCl volumetric concentrates (Sargent–Welch Acculate®, SC 10064, or equivalent) to 100 mL with deionized water. Alternately, the appropriate volume of concentrated HCl, suitable for trace metal analysis, can be diluted to 1000 mL with deionized water.
7. 0.2 N HCl wash solution—prepared as noted in Reagents, 6.
8. 0.2 N HCl/KCl solution—prepared by diluting the contents of two 0.1 N HCl concentrates (see Reagents, 6) and two 0.1 N KCl volumetric concentrates (Sargent-Welch Acculate®, SC 16942, or equivalent) to 1000 mL with deionized water. Alternately, the appropriate volume of concentrated HCl, suitable for trace metal analysis, and the appropriate weight of reagent-grade KCl can be diluted to 1000 mL with deionized water.
9. Sodium working solution (5.00 ppm Na)—Pipet 5 mL of the 1000-ppm sodium standard solution into a 1000-mL volumetric flask and dilute to volume with deionized water.
10. Calcium working solution (25.0 ppm Ca)—Pipet 25 mL of the 1000-ppm calcium standard solution into a 1000-mL volumetric flask and dilute to volume with deionized water.

PROCEDURE

1. Instrument setup (sodium)—
   Sodium lamp—10 ma
   Wavelength—589 nm
   Slit—0.7 mm
   Background correction—off
   Flame—air/acetylene (lean, blue)
2. Instrument setup (calcium)—
   Calcium lamp—12 ma
   Wavelength—422.7 nm
   Slit—0.7 mm
   Background correction—off
   Flame—air/acetylene (reducing, rich, slightly yellow)
3. Preparation of standard and sample—
   (a) Wash all glassware, first with dilute HCl (0.2 N) and then with copious amounts of deionized water.
   (b) Weigh 4.0 g [see Procedure, 4, (c) or Procedure, 5, (e) below] of sample to the nearest mg into an acid-washed screw-top culture tube.
   (c) Add 3.0 mL of methylene chloride to each tube.
   (d) Pipet 4.00 mL of 0.2 N HCl/KCl solution into each tube.
   (e) Cap and shake for 2 min.
   (f) Centrifuge for 30 sec.
   (g) Prepare 0.5-, 1.0- and 2.0-ppm sodium standards by pipetting 5, 10 and 20 mL, respectively, of the sodium working solution (Reagents, 9) into 50-mL volumetric flasks. These are then diluted to volume using the same matrix as the samples (0.2 N HCl/KCl).
   (h) Prepare 0.5-, 2.5- and 5.0-ppm calcium standards by pipetting 1, 5 and 10 mL, respectively, of the calcium working solution (Reagents, 10) into 50-mL volumetric flasks. These are then diluted to volume using the same matrix as the samples (0.2 N HCl/KCl).
4. Analysis for sodium—
   (a) Light the flame and optimize the nebulizer, burner and flame settings while aspirating a 2.0-ppm sodium standard.
   (b) Zero the instrument using the 0.2 N HCl/KCl blank.
   (c) Check the absorbances of the 0.5-, 1.0- and 2.0-ppm sodium standards for response and linearity.
   (d) Switch to the autoconcentration mode and calibrate at 0 (zero), 0.5 and 2.0 ppm sodium.

(e) Aspirate the aqueous layer of each sample tube and read the sodium concentrations directly from the instrument. For accuracy, analysis of samples containing >3 ppm sodium should be repeated, using an adjusted sample size. Recommended sample sizes are as follows:

Sodium content ppm	Sample mass, g
0.10 ≤ 3.0	4.0
3 ≤ 10	1.0
10 ≤ 20	0.5
> 20	0.2

5. Analysis for calcium—
   (a) Light the flame and optimize the nebulizer, burner and flame settings while aspirating 5.0-ppm calcium standard.
   (b) Zero the instrument using the 0.2 N HCl/KCl blank.
   (c) Check the absorbances of the 0.5-, 2.5- and 5.0-ppm calcium standards for response and linearity.
   (d) Switch to the autoconcentration mode and calibrate at 0 (zero), 0.5 and 5.0 ppm calcium.
   (e) Aspirate the aqueous layer of each sample tube and read the calcium concentration directly from the instrument. For accuracy, analysis of samples containing >5 ppm calcium should be repeated, using an adjusted sample size. Recommended sample sizes are as follows:

Sodium content ppm	Sample mass, g
0.10 ≤ 5.0	4.0
5 ≤ 10	1.0
10 ≤ 20	0.5
> 20	0.2

CALCULATIONS

1. Sodium content is calculated using the formulas

$$\frac{\text{ppm Na (reading)} \times 4}{\text{mass of sample, g}} = \text{ppm Na}$$

ppm sodium oleate = ppm Na × 13.2

2. Calcium content is calculated using the formulas

$$\frac{\text{ppm Ca (reading)} \times 4}{\text{mass of sample, g}} = \text{ppm Ca}$$

ppm calcium oleate = ppm Ca × 15

3. Soap is calculated using the formula

ppm soap = ppm calcium oleate + ppm sodium oleate

NOTES

Caution

Methylene chloride is toxic and is a carcinogen. The TLV is 100 ppm in air. A fume hood should be used at all times when using methylene chloride.

Hydrochloric acid is a strong acid and will cause severe burns. Protective clothing should be worn when working with this acid. It is toxic by ingestion and inhalationa and a strong irritant to eyes and skin. The use of a properly operating fume hood is recommended. When diluting the acid, always add the acid to the water, never the reverse.

NUMBERED NOTES

1. The calcium and sodium standards are stable for one week.

AOCS Official Method Ca 18-79
Reapproved 1997

Analysis for Chromium, Copper, Iron, Nickel and Manganese in Triglyceride Oils by Atomic Absorption Spectrophotometry Using a Graphite Furnace

DEFINITION

Vegetable and animal oils dissolved in methyl isobutyl ketone (MIBK) or MIBK + HNO_3 are analyzed for metals by direct injection into a graphite tube furnace. Calibration is based on MIBK or MIBK + HNO_3 solutions of organometallic reference compounds.

SCOPE

This method is intended for the analysis of crude and refined vegetable oils and animal fats that contain trace metals that may be present in the form of oil-soluble adducts. The detection limit of this method is approximately 0.1 mg/kg (0.1 ppm). This method has a lower detection limit than AOCS Method Ca 15-75, which has a detection limit of approximately 2 mg/kg (2 ppm). Therefore, when lower detection limits may be required, as in the case of contractual trade methods, AOCS Method Ca 18-79 should be used.

APPARATUS

1. Atomic absorption spectrophotometer—with Zeeman or Smith–Hieftje background correction, graphite tube furnace and appropriate hollow cathode lamps.
2. Vacuum desiccator—containing a suitable *fresh* desiccant (see AOCS Specification H 9-87).
3. Weighing bottles—10–15-mL capacity.
4. Hot plate—with rheostat temperature control.
5. Oven.
6. Beakers—25 and 100 mL.
7. Pipets—graduated, to deliver 5, 10 and 20 mL.
8. Eppendorf pipets—5, 10, 20, 30, 40 and 50 µL, with disposable tips.
9. Balance—analytical, 200-g capacity with ±0.0001 g sensitivity.

REAGENTS

1. Organometallic standards—National Institute of Testing (NIST); Conoco (Ponca City, OK), USA, or equivalent.
   (a) tris(1-phenyl-1,3-butanediono)chromium III.
   (b) bis(1-phenyl-1,3-butanediono)copper II.
   (c) tris(1-phenyl-1,3-butanediono)iron III.
   (d) Nickel cyclohexanebutyrate.
   (e) Manganous cyclohexanebutyrate.
2. 4-methyl-2-pentanone (methyl isobutyl ketone, MIBK)—reagent grade (see Notes, *Caution*).
3. Xylene—reagent grade (see Notes, *Caution*).
4. 2-Ethylhexanoic acid—practical grade.
5. 2-Ethylhexylamine—practical grade.
6. Nitric acid—Spectral or Ultrex grade (see Notes, *Caution*).

PREPARATION OF STANDARDS

1. Chromium stock standard, 100 µg/mL—Dry about 2 g tris(1-phenyl-1,3-butanediono)chromium III 1 hr at 110°C. Weigh 103.1 mg into a 25-mL beaker, add 5 mL xylene and 5 mL 2-ethylhexanoic acid and gently warm on hot plate without refluxing, swirling occasionally until the salt dissolves. Cool to ambient temperature. Transfer the solution to a 100-mL volumetric flask, rinsing the beaker with five 10-mL portions of MIBK, then dilute to exactly 100 mL, mix and store at 20 ± 2°C. This standard stock solution should be stable for at least 6 months.
2. Copper stock standard, 100 µg/mL—Dry about 0.13 g bis(1-phenyl-1,3-butanediono)copper II 30 min at 110°C. Weigh 60.6 mg into a 25-mL beaker, add 4 mL xylene and 4 mL ethylhexylamine and gently warm on hot plate without refluxing, swirling occasionally until the salt dissolves. Cool to ambient temperature. Transfer the solution to a 100-mL volumetric flask, rinsing the beaker with five 10-mL portions of MIBK, then dilute to exactly 100 mL, mix and store at 20 ± 2°C. This standard stock solution should be stable for at least 6 months.
3. Iron stock standard, 100 µg/mL—Dry about 0.2 g tris(1-phenyl-1,3-butanediono)iron III for 1 hr at 110°C. Weigh 97.1 mg into a 25-mL beaker, add 5 mL xylene and 5 mL 2-ethylhexanoic acid and gently warm on hot plate without refluxing, swirling occasionally until the salt dissolves. Cool to ambient temperature. Transfer the solution to a 100-mL volumetric flask, rinsing the beaker with five 10-mL portions of MIBK, then dilute to exactly 100 mL, mix and store at 20 ± 2°C. This standard stock solution should be stable for at least 6 months.
4. Nickel stock standard, 100 µg/mL—Desiccate about 0.15 g nickel cyclohexanebutyrate for 48 hr over suitable fresh desiccant. Rapidly weigh 67.6 mg into a 25-mL beaker, add 5 mL xylene and 5 mL 2-ethylhexanoic acid and gently warm on hot plate without refluxing, swirling occasionally until the salt dissolves. Cool to ambient temperature. Transfer the solution to a 100-mL volumet-

ric flask, rinsing the beaker with five 10-mL portions of MIBK, then dilute to exactly 100 mL, mix and store at 20 ± 2°C. This standard stock solution should be stable for at least 6 months.

5. Manganese stock standard, 100 µg/mL—Desiccate about 0.15 g manganese cyclohexanebutyrate for 48 hr over suitable fresh desiccant. Rapidly weigh 71.4 mg into a 25-mL beaker, add 5 mL xylene and 5 mL 2-ethylhexanoic acid and gently warm on hot plate without refluxing, swirling occasionally until the salt dissolves. Cool to ambient temperature. Transfer the solution to a 100-mL volumetric flask, rinsing the beaker with five 10-mL portions of MIBK, then dilute to exactly 100 mL, mix and store at 20 ± 2°C. This standard stock solution should be stable for at least 6 months.

WORKING STANDARDS

1. Fe—Pipet 10 µL of 100 ppm stock standard into a 25-mL volumetric flask. Add 15 mL MIBK, 12 drops concentrated HNO_3, swirl, dilute with MIBK to a final volume of exactly 25 mL and mix completely.
2. Cu—Pipet 10 µL of 100 ppm stock standard into a 25-mL volumetric flask, dilute with MIBK to a final volume of exactly 25 mL and mix completely.
3. Ni—Pipet 10 µL of 100 ppm stock standard into a 25-mL volumetric flask. Add 15 mL MIBK, 600 µL concentrated HNO_3, swirl, dilute with MIBK to a final volume of exactly 25 mL and mix completely.
4. Mn—Pipet 10 µL of 100 ppm stock standard into a 25-mL volumetric flask, dilute with MIBK to a final volume of exactly 25 mL and mix completely.
5. Cr—Pipet 10 µL of 100 ppm stock standard into a 25-mL volumetric flask, dilute with MIBK to a final volume of exactly 25 mL and mix completely.

PROCEDURE

1. Accurately weigh 1.0 g ± 0.1 mg of oil into a 10-mL volumetric flask, dilute to volume with appropriate solvent system [see (a) through (e) immediately following] and mix (see Notes, 1).
   (a) Fe–MIBK + 250 µL concd HNO_3/10 mL MIBK
   (b) Ni–MIBK + 250 µL concd HNO_3/10 mL MIBK
   (c) Cu–MIBK
   (d) Mn–MIBK
   (e) Cr–MIBK
2. Set up instrument for operation with the following graphite furnace conditions:

Dry	30 sec	100°C
Char	20 sec	800°C
Atomize	20 sec	2300°C[a]
Argon purge gas	5–6 L/min	

[a]2300°C is the upper limit for P-E HGA-2000 with grooved graphite tubes. If either HGA-2100 or HGA-2200 is available, atomization at 2500°C is preferred.

3. Install the appropriate hollow cathode lamp, turn on the instrument, turn on background corrector, adjust to the recommended operating lamp current, set the monochromator to the recommended wavelength (Table 1), set monochromator slit and allow the instrument to stabilize. After the instrument has stabilized, balance hollow cathode lamp and background corrector energy levels.

Table 1
Recommended wavelengths.

Element	Wavelength, nm
Fe	248.3
Cu	324.8
Ni	232.0
Mn	279.5
Cr	357.9

4. Record absorbance readings for the standard curve of interest by preparing at least a five-point curve. By varying injection volume, a single working standard solution can be used to establish the entire curve. Inject 10-, 20-, 30-, 40- and 50-µL volumes of working standard solution in which the absorbance of at least three determinations at each injection volume agree within ± 0.005 absorbance units. Plot the curve for the metal of interest using the average absorbance as the Y axis and concentration in µg as the X axis.
5. Sample analysis—
   (a) Inject 20 µL of sample as a first estimate. If an unacceptable absorbance value is obtained (either a low absorbance value or a high absorbance value that exceeds the working linear range of the standard curve), the sample injection volume must be adjusted accordingly (see Notes, 2). With P-E grooved graphite tubes, individual injection volumes must not exceed 50 µL.
   (b) For samples with unusually low metal content (see Notes, 3), multiple sample injections are permissible, if, after each injection, the sample is allowed to proceed through the dry and char cycle, and is then interrupted for each additional injection until the final aliquot is injected, in which case the dry/char/atomize cycle is not interrupted, but allowed to continue through atomization (see Notes, 4).
   (c) The absorbance value is then compared to the standard curve and the µg value for the sample is obtained from the X axis.
6. Repeat Procedure, 3–5 for each analysis using the appropriate source lamp (as noted in Table 1) and conditions.

CALCULATIONS

1. $$\text{Metal, ppm} = \frac{(1000)(C)(SV)(F)^*}{(IV)(SM)}$$

Where—
C = metal in mg from calibration graph
SV = final volume of sample flask
IV = volume, mL of sample injected into furnace
SM = sample mass in grams
F = dilution factor*, where F = volume of dilution flask/aliquot taken

*Use dilution factor only when dilution is made because initial sample preparation is too concentrated.

PRECISION

1. Several laboratories participated in the collaborative study of the graphite furnace technique used to determine copper, iron, nickel, chromium and manganese at levels below 1 ppm and mostly at 0.2 ppm and under. Results showed a relative standard deviation (RSD) of 15 and 30% at levels of 0.2 ppm and 0.1 ppm, respectively. At the 0.05 ppm level, RSD values of 50% and above were frequently observed.

NOTES

Caution

Methyl isobutyl ketone (MIBK) is flammable and a dangerous fire risk. Explosive limits in air are 1.4–7.5%. Avoid inhalation and ingestion. It is absorbed by the skin. The TLV is 50 ppm in air.

Xylene is flammable and a dangerous fire risk. The TLV is 100 ppm in air.

Nitric acid—Use effective acid-resistant fume-removal device whenever heating acids or performing reactions that liberate acid fumes. When diluting acids, always add acid to water unless otherwise directed in a method. Keep acids off skin and protect eyes when working with acids. If acids come in contact with skin or eyes, wash immediately with large amounts of water. Do not store oxidizing acids (perchloric, nitric, sulfuric) near organic materials. Mixing organic materials with these acids, particularly perchloric, could result in an explosion.

NUMBERED NOTES

1. Achieving a 10% wt/vol MIBK solution for C_{16} and C_{18} fatty acid triglycerides is readily accomplished down to an iodine value (IV) of 65–70. Below this IV, solubility decreases to approximately 2% wt/vol for triglycerides with an IV of 0–10. Lauric stearines, however, are soluble in MIBK at 7–8% wt/vol.
2. The following is a guide to the range of linear response to the metals of interest:

Fe	2.4 ng
Cu	8.0 ng
Ni	11.0 ng
Mn	2.0 ng
Cr	2.0 ng

3. For a sample with either an unusually low metal content or a troublesome sample matrix, the "standard method of addition" technique is recommended.
4. It is recommended that the purge gas *not* be interrupted during the atomization cycle. Past experience indicates that during the final stage of triglyceride decomposition when moving from the char to the atomization mode, a last surge of smoke is liberated from the sample. The background corrector is capable of compensating the magnitude of the smoke scatter effect. However, when operating in a purge gas interrupt mode during atomization, a nonuniform and poorly reproducible smoke pattern results in an apparent scatter of sample response. Operating in "purge gas on" mode during atomization may cause some loss in sensitivity, but with "purge gas on" it transforms or mixes the smoke into a uniform gray field that is reproducibly corrected.

REFERENCES

1. Olejko, J.T., *J. Am. Oil Chem. Soc. 53*:480 (1976).

AOCS Official Method Ca 18b-91

Reapproved 1997

Determination of Copper, Iron and Nickel by Direct Graphite Furnace Atomic Absorption Spectrophotometry

DEFINITION

This method is for the determination of trace amounts (µg/kg) of copper, iron and nickel. The method requires the vaporization of the oil or fat in a suitable graphite furnace connected to an atomic absorption spectrometer (previously calibrated using standard solutions of organocompounds of copper, iron and nickel), followed by the measurement of the metal content from the observed absorptions at selected wavelengths. The elements are analyzed sequentially (see References, 1 and 2).

SCOPE

Applicable to the determination of copper, iron and nickel in all types of crude or refined oils and fats, including contaminants from bleaching earth and/or catalysts. This method is equivalent to International Union of Pure and Applied Chemistry standard method 2.631 (References,1 and 2).

APPARATUS

1. Polyethylene- or polypropylene-capped bottles—20 and 50 mL, metal free (see Notes, 1).
2. Micropipets—20 and 50 µL.
3. Pipet tips.
4. Atomic absorption spectrometer—equipped with either "peak height" mode and printer, or "continuous" mode and pen recorder (full-scale response in 0.2 sec), together with appropriate hollow cathode tubes and deuterium background corrector. The spectrometer should be located in a dust-free atmosphere.

   Note—Zeeman or Smith–Hieftje background correction technique is considered superior to the deuterium arc technique.
5. Graphite furnace atomizer—equipped with facilities.
6. Graphite tubes—for use in a furnace atomizer (Apparatus, 5).
7. Chromatographic columns—diameter/height ratio 1/10 (see Notes, 2).
8. Electric oven—regulated at 60 ± 2°C and suitable for heating at 150°C.
9. Water bath.

REAGENTS

1. n-Heptane—analytical grade (see Notes, *Caution*).
2. Light petroleum—bp 40–60°C, analytical grade (see Notes, *Caution*).
3. Sunflower oil—refined, or a similar stable liquid oil with a low metal content (see Notes, 2).
4. Aluminum oxide—chromatographic grade (see Notes, 3).
5. Nitric acid—2 M, free from traces of iron, nickel and copper.
6. Niobium nitrate [$Nb(NO_3)_5$]—aqueous solution 1000 mg/L (see Notes, 4).
7. Standard stock solutions of Fe (10 mg/kg), Ni (10 mg/kg) and Cu (2 mg/kg)—prepared by appropriate dilution of organometallic standards with sunflower oil (Reagents, 3) (see Notes, 5).
8. Standard working solutions—prepared daily by diluting the stock solutions (Reagents, 7) with the low metal content oil (Reagents, 3) to give the following concentrations:
   Copper: 0.05, 0.1, 0.2 mg/kg
   Iron: 0.25, 0.5, 1.0 mg/kg
   Nickel: 0.25, 0.5, 1.0 mg/kg
9. Argon—minimum purity 99.99% (see Notes, 6).

PROCEDURE

1. Treatment of samples and solutions—
   (a) Place all samples and working solutions in the oven (Apparatus, 8) during the period of determination.
   (b) Shake samples vigorously before analysis.
   (c) If the metal content of a crude oil is known to be outside the range specified for the standards (Reagents, 8), dilute the sample with the low metal content oil (Reagents, 3) to bring the metal content within that range.
2. Preparation of apparatus—
   (a) Switch on the atomic absorption spectrometer (Apparatus, 4) and the deuterium (D_2) background corrector. In accordance with the instructions of the manufacturer, adjust lamp current, slit, wavelength and amplification. The required wavelengths (nm) are
   Copper: 324.7
   Iron: 302.1
   Nickel: 232.0
   (b) Optimize the position of the graphite furnace atomizer (Apparatus, 5) in the atomic absorption spectrometer (Apparatus, 4), and set the required programs for the furnace as follows:

	Step	Temp, °C	Ramp time, sec	Hold time, sec	Gas flow, mL/min
For Cu	1	900	50	30	300
	2	2700	1	5	50
For Fe, Ni	1	1200	50	30	300
	2	2700	1	5	50

361

If it is not possible to program the graphite furnace exactly as above, use a comparable program suitable for the equipment (see Notes, 7).

(c) Use a normal graphite tube (see Notes, 8).

(d) Pretreat the pipet tip (Apparatus, 3) by pipetting (using a micropipet; Apparatus, 2) and then discarding 20 µL heptane. The film of heptane remaining on the wall of the tip facilitates a reproducible transfer of the oil sample. The tip must be pretreated before each injection of an oil sample.

DETERMINATION

1. Graphite tube blank—Record the absorption, if any, of the graphite tube (Apparatus, 6) as such and autozero this absorption.
2. Liquid oil blank—By means of a micropipet (Apparatus, 2), inject 20 µL low metal content oil (Reagents, 3) into the graphite furnace (Apparatus, 5), initiate the temperature program and record the absorption.
3. Standardization of apparatus—By means of a micropipet (Apparatus, 2), inject 20 µL of the three standard working solutions of the metal under investigation (Reagents, 8) into the graphite furnace (Apparatus, 5) and record the absorptions.
4. Sample for analysis—
   (a) Oil (liquid) samples—By means of a micropipet (Apparatus, 2), inject 20 µL of the oil sample into the graphite furnace (Apparatus, 5), initiate the temperature program and record the absorption.
   (b) Fat samples (mp 40°C and higher)—By means of a micropipet (Apparatus, 2), introduce an extra temperature programming step: hold time 20 sec, temperature 60°C, internal gas flow 0 mL/min. Initiate the temperature program. Within the first program step introduce, by means of a micropipet (Apparatus, 2), 20 µL of the melted fat into the graphite furnace (Apparatus, 5), allow the tip to remain in the injection opening to liquify the fat and then inject. Record the absorption (see Notes, 9).
5. Number of determinations—Carry out two determinations in rapid succession.

CALCULATIONS

1. Measure the peak height on the recorder chart, or read from the display or printer.
2. Draw a calibration curve by plotting the absorption of the three standards (Determination, 3), corrected for the blank (Determination, 2), against their respective metal contents (see Notes, 10).
3. Read the metal content of the sample from the relevant calibration curve.
4. Report as the final result the mean of the results of the two determinations, provided the requirements for repeatability (Precision, 1) are met. If the requirements for repeatability are not met, discard the results and carry out two additional determinations on the test sample.

PRECISION

1. Repeatability value—The difference between the values obtained from two single determinations, carried out in rapid succession by the same operator, using the same apparatus for the analysis of the same test sample, should not be greater than the repeatability value (r) as calculated from the formulas in Table 1, which express the precision in relation to the determined mean value.
2. Reproducibility value—The difference between the values for the final result, obtained by two (or more) laboratories using this standard method for the analysis of the same laboratory sample, should not be greater than the reproducibility value (R) as calculated from the formulas in Table 1, which express the precision in relation to the determined mean value.

Table 1
Repeatability (r) and reproducibility (R) values.

Metal	Substrate	r	R
Cu	oil	0.0102 + 0.140 m	0.0085 + 0.358 m
	fat	0.0076 + 0.109 m	0.0028 + 0.492 m
Fe	oil	0.077 + 0.081 m	0.040 + 0.480 m
	fat	0.026 + 0.196 m	0.031 + 0.543 m
Ni	oil	0.056 + 0.127 m	0.027 + 0.442 m

m = corresponding mean concentration value.

NOTES

Caution

Heptane and light petroleum are flammable and a dangerous fire risk. Vapors may cause lung irritation and may produce neurotoxic effects. A fume hood should be used at all times when using these solvents.

NUMBERED NOTES

1. The polyethylene- or polypropylene-capped bottles are made metal free as follows: Clean the bottles thoroughly with warm nitric acid (Reagents, 5), rinse with distilled water and dry the bottles in a drying oven (Apparatus, 8) at about 80°C.
2. Low metal content oil is obtained as follows: Activate aluminum oxide (Reagents, 4) by heating in an oven (Apparatus, 8) at 150°C for 14 hr. Prepare a chromatographic column (Apparatus, 7), using twice the mass of activated aluminum oxide as the mass of the oil to be purified. Dissolve 1 part of the oil (Reagents, 3) by mass in 3 parts by volume of light petroleum (Reagents, 2). Add the oil solution to the column and elute with 5 parts by volume of light petroleum. Evaporate the light petroleum in the eluate on a heated water bath (Apparatus, 9), using a gentle stream of nitrogen (2–5 L/min). Under vacuum, remove the final traces of light petroleum from the purified oil.
3. Merck product no. 1077 is suitable.
4. Solution (code 88083) is available from Alfa Division, Danvers, MA, USA.
5. Suitable standards are available from Conoco, Ponca City, OK, USA (Conostan, 5000 mg/kg), or Merck, D-6100 Darmstadt, Germany (metal in standard oil, 1000 mg/kg).
6. If argon is not available, nitrogen may be used as purge gas. At temperatures above 2300°C, nitrogen forms

362

toxic cyanogen gas; therefore, continuous ventilation in the furnace area must be provided.

7. If in this case the background correction fails, dilute blank, standards and samples with an organic fat solvent, e.g., heptane, (Reagents, 1) to a maximum of 1:2 (m/m) and work at ambient temperature.
8. Separate graphite tubes should be used for each metal. The graphite tube for the determination of iron has to be coated with niobium to ensure that the total amount of iron is determined. With an uncoated tube the result will vary according to the type of iron compound present in the oil. A suitable coating procedure is as follows: By means of a micropipet (Apparatus, 2), inject 100 µL niobium nitrate solution (Reagents, 6) into the furnace. Start the temperature program to dry at 100°C for 60 sec, and then atomize at 2700°C for 5 sec. Repeat this procedure until 300 µL of niobium nitrate solution has been injected. Atomize at 2700°C until constant absorbance is attained (to remove any iron contamination).
9. The normal, minimum limit of detection can be improved by either a greater scale expansion or by repeated injections of the sample at the end of the ashing operation, then allowing the program to proceed to completion. If the metal content is too high (i.e., exceeds the calibration curve), measure the absorption after a further dilution of the sample with sunflower oil.

   Better precision can be obtained with the use of an autosampler, with sample tray held at 60°C. At 60°C, delivery of different amounts of sample is minimized, because all edible oils have nearly the same density at 60°C. While solvents may be used for sample dilution, their use is discouraged; direct injection is preferred.
10. With the use of sophisticated equipment, autocalibration can be applied.

REFERENCES

1. *Standard Methods for the Analysis of Oils, Fats and Derivatives*, International Union of Pure and Applied Chemistry, 7th edn., Blackwell Scientific Publications, 1987, IUPAC Method 2.631.
2. *Pure Appl. Chem. 60*:893 (1988).

AOCS Official Method Ca 18c-91

Reapproved 1997

Determination of Lead by Direct Graphite Furnace Atomic Absorption Spectrophotometry

DEFINITION

This method is used for the determination of trace amounts (µg/kg) of lead. The method requires the vaporization of the oil or fat in a suitable graphite furnace, with or without a platform, connected to an atomic absorption spectrometer (previously calibrated using standard solutions of an organocompound of lead), followed by the measurement of the metal content from the observed absorption at a wavelength of 283.3 nm (see References, 1 and 2).

SCOPE

Applicable to the determination of lead in all types of crude or refined edible oils and fats. This method is equivalent to International Union of Pure and Applied Chemistry standard method 2.632 (References, 1 and 2).

APPARATUS

1. Polyethylene- or polypropylene-capped bottles—20 mL, metal free (see Notes, 1).
2. Micropipet—20 µL.
3. Pipet tips.
4. Electric oven—regulated at 60 ± 2°C.
5. Atomic absorption spectrometer—equipped with either "peak height" mode and printer, or "continuous" mode and pen recorder (full-scale response in 0.2 sec), together with the appropriate electrode-less discharge lamp (or hollow cathode lamp) and deuterium background corrector (or Zeeman atomic absorption spectrometer).

   Note—Zeeman or Smith–Hieftje background correction technique is considered superior to the deuterium arc technique.
6. Graphite furnace atomizer—placed in the atomic absorption spectrometer (Apparatus, 5), equipped with a control unit for temperature programming.
7. Graphite tube—normal (uncoated).
8. Platform—pyrolytic, in combination with uncoated or pyrolytically coated graphite tube (see Notes, 2).

REAGENTS

1. Cyclohexane—analytical grade (see Notes, *Caution*).
2. Lecithin—well defined, containing 2% phosphorus.
3. Matrix modifier—2% (m/v) Lecithin solution prepared by dissolving 2 g lecithin (Reagents, 2) in 100 mL cyclohexane (Reagents, 1).
4. Blank oil—refined; any edible oil is suitable. Store in a metal-free polyethylene bottle. The lead content of oil should not be greater than 1 µg/kg.
5. Standard stock solution of Pb (10 mg/kg)—prepared by appropriate dilution of an organometallic standard (see Notes, 3) with the blank oil (Reagents, 4).
6. Standard working solutions—prepared daily by diluting the stock solution (Reagents, 5) with the blank oil (Reagents, 4) to give the following concentrations of Pb: 0.020 mg/kg, 0.050 mg/kg and 0.100 mg/kg.
7. Argon—minimum purity 99.99%.

PROCEDURE

1. Treatment of samples, blank and standards—
   (a) Place all samples and standard working solutions in the oven (Apparatus, 4), regulated at 60 ± 2°C.
   (b) Shake samples vigorously.
   (c) Weigh 5.00 g sample in a 20-mL bottle (Apparatus, 1) together with 5.00 g matrix modifier (Reagents, 3) and mix thoroughly.
   (d) Repeat steps (b) and (c) for the three standard working solutions (Reagents, 6) and the blank oil (Reagents, 4).
2. Preparation of apparatus—
   (a) Switch on the atomic absorption spectrometer and the deuterium background corrector. In accordance with the manufacturer's instructions supplied with the spectrometer, adjust the lamp current, slit width, wavelength and amplification. The required wavelength is 283.3 nm.
   (b) Optimize the position of the graphite furnace atomizer (Apparatus, 6) in the atomic absorption spectrometer (Apparatus, 5), and set the required program on the control unit of the furnace. If available, place platform in graphite tube (see Notes, 2).
   (c) Before each injection, pretreat the pipet tip (Apparatus, 3) by pipetting and then discarding 20 µL of cyclohexane.
3. Programs for the graphite furnace atomizer are given in Table 1 (for Varian Spectrometer, see Notes, 4).

DETERMINATION

1. Measurement of the blank—Inject 20 µL of the blank solution prepared according to Procedure, 1 into the graphite furnace, initiate the temperature program and record the absorption.
2. Measurement of standards—Inject 20 µL of the three standard solutions prepared according to Procedure, 1 into the graphite furnace and record the absorptions.
3. Measurement of sample solutions—Inject 20 µL of the sample solution prepared according to Procedure, 1 into the graphite furnace, initiate the temperature program and record the absorption.

CALCULATIONS

1. Measure the peak height on the chart recorder, or take the reading of the display or printer.
2. Draw a calibration curve by plotting the absorption of the three standards (Determination, 2), corrected for the blank (Determination, 1), against their respective metal content (see Notes, 4, 5).
3. Read the metal content of the sample from the calibration curve.

364

Table 1
Programs for the graphite furnace atomizer.

(a) Uncoated tube (Apparatus, 7), off the wall, maximum power heating, gas stop.

Step	Temp, °C	Ramp time, sec	Hold time, sec	Gas flow, mL/min
1	100	10	20	300
2	650	60	40	300
3	1900	0	5	0
4	2700	1	3	50

(b) Pyro-coated tube (Apparatus, 8) with platform, maximum power heating, gas stop.

Step	Temp, °C	Ramp time, sec	Hold time, sec	Gas flow, mL/min
1	200	10	20	300
2	650	60	40	300
3	1700	0	5	0
4	2700	1	3	50

Table 2
Repeatability (r) and reproducibility (R) values.

Pb in edible oil	r = 0.19 m	R = 0.30 m
Pb in cocoa butter	r = 0.15 m	R = 0.68 m

m = corresponding mean concentration value.

4. Report as the final result the mean of the results of two single determinations, provided the requirements for repeatability (see Precision, 1) are met. If the requirements for repeatability are not met, discard the results and repeat two determinations on the test sample.

PRECISION

1. Repeatability value—The differences between the values obtained from two single determinations, carried out in rapid succession by the same operator, using the same apparatus for the analysis of the same test sample, should not be greater than the repeatability value (r) as calculated from the formulas in Table 2, which express the precision in relation to the determined mean value (see Notes, 6).
2. Reproducibility value—The difference between the values for the final result, obtained by two (or more) laboratories using this standard method for the analysis of the same laboratory sample, should not be greater than the reproducibility value (R) as calculated from the formulas in Table 2 which express the precision in relation to the determined mean value (Table 3).

NOTES

Caution

Cyclohexane is flammable and a dangerous fire risk. It is moderately toxic by inhalation and skin contact. The TLV in air is 300 ppm.

Table 3
The interlaboratory test carried out at the international level in 1991 by the International Union of Pure and Applied Chemistry (IUPAC) Commission on Oils, Fats and Derivatives, in which 20 laboratories participated, each obtaining two test results for each sample, gave the statistical results [evaluated in accordance with International Organization for Standardization (ISO) 5725-1986] summarized in the following table.

Statistic	Level 1		Level 2		Level 3	
	Batch 1	Batch 2	Batch 1	Batch 2	Batch 1	Batch 2
Edible Oil (Soybean Oil)						
No. Labs	14	16	16	14	16	16
Pb (mg/kg)[a]	0.080	0.090	0.047	0.054	0.018	0.023
Mean	0.083	0.086	0.046	0.057	0.018	0.022
S_r	0.005	0.006	0.003	0.004	0.002	0.002
S_R	0.007	0.017	0.010	0.005	0.005	0.006
RSD_r	6.1	7.3	5.9	6.7	11.3	11.2
RSD_R	9.0	19.3	22.6	8.5	30.2	28.2
r[b]	0.014	0.018	0.008	0.011	0.006	0.010
R[c]	0.021	0.046	0.029	0.013	0.015	0.020
Cocoa Butter						
No. Labs	15	16	16	15	15	16
Pb (mg/kg)[a]	0.080	0.090	0.047	0.054	0.018	0.023
Mean	0.084	0.090	0.052	0.058	0.024	0.029
S_r	0.004	0.003	0.004	0.002	0.001	0.001
S_R	0.018	0.019	0.012	0.013	0.007	0.008
RSD_r	5.3	3.7	7.0	3.5	4.9	5.0
RSD_R	21.1	20.8	22.3	22.8	29.7	26.9
r[b]	0.012	0.009	0.010	0.006	0.003	0.004
R[c]	0.050	0.053	0.032	0.037	0.020	0.022

[a]Actual values.
[b]$2.8 \times S_r$.
[c]$2.8 \times S_R$.

365

NUMBERED NOTES

1. The polyethylene- or polypropylene-capped bottles are made metal free in the following way: Clean the bottles thoroughly with warm nitric acid, rinse with distilled water and dry the bottles in a drying oven at about 80°C.
2. Both atomization off the wall and atomization off the platform can be used. The accuracy and the sensitivity off the platform is two times higher than off the wall.
3. A suitable standard (Conostan, 5000 mg/kg) is available from Conoco, Ponca City, OK, USA.
4. For those having a Varian spectrophotometer, the modifications shown in Table 4 should be made.
5. With the use of sophisticated equipment, autocalibration can be applied.

REFERENCES

1. *Standard Methods for the Analysis of Oils, Fats and Derivatives*, International Union of Pure and Applied Chemistry, 7th edn., Blackwell Scientific Publications, 1987, IUPAC Method 2.632.
2. *Pure Appl. Chem. 60:*893 (1988).
3. Hendrikse, P.W., Slikkerveer, F.J., Folkersma, A., Dieffenbacher, A., *Pure and Appl. Chem. 63*: 1183–1192 (1991). Report on the collaborative study.

OTHER REFERENCES

*Anal. Chem. 54:*67A (1982).
*At. Spectrosc. 1:*30 (1980).
*J. Assoc. Off. Anal. Chem. 72:*34 (1989).

Table 4
Modifications for Varian spectrophotometer.

(a) Program for uncoated tube (Apparatus, 7), off the wall.

Step	Temp, °C	Time, sec	Gas flow, L/min	Gas type	Read command
1	100	20.0	3.0	Normal	No
2	100	40.0	3.0	Normal	No
3	550	60.0	3.0	Normal	No
4	550	40.0	3.0	Normal	No
5	550	1.0	0.0	Normal	No
6	2500	1.0	0.0	Normal	Yes
7	2500	5.0	0.0	Normal	Yes
8	2500	3.0	3.0	Normal	No

Use 10 µL for injection.

(b) Program for pyro-coated tube (Apparatus, 8), with platform.

Step	Temp, °C	Time, sec	Gas flow, L/min	Gas type	Read command
1	200	10.0	3.0	Normal	No
2	200	20.0	3.0	Normal	No
3	650	60.0	3.0	Normal	No
4	650	40.0	3.0	Normal	No
5	650	1.0	0.0	Normal	No
6	2000	0.7	0.0	Normal	Yes
7	2000	5.0	0.0	Normal	Yes
8	2000	3.0	3.0	Normal	No

Use 10 µL for injection.

3/6

AOCS Official Method Ca 12b-92

Replaces Ca 12b-87 • Reapproved 1997

Phosphorus by Direct Graphite Furnace Atomic Absorption Spectrometry

DEFINITION

This method, based on International Union of Pure and Applied Chemistry (IUPAC) Method 2.423 (see References, 1), determines the total phosphorus present in oils and fats, expressing the value as mg/kg (parts per million, ppm). The method requires the vaporization of the oil or fat in a suitable graphite furnace, with or without a L'vov platform, connected to an atomic absorption spectrometer, previously calibrated using standard solutions of soy lecithin and the measurement of the phosphorus content from the observed absorption at a wavelength of 213.6 nm.

SCOPE

This method is for the determination of trace amounts (mg/kg) of phosphorus in all types of crude or refined edible oils and fats. Spectral interference by other elements is not to be expected, provided the measurements are carried out at the wavelength specific for phosphorus (213.6 nm).

APPARATUS

1. Test tubes—10 mL.
2. Micropipet—20 µL.
3. Pipet tips.
4. Electric oven—regulated at 60 ± 2°C.
5. Atomic absorption spectrometer—equipped with either "peak height" mode and printer, or "continuous" mode and pen recorder (full-scale response in 0.2 sec), together with the appropriate electrode-less discharge lamp (or hollow cathode lamp) and deuterium background corrector (or Zeeman atomic absorption spectrometer).
6. Graphite furnace atomizer—placed in the atomic absorption spectrometer (Apparatus, 5), equipped with a control unit for temperature programming.
7. Graphite tube—normal (uncoated).
8. Platform—pyrolytic, in combination with uncoated graphite tube (see Notes, 1).

REAGENTS

1. Cyclohexane—analytical grade (see Notes, *Caution*).
2. Lanthanum organometallic standard—such as lanthanum-2,4-pentadionate (see Notes, 2). A suitable standard (Conostan, 5000 mg/kg) is available from Conoco, Ponca City, OK, USA.
3. Matrix modifier (see Notes, 2)—0.05% (m/v) Lanthanum solution is prepared by dissolving 5 g lanthanum standard (Reagents, 2) in 50 mL cyclohexane (Reagents, 1), [see Procedure, 1, (c) for use of matrix modifier].
4. Blank oil—refined liquid edible oil with a phosphorus content not greater than 1 mg/kg (see Notes, 3).
5. Lecithin—a well-defined lecithin containing 2% phosphorus, e.g., soy lecithin (see Notes, 3).
6. Standard stock solution—A stock solution of 400 mg P/kg is prepared by dissolving 1 g of lecithin (Reagents, 5) in 4 g cyclohexane (Reagents, 1) and 45 g of blank oil (Reagents, 4).
7. Standard working solutions—10 mg P/kg, 20 mg P/kg and 40 mg P/kg are prepared by diluting the 400 mg/kg stock solution (Reagents, 6) with blank oil (Reagents, 4).
8. Argon—minimum purity 99.99%.

PROCEDURE

1. Treatment of samples, blank and standards—
   (a) Place all samples, blank oil and standard working solutions in the oven (Apparatus, 4), regulated at 60 ± 2°C.
   (b) Shake samples vigorously.
   (c) Weigh 1.00 g sample in a 10-mL test tube together with 1.00 g matrix modifier (Reagents, 3) and mix thoroughly (see Notes, 4).
   (d) Apply steps (b) and (c) to the three standard working solutions (Reagents, 7) and the blank oil (Reagents, 4).
2. Preparation of apparatus—
   (a) In accordance with the manufacturer's instructions supplied with the spectrometer, switch on the atomic absorption spectrometer system and adjust the lamp current, slit width, wavelength and amplification. The required wavelength is 213.6 nm.
   (b) Optimize the position of the graphite furnace atomizer (Apparatus, 6) in the atomic absorption spectrometer (Apparatus, 5), and set the required program on the control unit of the furnace. If available, place platform in graphite tube.
   (c) Pretreat the pipet tip (Apparatus, 3) before each injection by pipetting and then discarding 20 µL cyclohexane.
   (d) Inject 20 µL of the standard working solution with a content of 40 mg P/kg (Reagents, 7) with the micropipet (Apparatus, 2) into the graphite furnace, initiate the temperature program and record the absorption.
   (e) Repeat the previous step until the absorption is constant (see Notes, 5)
3. Program for the graphite furnace atomizer (see Notes, 6):

Step no.	Temp, °C	Ramp time, sec	Hold time, sec	Initial gas flow, mL/min.
1	600	40	20	300
2	1600	50	40	300
3	2800	0	5	0
4	2800	1	3	50

367

DETERMINATION

1. Measurement of the graphite tube blank—Record the absorption, if any, of the graphite tube as such and autozero this absorption (see Notes, 5).
2. Measurement of the blank—Inject 20 µL of the blank solution prepared according to Procedure, 4 into the graphite furnace, initiate the temperature program and record the absorption.
3. Measurement of the working standards—Inject 20 µL of the three standard solutions prepared according to Procedure, 1, (d) into the graphite furnace and record the absorptions.
4. Measurement of sample solutions—Inject 20 µL of the sample solution prepared according to Procedure, 1, (c) into the graphite furnace, initiate the temperature program and record the absorption.

CALCULATIONS

1. Measure the peak height on the recorder chart, or take the reading of the display or printer.
2. Draw a calibration curve by plotting the absorption of the three standards (Determination, 2), corrected for the blank (Determination, 1), against their respective phosphorus content (see Notes, 3).
3. Measure the absorption of the sample and correct for the blank.
4. Read the phosphorus content of the sample from the calibration curve.
5. Express the results as mg/kg (to two significant figures).

PRECISION

1. Repeatability limit—The absolute difference between two independent single test results, obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within short intervals of time, should not be greater than the repeatability limit (r) as calculated from the formulas in Table 1.
2. Reproducibility limit—The absolute difference between two single test results, obtained with the same method on identical test material in different laboratories with different operators using different equipment, should not be greater than the reproducibility limit (R) as calculated from the formulas in Table 1.
3. Trueness (bias)—The bias of the method was demonstrated in the collaborative study of the method (see Table 2) to be negligible when used for the determination of concentrations of phosphorus in the range 10–30 mg/kg.
4. Sensitivity—The sensitivity of the method is demonstrated by the low values for r and R at the low absorption levels studied (see Table 2). The limit of detection is 0.1 mg/kg; the limit of determination is 1 mg/kg.

Table 1
Repeatability (r) and reproducibility (R) limits.

P in edible oil:	$r = 0.19 \times m$	$R = 0.30 \times m$
Where—		
	m = corresponding mean concentration value	

Table 2
Statistical and other data derived from the results of the interlaboratory test.

Sample:	Sunflower oil		
Batch:	A (high)	B (medium)	C (low)
Number of laboratories retained after eliminating outliers	17	17	17
Number of outliers, laboratories	2	1	1
Number of accepted results	32	34	34
Mean value, mg/kg	29.55	20.00	10.18
True or accepted value, mg/kg	28.50	19.00	9.00
Repeatability standard deviation, S_r in mg/kg	2.30	1.68	0.91
Repeatability relative standard deviation, RSD_r, %	7.8	8.4	9.0
Repeatability limit, r, $2.8 \times S_r$	6.45	4.71	2.56
Reproducibility standard deviation, S_R in mg/kg	3.51	2.33	1.24
Reproducibility relative standard deviation, RSD_R, %	11.9	11.7	12.2
Reproducibility limit, R, $2.8 \times S_R$	9.82	6.63	3.48

5. An interlaboratory test carried out at the international level in 1989 by the IUPAC Commission on Oils, Fats and Derivatives, in which 21 laboratories participated, each obtaining two test results for each sample, gave the statistical results [evaluated in accordance with International Organization for Standardization (ISO) 5725-1986] summarized in Table 2 (References, 2).

NOTES

Caution

Cyclohexane is flammable and a dangerous fire risk. It is moderately toxic by inhalation and skin contact. The TLV in air is 300 ppm.

NUMBERED NOTES

1. Both atomization off the wall and atomization off the platform can be used. It is not necessary to change the temperature program for the graphite furnace.
2. The amount of phosphorus found depends on the types of phosphatide present in the oil. The addition of lanthanum proved to be essential to find the total amount of phosphorus. The lanthanum standard is specified to enhance the phosphorus signal and stabilize the phosphorus so it is not lost. Instead of lanthanum, calcium may be used. Conostan metallo-organic standards, including lanthanum, are available from Interchim, France and Conoco, Ponca City, OK, USA.
3. The concentration of phosphorus in the blank oil and in the lecithin is determined by an accepted digestion/colori-

368

metric method, IUPAC 2.421 or AOCS Official Method Ca 12-55. As an alternative to lecithin, a phosphatide of known purity, e.g., phosphatidyl serine, could be used.

4. If the expected or found concentration of phosphorus is higher than 40 mg/kg, dilute the sample with blank oil (Reagents, 4). In that case, multiply the observed concentration (Determination, 4) with the correct dilution factor.
5. With a new graphite tube, three to four "determinations" using one and the same working standard have to be carried out in order to obtain an acceptable state of equilibrium.
6. For those having a Varian apparatus, the following temperature program should be used:

Step no.	Temp, °C	Time, sec	Gas flow, L/min
1	120	20	3.0
2	120	20	3.0
3	500	30	3.0
4	500	10	3.0
5	1600	50	3.0
6	1600	20	3.0
7	1600	2	0.0
8	2800	1	0.0
9	2800	2	0.0
10	2800	5	3.0
11	40	20	3.0

REFERENCES

1. AOCS Official Method Ca 12b-92 replaces AOCS Recommended Practice Ca 12b-87, Phosphorus by Atomic Absorption Spectroscopy. Method Ca 12b-92 was adopted from *Standard Methods for the Analysis of Oils, Fats and Derivatives*, International Union of Pure and Applied Chemistry, 7th edn., Blackwell Scientific Publications, 1987, IUPAC Method 2.423.
2. Collaborative study results published in *Pure Appl. Chem. 63:*1191 (1991).

369

CONSUMER'S GUIDE TO FATS

U. S. Food and Drug Administration
FDA Consumer
May 1994; Revised November 1994 & January 1999

Email this Page To a Friend

A Consumer's Guide to Fats

by Eleanor Mayfield

Once upon a time, we didn't know anything about fat except that it made foods tastier. We cooked our food in lard or shortening. We spread butter on our breakfast toast and plopped sour cream on our baked potatoes. Farmers bred their animals to produce milk with high butterfat content and meat "marbled" with fat because that was what most people wanted to eat.

But ever since word got out that diets high in fat are related to heart disease, things have become more complicated. Experts tell us there are several different kinds of fat, some of them worse for us than others. In addition to saturated, monounsaturated and polyunsaturated fats, there are triglycerides, trans fatty acids, and omega 3 and omega 6 fatty acids.

Most people have learned something about cholesterol, and many of us have been to the doctor for a blood test to learn our cholesterol "number." Now, however, it turns out that there's more than one kind of cholesterol, too.

Almost every day there are newspaper reports of new studies or recommendations about what to eat or what not to eat: Lard is bad, olive oil is good, margarine is better for you than butter--then again, maybe it's not.

Amid the welter of confusing terms and conflicting details, consumers are often baffled about how to improve their diets. Clearly, though, consumers are interested in obtaining this information. In a poll conducted by Nielsen Marketing Research, people were asked to select the food qualities that were "very important" to them, and knowing which foods were low in fat and cholesterol ranked highest:

Percentage of people who said these food qualities were "very important" to them:

food quality	percentage
low caffeine	31.2%
low calorie	38.2%
low sodium	41.3%
low fat/cholesterol	58.6%

Text version of the table.

FDA regulations enable consumers to see clearly on a food product's label how much and what kind of fat the product contains. (See "A Little 'Lite' Reading" in the June 1993 FDA Consumer.) Understanding the terms used to discuss fat is crucial if you want to make sure your diet is within recommended guidelines. (See "Fat Words.")

Fats and Fatty Acids

Fats are a group of chemical compounds that contain fatty acids. Energy is stored in the body mostly in the form of fat. Fat is needed in the diet to supply essential fatty acids, substances essential for growth but not produced by the body itself.

There are three main types of fatty acids: saturated, monounsaturated and polyunsaturated. All fatty acids are molecules composed mostly of carbon and hydrogen atoms. A saturated fatty acid has the maximum possible number of hydrogen atoms attached to every carbon atom. It is therefore said to be "saturated" with hydrogen atoms.

Some fatty acids are missing one pair of hydrogen atoms in the middle of the molecule. This gap is called an "unsaturation" and the fatty acid is said to be "monounsaturated" because it has one gap. Fatty acids that are missing more than one pair of hydrogen atoms are called "polyunsaturated."

Saturated fatty acids are mostly found in foods of animal origin. Monounsaturated and polyunsaturated fatty acids are mostly found in foods of plant origin and some seafoods. Polyunsaturated fatty acids are of two kinds, omega-3 or omega-6. Scientists tell them apart by where in the molecule the "unsaturations," or missing hydrogen atoms, occur.

371

Recently a new term has been added to the fat lexicon: trans fatty acids. These are byproducts of partial hydrogenation, a process in which some of the missing hydrogen atoms are put back into polyunsaturated fats. Some of the hydrogenated fatty acids take on a "straighter" structure: these are the trans fatty acids. "Hydrogenated vegetable oils," such as vegetable shortening and margarine, are solid at room temperature because straightening fatty acids allows them to pack more tightly.

Cholesterol

Cholesterol is sort of a "cousin" of fat. Both fat and cholesterol belong to a larger family of chemical compounds called lipids. All the cholesterol the body needs is made by the liver. It is used to build cell membranes and brain and nerve tissues. Cholesterol also helps the body produce steroid hormones needed for body regulation, including processing food, and bile acids needed for digestion.

People don't need to consume dietary cholesterol because the body can make enough cholesterol for its needs. But the typical U.S. diet contains substantial amounts of cholesterol, found in foods such as egg yolks, liver, meat, some shellfish, and whole-milk dairy products. Only foods of animal origin contain cholesterol.

Cholesterol is transported in the bloodstream in large molecules of fat and protein called lipoproteins. Cholesterol carried in low-density lipoproteins is called LDL-cholesterol; most cholesterol is of this type. Cholesterol carried in high-density lipoproteins is called HDL-cholesterol.

A person's cholesterol "number" refers to the total amount of cholesterol in the blood. Cholesterol is measured in milligrams per deciliter (mg/dl) of blood. (A deciliter is a tenth of a liter.) Doctors recommend that total blood cholesterol be kept below 200 mg/dl. The average level in adults in this country is 205 to 215 mg/dl. Studies in the United States and other countries have consistently shown that total cholesterol levels above 200 to 220 mg/dl are linked with an increased risk of coronary heart disease. (See "Lowering Cholesterol" in the March 1994 FDA Consumer.)

LDL-cholesterol and HDL-cholesterol act differently in the body. A high level of LDL-cholesterol in the blood increases the risk of fatty deposits forming in the arteries, which in turn increases the risk of a heart attack. Thus, LDL-cholesterol has been dubbed "bad" cholesterol.

On the other hand, an elevated level of HDL-cholesterol seems to have a protective effect against heart disease. For this reason, HDL-cholesterol is often called "good" cholesterol.

In 1992, a panel of medical experts convened by the National Institutes of Health (NIH) recommended that individuals should have their level of HDL-cholesterol checked along with their total cholesterol.

According to the National Heart, Lung, and Blood Institute (NHLBI), a component of NIH, a healthy person who is not at high risk for heart disease and whose total cholesterol level is in the normal range (around 200 mg/dl) should have an HDL-cholesterol level of more than 35 mg/dl. NHLBI also says that an LDL-cholesterol level of less than 130 mg/dl is "desirable" to minimize the risk of heart disease.

Some very recent studies have suggested that LDL-cholesterol is more likely to cause fatty deposits in the arteries if it has been through a chemical change known as oxidation. However, these findings are not accepted by all scientists.

The NIH panel also advised that individuals with high total cholesterol or other risk factors for coronary heart disease should have their triglyceride levels checked along with their HDL-cholesterol levels.

Triglycerides and VLDL

Triglyceride is another form in which fat is transported through the blood to the body tissues. Most of the body's stored fat is in the form of triglycerides. Another lipoprotein--very low-density lipoprotein, or VLDL--has the job of carrying triglycerides in the blood. NHLBI considers a triglyceride level below 200 mg/dl to be normal.

It is not clear whether high levels of triglycerides alone increase an individual's risk of heart disease. However, they may be an important clue that someone is at risk of heart disease for other reasons. Many people who have elevated triglycerides also have high LDL-cholesterol or low HDL-cholesterol. People with diabetes or kidney disease--two conditions that increase the risk of heart disease--are also prone to high triglycerides.

Dietary Fat and Cholesterol Levels

Many people are confused about the effect of dietary fats on cholesterol levels. At first glance, it seems reasonable to think that eating less cholesterol would reduce a person's cholesterol level. In fact, eating less cholesterol has less effect on blood cholesterol levels than eating less saturated fat. However, some studies have found that eating cholesterol increases the risk of heart disease even if it doesn't increase blood cholesterol levels.

Another misconception is that people can improve their cholesterol numbers by eating "good" cholesterol. In food, all cholesterol is the same. In the blood, whether cholesterol is "good" or "bad" depends on the type of lipoprotein that's carrying it.

Polyunsaturated and monounsaturated fats do not promote the formation of

artery-clogging fatty deposits the way saturated fats do. Some studies show that eating foods that contain these fats can reduce levels of LDL-cholesterol in the blood. Polyunsaturated fats, such as safflower and corn oil, tend to lower both HDL- and LDL-cholesterol. Edible oils rich in monounsaturated fats, such as olive and canola oil, however, tend to lower LDL-cholesterol without affecting HDL levels.

How Do We Know Fat's a Problem?

In 1908, scientists first observed that rabbits fed a diet of meat, whole milk, and eggs developed fatty deposits on the walls of their arteries that constricted the flow of blood. Narrowing of the arteries by these fatty deposits is called atherosclerosis. It is a slowly progressing disease that can begin early in life but not show symptoms for many years. In 1913, scientists identified the substance responsible for the fatty deposits in the rabbits' arteries as cholesterol.

In 1916, Cornelius de Langen, a Dutch physician working in Java, Indonesia, noticed that native Indonesians had much lower rates of heart disease than Dutch colonists living on the island. He reported this finding to a medical journal, speculating that the Indonesians' healthy hearts were linked with their low levels of blood cholesterol.

De Langen also noticed that both blood cholesterol levels and rates of heart disease soared among Indonesians who abandoned their native diet of mostly plant foods and ate a typical Dutch diet containing a lot of meat and dairy products. This was the first recorded suggestion that diet, cholesterol levels, and heart disease were related in humans. But de Langen's observations lay unnoticed in an obscure medical journal for more than 40 years.

After World War II, medical researchers in Scandinavia noticed that deaths from heart disease had declined dramatically during the war, when food was rationed and meat, dairy products, and eggs were scarce. At about the same time, other researchers found that people who suffered heart attacks had higher levels of blood cholesterol than people who did not have heart attacks.

Since then, a large body of scientific evidence has been gathered linking high blood cholesterol and a diet high in animal fats with an elevated risk of heart attack. In countries where the average person's blood cholesterol level is less than 180 mg/dl, very few people develop atherosclerosis or have heart attacks. In many countries where a lot of people have blood cholesterol levels above 220 mg/dl, such as the United States, heart disease is the leading cause of death.

High rates of heart disease are commonly found in countries where the diet is heavy with meat and dairy products containing a lot of saturated fats. However, high-fat diets and high rates of heart disease don't inevitably go hand-in-hand.

Learning from Other Cultures

People living on the Greek island of Crete have very low rates of heart disease even though their diet is high in fat. Most of their dietary fat comes from olive oil, a monounsaturated fat that tends to lower levels of "bad" LDL-cholesterol and maintain levels of "good" HDL-cholesterol.

The Inuit, or Eskimo, people of Alaska and Greenland also are relatively free of heart disease despite a high-fat, high-cholesterol diet. The staple food in their diet is fish rich in omega-3 polyunsaturated fatty acids.

Some research has shown that omega-3 fatty acids, found in fish such as salmon and mackerel as well as in soybean and canola oil, lower both LDL-cholesterol and triglyceride levels in the blood. Some nutrition experts recommend eating fish once or twice a week to reduce heart disease risk. However, dietary supplements containing concentrated fish oil are not recommended because there is insufficient evidence that they are beneficial and little is known about their long-term effects.

Omega-6 polyunsaturated fatty acids have also been found in some studies to reduce both LDL- and HDL-cholesterol levels in the blood. Linoleic acid, an essential nutrient (one that the body cannot make for itself) and a component of corn, soybean and safflower oil, is an omega-6 fatty acid.

At one time, many nutrition experts recommended increasing consumption of monounsaturated and polyunsaturated fats because of their cholesterol-lowering effects. Now, however, the advice is simply to reduce dietary intake of all types of fat. (Infants and young children, however, should not restrict dietary fat.)

The available information on fats may be voluminous and is sometimes confusing. But sorting through the information becomes easier once you know the terms and some of the history.

The "bottom line" is actually quite simple, according to John E. Vanderveen, Ph.D., director of the Office of Plant and Dairy Foods and

373

Beverages in FDA's Center for Food Safety and Applied Nutrition. "What we should be doing is removing as much of the saturated fat from our diet as we can. We need to select foods that are lower in total fat and especially in saturated fat." In a nutshell, that means eating fewer foods of animal origin, such as meat and whole-milk dairy products, and more plant foods such as vegetables and grains.

Eleanor Mayfield is a writer in Silver Spring, Md.

Fat Words

Here are brief definitions of the key terms important to an understanding of the role of fat in the diet.

Cholesterol
A chemical compound manufactured in the body. It is used to build cell membranes and brain and nerve tissues. Cholesterol also helps the body make steroid hormones and bile acids.

Dietary cholesterol
Cholesterol found in animal products that are part of the human diet. Egg yolks, liver, meat, some shellfish, and whole-milk dairy products are all sources of dietary cholesterol.

Fatty acid
A molecule composed mostly of carbon and hydrogen atoms. Fatty acids are the building blocks of fats.

Fat
A chemical compound containing one or more fatty acids. Fat is one of the three main constituents of food (the others are protein and carbohydrate). It is also the principal form in which energy is stored in the body.

Hydrogenated fat
A fat that has been chemically altered by the addition of hydrogen atoms (see trans fatty acid). Vegetable shortening and margarine are hydrogenated fats.

Lipid
A chemical compound characterized by the fact that it is insoluble in water. Both fat and cholesterol are members of the lipid family.

Lipoprotein
A chemical compound made of fat and protein. Lipoproteins that have more fat than protein are called low-density lipoproteins (LDLs). Lipoproteins that have more protein than fat are called high-density lipoproteins (HDLs). Lipoproteins are found in the blood, where their main function is to carry cholesterol.

Monounsaturated fatty acid
A fatty acid that is missing one pair of hydrogen atoms in the middle of the molecule. The gap is called an "unsaturation." Monounsaturated fatty acids are found mostly in plant and sea foods. Olive oil and canola oil are high in monounsaturated fatty acids. Monounsaturated fatty acids tend to lower levels of LDL-cholesterol in the blood.

Polyunsaturated fatty acid
A fatty acid that is missing more than one pair of hydrogen atoms. Polyunsaturated fatty acids are mostly found in plant and sea foods. Safflower oil and corn oil are high in polyunsaturated fatty acids. Polyunsaturated fatty acids tend to lower levels of both HDL-cholesterol and LDL-cholesterol in the blood.

Saturated fatty acid
A fatty acid that has the maximum possible number of hydrogen atoms attached to every carbon atom. It is said to be "saturated" with hydrogen atoms. Saturated fatty acids are mostly found in animal products such as meat and whole milk. Butter and lard are high in saturated fatty acids. Saturated fatty acids tend to raise levels of LDL-cholesterol ("bad" cholesterol) in the blood. Elevated levels of LDL-cholesterol are associated with heart disease.

Trans fatty acid
A polyunsaturated fatty acid in which some of the missing hydrogen atoms have been put back in a chemical process called hydrogenation, resulting in "straighter" fatty acids that solidify at higher temperatures. Trans fatty acids are under study to determine their effects on cholesterol.

--E.M.

374

Government Advice

Dietary guidelines endorsed by the U.S. Department of Agriculture and the U.S. Department of Health and Human Services advise consumers to:

- Reduce total dietary fat intake to 30 percent or less of total calories.
- Reduce saturated fat intake to less than 10 percent of calories.
- Reduce cholesterol intake to less than 300 milligrams daily.

Approval of Olestra

On Jan. 24, 1996, FDA approved olestra, a fat-based substitute for conventional fats. For more about this new substance, see:

- Olestra and Other Fat Substitutes (FDA backgrounder)
- FDA Approves Fat Substitute, Olestra (a 1996 FDA Press Release)
- Olestra Approved with Special Labeling (a sidebar that appeared in an article on snack foods in the April 1996 FDA Consumer)
- Taking the Fat Out of Food (an FDA Consumer article)

Publication No. (FDA) 99-2286

This is a mirror of the page at HTTP://www.fda.gov/opacom/catalog/fatguide.html

RAPID METHODS OF ANALYSIS

QUICK TESTS FOR FATS AND OILS

Richard F. Stier, Consulting Food Scientist

Introduction

Fats and oils are an integral component of many bakery products and snack foods. They are used as ingredients, as a heat transfer medium for frying, as a release agent, for spraying flavors or coatings, as a functional component providing mouthfeel and as a carrier for flavors. Fryers are probably the largest users of oils and include operations such as industrial frying (snacks, doughnuts, filled pastries), in-store bakeries and the foodservice and restaurant industries. The amount of frying fats and oils used in the food industry is incredible. Frito-Lay, the largest snack producer in this country, uses something in the range of 1,000,000 pounds of oils per day in all their plants. The city of New Orleans alone discards up to 2,000,000 pounds of used cooking oil each year. Yet despite the dire predictions and warnings of nutritionists and consumer activists, usage is increasing. Why? Fried foods taste good. Frying imparts wonderful flavors and textures to foods; assuming the oil is properly maintained.

With fats and oils enjoying such widespread use, it would seem that a quick test for quality and/or safety would be a natural. For example, in industrial applications, the majority of the oils used are delivered in bulk, so there is an opportunity to employ rapid tests as a means of assuring the quality of lots as they are delivered. The second real application for rapid tests for fats and oils is during deep-fat frying as a means for monitoring oil quality changes to assure that only the highest quality fried product is produced (1).

Why Rapid Tests for Fats and Oils

Rapid tests provide a quick and easy means to monitor the quality of systems. With oils, the object might be to "clear" incoming lots or test a frying oil to determine whether it is still acceptable for frying, that is, it will produce good food. This is the really bottom line for food processors. The rapid tests can provide processors, doughnut shop mangers or restaurant operators with an inexpensive means of assuring quality and of saving or making money. In the late 1980's, a major doughnut chain evaluated the use of quick tests in one region of the country. They found that the tests actually increased sales in some stores. Why? Oil was not overused which increased quality which lead to increased sales.

Stier and Blumenthal proposed criteria for the ideal frying oil quick test (2). These criteria can in fact be expanded to cover any rapid test (Fig. 1). Another

FIGURE 1 IDEAL RAPID TEST

- CORRELATE WITH OFFICIAL/RECOGNIZED METHODS
- PROVIDE AN OBJECTIVE INDEX
- EASY TO USE
- SAFE FOR USE IN FOOD PREPARATION/PRODUCTION AREA
- CORRELATE TO FOOD QUALITY/SAFETY
- FIELD RUGGED
- ALLOW FOR REMOTE REINSPECTION

Copyright 1999, Richard F. Stier
Used with Permission of the Author

criteria not mentioned in the figure is cost. Rapid tests must provide good value for their cost. A rapid test with potential applications in foodservice must be an especially good value. For these operations, the test must also be easy to use, that is, relatively "idiot-proof".

Rapid Tests for Fats and Oils

Stier and Blumenthal also defined four basic kinds of on-or at-line tests or systems available to food processors for assuring quality of their operations. These are;

- Rapid tests conducted by an operator who must then take action based on results
- Automatic monitoring devices requiring operator adjustment
- Automatic devices with no control capabilities
- Automatic devices with control capabilities

This dscussion will examine only the first of these four categories, that is, the rapid tests conducted by an operator. There are several instruments on the market today which can automatically sample and evaluate oils, but they will be left for another time. The different technologies this piece will focus on are summarized in Fig.2 (3). Additional information on how quick tests can be used for quality monitoring, troubleshooting and other applications may be found in Snack Professional (4).

Figure 2 Rapid Tests for Fats and Oils (3)

TYPE OF TEST	MANUFACTURER
PHYSICAL TESTS - NO INSTRUMENT	
FOAM HEIGHT	
COLOR WANDS	
CLARITY	
PHYSICAL TEST - INSTRUMENT	
FRI-CHECK	FRI-CHECK
VISCOSITY METER	LEATHERHEAD
CHEMICAL TESTS - INSTRUMENT	
COAT	LANSON INDUSTRIES
RULER	LANSON INDUSTRIES
PCT 120	3M
FOOD OIL SENSOR	NORTHERN INSTRUMENTS
CHEMICAL QUICK TESTS	
OXIFRIT	MERCK
FRITEST	MERCK
ACM/PCM	MIR-OIL
TPM, FFA, WET	TEST KIT TECHNOLOGIES
AV-CHECK	ADVANTEC
SHORTENING MONITOR	3M

The bottom line with rapid tests is that the operator must do the test on-line and based on the results, make adjustments to the process and/or food handling system. As Figure 1 says, this implies that the test should be easy to conduct and safe to use in the process environment. For example, tests incorporating glassware and toxic chemicals are really not what one wants to use in the plant or in a foodservice operation.

Let's examine the existing quick tests, instruments and methods for evaluating fats for fats and oils which are available to today's processor.

Copyright 1999, Richard F. Stier
Used with Permission of the Author

Copyright 1999, Richard F. Stier
Used with Permission of the Author

PHYSICAL TESTS (No Instrument)

There are several simple physical tests used by the industry. These are used to "measure" foam, oil color and oil clarity. Degrading oils begin to foam due the formation of polymers, soaps and other surfactant materials. In continuous industrial processing, foaming is rarely a concern. It is a much greater problem in foodservice operations, doughnut frying or operations using batch fryers. There are operators who discard oils when the foam reaches a certain height. Some fryers have a "grid" or ruler (much like the depth markers on the bow of a ship) etched on the back side which is used. Others insert a marker or ruler in the oil and measure foam height. This is a simple and inexpensive test, but there is no real correlation between foam and food quality.

Oil clarity is another "quick test" which is used. Some operators feel that as oils become darker and less clear, it is no longer fit for use. A clarity test, called the visibility tester, is used by Kentucky Fried Chicken (KFC). They use a stainless steel rod attached to shiny silver disk or plate. The rod has a scale showing three indented marks which are used to judge shortening quality. The shiny disk is lowered into the oil with the depth of insertion monitored on the rod. A penlight is held next to the rod, turned on and the disk observed. If the disk is invisible at or above a certain depth the oil is discarded (5).

The third physical test is oil color. Oil suppliers provide users with a dark colored wand. Oils which reach the color of the wand are considered unfit for use and should be discarded. To conduct the test, an operator removes a sample of oil from the fryer with a dropper. The color of the oil in the dropper is compared to the wand and a decision is then made. These tests have no real relationship to actual oil quality. They are also very subjective. A good oil can be dark and a bad oil light in color. The greatest argument against color is that it has not been adopted as a regulatory tool by any of the nations who have established regulations for restaurant frying oils despite its ease of use (1,6,7).

PHYSICAL TESTS (Instrument)

GEC Marconi of Chelmsford, England has developed, with assistance of the scientists at Leatherhead Food Research, an instrument to measure viscosity (8,9,10). The instrument was designed to measure viscosity of used frying oils based on the formation of *"polymerized and oxidized matter"* or POM. The inventors selected viscosity as the parameter to measure because they felt the formation of degradation products during frying would result in an increase in this particular index. These researchers determined that an oil in excess of 15% POM was unfit for human consumption. Within the instrument itself are two short vibrating steel tubes which are excited by internally mounted pizocystals. The mode of operation may be compared to that of a tuning fork. Dampening depends on the viscosity of the surrounding fluid and resonance depends on density. The probe gives continuous readings of these two parameters. The probe also contains an internal thermocouple to compensate for changes in viscosity due to temperature. A common cut-off for the probe was defined for which the reference value fell between the limit values for this reference value to indicate the discard point. The developers feel that the instrument could be used in both industrial and foodservice frying operations providing rapid results for the user. The instrument can be modified to allow

Copyright 1999, Richard F. Stier
Used with Permission of the Author

Copyright 1999, Richard F. Stier
Used with Permission of the Author

a digital readout of results (industrial frying) or red, yellow and green lights for restaurant operations. The latter instrument would be considerably less expensive.

The latest rapid test to enter the marketplace is FRI-HECK. This test was developed by Dr. Christian Gertz of Hagen, Germany (21). The test relates the viscosity and density of the oil to oil degradation as determined by polar materials and polymers. The FRI_CHECK unit consists of an electronic box with a removable steel tube. The tube is filled with the oil to be tested and maintained at constant temperataure. A piston-like body is then dropped. The falling time may be related to percent polar materials. Tests comparing the FRI-CHECK to standard methods such as polar materials, polymers and free fatty acids showed excellent correlation. The system can be used for monitoring degradation of used frying fats.

CHEMICAL TESTS (Instrument)

There are two companies who have developed instruments for measuring oil quality which have been designed to measure chemical components in degrading frying oils. Lanson Industries in Boise, Idaho markets two instruments called COAT and RULER and Northern Instruments of Lino Lakes, Minnesota manufactures and markets the Food Oil Sensor (FOS).

The COAT (Cooking Oil Analysis Technique) and RULER (Remaining Useful Life Evaluation Routine) sprang from technology developed at the University of Dayton and Wright Laboratory at Wright-Patterson Air Force base to monitor the condition of engine oil lubricants. The researchers felt that the technology could be expanded to be used as a monitor for cooking oil quality. The RULER was developed for determining the presence of antioxidants, such as BHA, BHT, TBHQ and tocopherols) and was designed to be used "off-line". Lanson personnel feel that this instrument is more suitable for industrial operations. COAT is also known s the "Smart Dipstick". It is a simple instrument which uses solid state technology that yields an oil quality value when the oil probes on the instrument is inserted in hot oil. COAT represents an overall oil quality value. In-house tests conducted by Lanson staff have demonstrated a good correlation with free fatty acids, oil color and smoke point. The instrument was also able to detect when fresh oil was added to fryers. They also feel that COAT can also be used as a tool to monitor performance of filter materials. The manufacturers imply that use of their instrument will prevent pre-mature disposal of cooking oils, leading to overall cost savings and that their instrument will provide more consistent end point readings than other methods (11) . Unfortunately, the only available literature on the product is that which has been developed internally.

The Food Oil Sensor has been on the market since the late 1970's. This sensor monitors the change in dielectric constant of degrading frying fats. A sensitive bridge circuit detects minute dielectric changes in the oil (12). Operating the instrument is a two step protocol. In the first step, calibration, the instrument is turned on and the sensor cup should be thoroughly cleaned. The cup must then be filled with fresh shortening of the same make and type used in the fryer. Turn the instrument on and holding down the test switch, "zero" the instrument. To run a test, place the test shortening in the sensor cup. When the "green" light goes on, depress and hold the test switch down. Read the shortening status number.

Copyright 1999, Richard F. Stier
Used with Permission of the Author

380

Copyright 1999, Richard F. Stier
Used with Permission of the Author

This instrument has been evaluated by many workers the world over. It is, in fact, used rather extensively in Europe. Fritsch et al (13) acknowledged that the FOS provided a good measure of oil deterioration, but acknowledged that there were concerns, such as with calibration. Studies by Croon et al (14) found that the FOS correlated well with polar materials (the best index of oil quality) and triglyceride dimers as a means of evaluating oil quality. Saltmarsh (15) described other concerns with the instrument including sensitivity to air currents, long warm up period, possible adverse effects of water on results and effects of oils leaching from the foods on readings. Northern Instruments is now working to make the instrument easier to use by building a self-calibrating feature into it (16).

3M recently introduced an instrument called the Polar Compound Tester or PCT 120 (22) . This unit (Figure 3) allows users to evaluate % polar materials in degrading oils. According to the manufacturer, calibration of the unit allows accurate determination of polar compounds in the samples. The user places a treated strip in the metallic well of the instrument and pours oil through a funnel so it contacts the strip. The operator pushes the "Start" button and reads the result when a flashing green light indicates that the sample is ready. Results are read using an embossed scale on the lid. 3M believes that the unit is easy to use, precise and will help users assure that they satisfy European regulations on restaurant frying oils.

CHEMICAL TESTS (Quick Tests)

Chemical quick tests can be a very useful quality monitoring tool. What potential users must first do, however, is establish a relationship between what the test is measuring and the quality of the food they want to produce (1). This is something which may be determined "in-house" or with help from the supplier. As with any product or service available on the market today, look at the customer service side of the quick test product. The supplier's responsibilty should be to not only provide detailed instruction on how to use the test, but also how to calibrate it to provide the best results. Remember, it is the user, not the manufacturer of rapid test technology, who best understands what defines the quality of the products they are manufacturing.

OXIFRIT & FRITEST (Merck) - The Merck Company of Darmstadt, Germany developed these oil quality tests in response to regulatory concerns regarding frying oil quality. In 1973, the DGF (17) issued recommendations for control of frying oil quality. Among the recommendations was that oxidized fatty acids (OFA) be used as an endpoint index for restaurant frying oils. In 1979 (18), the organization suggested that total polar materials be used instead of the OFA test. These tests were developed to provide operators and regulators with a quick and easy means monitor oil quality. The Fritest measures the alkali color number and the Oxifrit Test oxidation products. Both tests are colorimetric tests which use a solvent based reagent system. The tests are being or have been used by regulators in Switzerland, Finland, Denmark, Austria, Luxembourg, Portugal, Norway and Sweden as a monitoring tool (4). To use the Fritest, the test reagent is placed into a sample tube to the fill line and hot oil is added to a second line. The tube is stoppered, gently shaken and read against the color scale after one minute. The Oxifrit Test is slightly different in taht five drops of a second reagent are added to the sample tube before the addition of oil. The colors for each test are used to judge the quality of the oil. The Fritest has a four color scale and the Oxifrit three. For Fritest, Merck recommends that the oil be changed at color three and that it has "gone off" at color four. The

Copyright 1999, Richard F. Stier
Used with Permission of the Author

Copyright 1999, Richard F. Stier
Used with Permission of the Author

three color Oxifrit test recommends a change at color two (green) and that at color three (olive) the fat is unfit for use.

ACM/PCM (Oil Process Systems) -The ACM and PCM tests were developed and patented by Libra Laboratories (19). Patent and distribution rights are now owned by Oil Process Systems of Allentown, Pennsylvania. Oil Process Systems, also known by the name of Mir-Oil, manufactures and distributes filter materials. The quick tests were developed as a means of supporting the filter business. The ACM test measures alkaline contaminant materials, which include soaps. The Mir-Oil product is designed to reduce the ACM load in used cooking oils, so this test serves as means to demonstrate efficacy of the system. The PCM measures polar contaminant materials (accumulated polar) and was developed as an endpoint test. These tests are colorimetric solvent based tests. To conduct a test, tubes are placed on a flat surface and hot oil is added to a fill line using a glass dropper. The appropriate solvent is then added to the second line and tube is capped and shaken. The colors which form are then compared to a color card. The color changes which occur with the ACM are very dramatic going from yellow to dark blue.

SHORTENING MONITOR (3M) - The Shortening Monitor was developed by the 3M Company of St. Paul, Minnesota. The test consists of a white paper strip measuring 1.0 x 10 cm (0.3 x 3.75 inches) which has four blue bands across it. They have are used as a dip test to measure accumulated free fatty acids, similar to commonly used pH papers. The tests were developed to provide users, especially those in the fast-food industry with an inexpensive means to objectively measure cooking oil quality (20). The test strips are delivered in a stoppered vial. To conduct a test, the user opens the vial and removes a single strip. The operator grips the strip at the top and immerses it in the hot oil for 1-2 seconds. All four bands must be covered. The strip is removed and excess oil is alowed to drain into the fryer. After 15 seconds the strip is held up to the light and the number of bands which have changed to yellow are counted. The approximate free fatty acid (FFA) values for the bands are >2.0, > 3.5, >5.5 and >7.0%. The test, therefore, would have little value in a chip operation in which operators generally strive to maintain FFA levels at or below 0.50%. This 0.50% level is not cast in stone, however. Some operators have lower values and others higher. Other operations, such as batter and/or breaded products which use higher FFA levels for control, may utilize the test. Ms. Gretchen Schildwachter of Fishking Processors, Inc., in Los Angeles has found the test to be an excellent quality tool in their operations.

AV-CHECK (Advantec) - Advantec manufactures a test similar to the #3M test strip. The test is distributed in Asia by Ajinomoto.This test is used to monitor acid value. The operator places a plastic strip with a indicator on the tip into the cooking oil. Based on the degree of abuse, the color indicator changes fromdark blue to a light olive green. The dark blue color indicates that the acid vaalue is "0", whereas the light green is 4.0. The color chart goes as follows: 0, 0.5. 1.0. 2.0, 3.0 and 4.0. Corresponding colors are blue, dark green, light green, olive, light olive and very light olive.

TPM, FFA, & WET (Test Kit Technologies) - Test Kit Technologies has been manufacturing and marketing rapid tests for fats and oils for almost ten years. Their first generation test kits utilized the solvent technology and color cards employed by the the Mir-Oil and Merck tests. Since that time, they have developed and patented a system called GiTIC or Gel-in-Tube

Copyright 1999, Richard F. Stier
Used with Permission of the Author

Copyright 1999, Richard F. Stier
Used with Permission of the Author

Instant Chemistry. With the GiTIC technology, hot oil samples are collected from the fryer, filtered and added directly to the gel. The oil melts the gel and the components of the gel react with the oil to produce a color. While still hot the tube is placed in a small colorimeter and a reading is obtained. The reading may be directly related to oil quality and used for quality monitoring. Tests are available for total polar materials (TPM), free fatty acids (FFA) and surfactant materials or WET for water emulsion titratables. The TPM test is a single phase test whereas the FFA and WET tests are two-phase, producing a colored bottom layer, all of which are read by the instrument. Tests which may be used with a color card may be specially produced for select customers. The FFA and TPM tests are currently under scrutiny by the AOAC (Association of Official Analytical Chemists) and could become the first rapid tests for fats and oils which receive "official method" status. The potential AOAC status of the FFA and TPM tests supports the accuracy the technology, but the tests are not perfect. Tubes must be tested quickly or the gels set up, providing false readings and the filtration step adds to the handling required. Test Kit Technology staff recommend that users establish their own quality parameters or action numbers using the tests, that is, food quality should be related to the values obtained with the tests.

Summary

The use of rapid tests or instruments for monitoring fats and oils can be of enormous economic benefits to food processors. The potential benefits of improved monitoring and control include reduced lab work and all activities involved with laboratory testing, more rapid detection and response to process deviations, reduced product costs thanks to enhanced operating efficiencies, reduced waste through faster startups and lower rework volumes, faster release and clearance of finished goods, lower labor costs, and most important happier customers; customers who will continue to buy your products and ingredients. There are oil chemists who have some concerns about these tests, however. Perhaps their chief wory is that potential users do not understand that it is up to them to develop the relationship between test results and food/oil quality.

Rapid tests for fats and oils have also not received the attention that those for microbiology have, however. Whereas the microbiological quick tests can be used to test for organisms which are acknowledged great public health problems or can create major economic concerns, the overall benefits of oil testing have not been clearly defined. There is also debate over whether abused oils are really a public health issue. Much of Europe seems to think so (at least for restaurant frying oils), but the issue is not even an issue here in the United States. So can rapid tests pay off for your operation? Those operations who have adopted such systems have reported paybacks, but this is one area that each and every company must look at for themselves.

Copyright 1999, Richard F. Stier
Used with Permission of the Author

REFERENCES

1. Stier, R.F. and M.M. Blumenthal (1994), "Quality in Frying", Baking & Snack, 16:11, p.38-42.

2. Stier, R.F. and M.M. Blumenthal (1992), "The Use of Rapid Methods for On-Line Monitoring", Baking & Snack, 14:5, 30.

3. Stier, R.F. (1995), "Rapid Tests for Fats and Oils", Presented at IFT Sponsored Short Course Rapid Methods for Quality Monitoring in the Food Industry, June 2-3, Anaheim, CA

4. Anonymous, (1995), "Oil: Quick Testing for Quality", Snack Professional, Vol.3:1, p.16-19.

5. Anonymous, (1995), "Viscosity Tester Method", KFC Corporation R & D/QC

6. Firestone, D., R.F. Stier and M.M. Blumenthal, (1991), "Regulation of Frying Fats and Oils", Food Technology, 45:2, 90-94.

7. Stier, R.F. and M.M. Blumenthal (1991), "Regulate Frying Fats", Baking & Snack, 13:11, 11-18.

8. Gillatt, P., Kress-Rogers, E.., and Rossell, B., (1991), "A Novel Sensor for Measurement of Frying Oil Quality", Lipid Technology, July-September, p. 78-82.

9. Kress-Rogers, E., Gillatt, P.N., and Rossell, J.B., (1990), "Development and Evaluation of a Novel Sensor for In Situ Assessment of Frying Oil Quality", Food Control, July, p.163-178

10. Anonymous, (1992), "Oils and Fats research at Leatherhead Food RA", Inform, Vol.3:5, 586-593.

11. Lanson Industries, (1992), "Smart Dipstick Takes Guesswork Out of Oil Changing", Press Release, Sept. 24.

12. Graziano, V.J., (1979), "Portable Instrument Rapidly Measures Quality of Frying Fat in Food Service Operations", Food Technology, 33:9, 52-57.

13. Fritsch, C.W., D.C. Egberg, and J.S. Magnuson, (1979), "Changes in Dielectric Constant as a Measure of Frying", JAOCS, 56:8, 746.

14. Croon, L.B., A. Rogstad, T. Leth, and T. Kiutamo, " A Comparative Study of Analytical Methods for Quality Evaluation of Frying Fats", Fette Seifen Anstrichm., 88:3, 87-91.

15. Saltmarsh, M., (1990), "Rapid Analysis of Fats and Oils: Thermal Degradation", Presentation at Western Food Industries Conference, UC Davis, March 1990.

16. Peake, E.R., (1996), Personal Communication, July 26.

17. DGF, (1973), Meeting Summary, DGF (German Society for Fat Research) Symposium on Frying & Cooking Fats, Fette Seifen Anstrichm., 75:49.

18. DGF, (1979), Meeting Summary, DGF Symposium on Frying & Cooking Fats, Fette Seifen Anstrichm., Special Isue 81: 493.

19. Blumenthal, M.M., J.R. Stockler, and P.J. Summers, (1985), "Alkaline Contaminant Materials in Used Frying Oils: A New Quick Test", JAOCS, 62:9, 1373-1374.

20. Anonymous, (1986), "Test Strip for Frying Fats", JAOCS, 63:7, 838-839.

21. Gertz, C., (2000), "Chemical and Physical Parameters as Quality Indicators of Used Frying Oils", European Journal of Lipid Technology, 102:8-9, 566-572.

22. Truong, J. G., (2000), "A Universal, Easy to Use and Direct Method for Measuring % Total Polar Compounds in Degrading Oil", European Journal of Lipid Technology, 102:8-9, 586.

SPEECHES\QUICK.doc

Copyright 1999, Richard F. Stier
Used with Permission of the Author

AOCS Official Method Ca 12-55

Replaced Ca 12-53 • Reapproved 1997

Phosphorus

DEFINITION

This method determines phosphorus or the equivalent phosphatide content by ashing the sample in the presence of zinc oxide, followed by the spectrophotometric measurement of phosphorus as a blue phosphomolybdic acid complex.

SCOPE

Applicable to crude, degummed and refined vegetable oils.

APPARATUS

1. Vycor crucibles, or equivalent—capable of withstanding a muffle furnace temperature of at least 600°C, 50 mL.
2. Watch glasses—75-mm diameter.
3. Electric hot plate—with rheostat control.
4. Muffle furnace—with pyrometer and suitable controls to maintain a temperature of 550–600°C.
5. Funnel—glass, short stem, 50-mm diameter.
6. Filter paper—ashless, 90-mm diameter, Whatman no. 42, or equivalent.
7. Wash bottle—1 liter, for use with hot water.
8. Volumetric flasks—50, 100, 250 and 500 mL, with glass stoppers.
9. Transfer pipets—2, 5, 10 and 25 mL.
10. Pipet—Mohr type, 10 mL, with 0.1-mL subdivisions.
11. Spectrophotometer—meeting the requirements of AOCS Official Method Cc 13c-50, at a minimum, capable of measuring absorbance at 650 nm with a repeatability of 0.5%.
12. Cuvettes—1.000 ± 0.005 cm, for use in the visible region of the spectrum.

REAGENTS

1. Hydrochloric acid (HCl)—concentrated, sp gr 1.19 (see Notes, *Caution*).
2. Zinc oxide—commercial grade or reagent grade.
3. Potassium hydroxide (KOH) pellets—reagent grade (see Notes, *Caution*).
4. Sulfuric acid (H_2SO_4)—concentrated, sp gr 1.84 (see Notes, *Caution*).
5. Sodium molybdate—reagent grade.
6. Hydrazine sulfate—reagent grade (see Notes, *Caution*).
7. Potassium dihydrogen phosphate—reagent grade, dried at 101°C for 2 hr before use.

SOLUTIONS

1. Sodium molybdate—Carefully add 140 mL of concentrated H_2SO_4 to 300 mL of distilled water. Cool to room temperature and add 12.5 g of sodium molybdate. Dilute to 500 mL with distilled water, mix thoroughly and allow the solution to stand for at least 24 hr before use.
2. Hydrazine sulfate, 0.015%—Dissolve 0.150 g of hydrazine sulfate in 1 liter of distilled water.
3. Potassium hydroxide, 50% solution—Dissolve 50 g of KOH in 50 mL of distilled water with cooling (see Notes, *Caution*).
4. Standard phosphate solution—
   (a) Standard stock solution—prepared by dissolving 1.0967 g of dry potassium dihydrogen phosphate (Reagents, 7) in distilled water and diluting to 250 mL in a volumetric flask. This solution contains 1 mg of phosphorus per mL.
   (b) Standard working solution—prepared by pipetting 5 mL of the standard stock solution into a 500-mL volumetric flask and diluting to volume with distilled water. This solution contains 0.01 mg of phosphorus per mL.

PROCEDURE

1. Weigh 3.0–3.2 ± 0.001 g of sample into a Vycor crucible. Add 0.5 g of zinc oxide.
2. Heat slowly on a hot plate until the sample thickens, then gradually increase the heat until the mass is completely charred.
3. Place the crucible in a muffle furnace at 550–600°C and hold for 2 hr. Remove and cool to room temperature.
4. Add 5 mL of distilled water and 5 mL of concentrated HCl to the ash.
5. Cover the crucible with a watch glass and heat to gentle boil for 5 min.
6. Filter the solution into a 100-mL volumetric flask. Wash the inside of the watch glass and the sides of the crucible with about 5 mL of hot distilled water, using a wash bottle with a fine stream of water. Wash the crucible and the filter paper with four additional 5-mL portions of hot distilled water.
7. Cool the solution to room temperature and neutralize to a faint turbidity by the dropwise addition of 50% KOH solution. Add concentrated HCl dropwise until the zinc oxide precipitate is just dissolved, then add 2 additional drops. Dilute to volume with distilled water and mix thoroughly.
8. Pipet 10 mL of this solution into a clean, dry 50-mL volumetric flask (see Notes, 1).
9. Add 8.0 mL of hydrazine sulfate solution and 2.0 mL of sodium molybdate solution, in the order given.
10. Stopper and invert three or four times. Loosen the stopper and heat for 10 ± 0.5 min in a vigorously boiling water bath.
11. Remove from the bath, cool to 25 ± 5°C in a water bath, dilute to volume and mix thoroughly (see Notes, 2).
12. Transfer the solution to a clean, dry cuvette. Measure the absorbance at 650 nm with the instrument adjusted

to read 0% absorbance (100% transmittance) for a cuvette containing distilled water (see Notes, 1).

13. Prepare a reagent blank with no added oil by following the procedure as described in Procedure, 1–12.
14. Measure the phosphorus content of the sample and the blank by comparison with the standard curve, obtained as follows:

Preparation of Standard Curve

(a) Pipet 0.0, 1.0, 2.0, 4.0, 6.0, 8.0 and 10.0 mL of the standard working solution [Solutions, 4, (b)] into 50-mL volumetric flasks. Dilute each to 10 mL with distilled water, using a measuring pipet. Proceed as directed in Procedure, 9–12. The aliquots respond, respectively, to 0.0, 0.01, 0.02, 0.04, 0.06, 0.08 and 0.10 mg of phosphorus.

(b) Plot the absorbance of each standard against its phosphorus content in milligrams on linear graph paper.

CALCULATIONS

1. Phosphorus, % = $\frac{10\,(A - B)}{W \times V}$

Where—

A = phosphorus content of sample aliquot in mg
B = phosphorus content of blank aliquot in mg
W = mass of sample in g
V = volume of aliquot taken in Procedure, 8.

2. Equivalent phosphatides, % = % phosphorus × 30 (see Notes, 4)

NOTES

Caution

Hydrochloric acid is a strong acid and will cause severe burns. Protective clothing should be worn when working with this acid. It is toxic by ingestion and inhalation and a strong irritant to eyes and skin. The use of a properly operating fume hood is recommended. When diluting the acid, always add the acid to the water, never the reverse.

Potassium hydroxide, like all alkalies, can burn skin, eyes and respiratory tract severely. Wear heavy rubber gloves and face shield to protect against concentrated alkali liquids. Use effective fume-removal device or gas mask to protect respiratory tract against alkali dusts or vapors. When working with extremely caustic materials, such as potassium hydroxide, always add pellets to water and not the reverse. Alkalies are extremely exothermic when mixed with water. Take precautions to contain the caustic solution in the event that the mixing container breaks from the extreme heat generated.

Sulfuric acid is a strong acid and will cause severe burns. Protective clothing should be worn when working with this acid. It is an oxidizing agent and should not be stored in the vicinity of organic materials. Use great caution in mixing with water due to heat evolution that can cause explosive splattering. Always add the acid to water, never the reverse.

Hydrazine sulfate can cause eye, skin and mucous membrane irritation, and liver and kidney damage. This compound is a known carcinogen in laboratory animals, causing lung and liver tumors in rats. It is a suspected human carcinogen. Precautions in handling this compound include use of gloves, eye protection and respiratory protection. Avoid the inhalation of dust and powder. Dispose of waste material and waste solutions in a proper and safe manner.

NUMBERED NOTES

1. If the absorbance of the colored solution measured in Procedure, 12 is too high (>0.9), take a smaller size aliquot of the sample solution than specified in Procedure, 8 (e.g., 2.0 mL), dilute to 10 mL with distilled water using a measuring pipet and continue as directed in Procedure, 9–12.
2. Samples of high phosphorus content may still give absorbencies >0.9. If this is the case, pipet 10 mL of the sample solution prepared in Procedure, 7 into a 100-mL volumetric flask and dilute to volume with distilled water. Carry out the color development sequence noted in 8–12, using an appropriate size aliquot diluted to 10 mL with distilled water. Multiply the phosphorus content obtained, using the equation in Calculations, by the dilution factor (10, if following the procedure described in this paragraph).
3. No unreasonable delay should be incurred between developing the color in Procedure, 11 and measuring the absorbance in Procedure, 14.
4. This is an approximation for converting percent phosphorus to percent phosphatides in soybean oil.

386

AOCS Recommended Practice Cg 1-83

Reapproved 1997

Correlation of Oil Volatiles with Flavor Scores of Edible Oils

DEFINITION

This method provides a representative condition for sample preparation and procedures for the analysis of gas chromatographic volatiles, such as direct injection, static headspace and dynamic headspace (purge and trap), in order to permit the generation of correlation factors for vegetable oil flavor, utilizing a trained flavor panel.

SCOPE

Applicable to all domestic deodorized edible vegetable oils with potential application to other untested edible oils.

PRINCIPLE

1. Volatiles isolated and measured from edible oils have been shown to be related to oil quality (see References, 1–7).
2. The isolated volatiles represent those formed by the breakdown of hydroperoxides and other flavor precursors as well as other free volatiles present in the samples.
3. The volatile procedure offers an objective means of evaluating oil quality and stability, if used under standardized conditions.
4. Correlation studies between volatiles and taste panel results for each particular oil, or processing treatment, such as hydrogenation, are necessary.

PROCEDURE

1. Specific samples should be stored under standardized conditions of light or absence of light, temperature and time prior to analysis by a formal taste panel and one of the volatile measurement techniques.
2. A typical procedure, given only as a reference to show the conditions of control of storage history, might be as follows—
   (a) The sample of oil is stored in a 240-mL clear glass bottle with 2.25 cm of headspace. A screw cap is tightened to a consistent torque.
   (b) The sample is then maintained at room temperature (22–25°C) or under Schaal oven conditions (60°C). Storage time would normally be 2–6 months at room temperature (22–25°C) and 2–8 days at 60°C.
   (c) A separate sample bottle (for each volatile and taste analysis) is taken at time zero and then repeated once a week for a minimum of three analyses during the storage period.
   (d) See Method Cg 4-94 on Volatile Organic Compounds (VOC) in fats and oils by gas chromatography for descriptions of three gas chromatographic methods: direct injection, static headspace, and purge and trap.
   (e) See Method Cg 2-83 on Flavor Panel Evaluation of Vegetable Oils for descriptions of sensory methods.
   (f) Volatiles should be measured against a known internal standard, or if not feasible, an external standard for final quantitation. Specific components, such as hexanal, might be used for correlation; however, total volatiles are recommended for most applications. Results are usually expressed as total parts per million relative to a standard, although integrator counts may be used.
   (g) Correlation of volatiles with taste panel scores can be established by using linear multiple regression analysis on a series of samples taken over the storage period. Total volatiles converted to their logarithms have been preferred for correlation in some procedures vs. taste panel scores (References, 1 and 3). In either respect, the larger the database is, the more reliable will be the correlations.
   (h) The precision of the analyses should also be calculated at different levels of volatiles to establish the level of expected error. Such reproducibility studies should be established prior to attempts to establish taste panel correlations.

REFERENCES

For a list of references for flavor volatile methods successfully utilized in collaborative studies of the Flavor Nomenclature Committee see References, 6.

1. DuPuy, H.P., E.T. Rayner, J.F. Wadsworth, and M.G. Legendre, *JAOCS 54:*445 (1977).
2. Waltking, A.E., and H. Zmachinski, *JAOCS 54:*454 (1977).
3. Jackson, H.W., and D. Giacherio, *JAOCS 54:*458 (1977).
4. Warner, K., C.D. Evans, G.R. List, H.P. DuPuy, J.I. Wadsworth, and G. E. Goheen, *JAOCS 55:*252 (1978).
5. Min, D.B., *J. Food Sci. 46:*1453 (1981).
6. Waltking, A.E., *JAOCS 59:*116A (1982).
7. Snyder, J.M., E.N. Frankel, E. Selke, and K. Warner, *JAOCS 65:*1617 (1988).

AOCS Recommended Practice Cg 2-83

Reapproved 1997

Flavor Panel Evaluation of Vegetable Oils

DEFINITION

This method provides a standardized technique for the sensory evaluation of edible vegetable oils. It encompasses standard preparation and presentation of samples, as well as the procedures for tasting and scoring the oils.

SCOPE

Applicable to all deodorized edible vegetable oils, with potential application to other untested edible oils (see Notes, 1). The scales for both intensity and quality are applicable to most testing situations from quality control to research studies.

APPARATUS

1. Isolated taste panel room—
   (a) Temperature range, 24 ± 1°C.
   (b) Lighting, white and red fluorescent light, with white light to provide 80 foot-candles of light on table area of individual booth and red light and white light to be operated separately or together.
   (c) Filtered air, under positive pressure to create an airflow out of panel room to adjacent areas.
   (d) Individual booths for panelists or tables with 45-cm (18-in.) high dividers to separate panelists.
2. Sample presentation utensils—
   (a) 50-mL covered glass beakers.
   (b) Aluminum blocks, drilled to ½ the height of the glass beakers, to comfortably hold 4 beakers.
   (c) Hot plate or oven capable of heating the aluminum blocks to a constant temperature such that the oil attains a temperature of 50 ± 1°C.

REAGENTS

1. Carbon-filtered tap water—heated to 38°C.
2. Reference standards for oil quality and intensity—consisting of one or a group of the following, which, immediately after preparation, were constantly flushed with nitrogen until stored frozen (≤10°C) in glass jars completely filled with no headspace for up to 1 year. The jar size should be adequate for the needs of the panel for a single day's evaluation. Any excess reference oil from an opened jar should be discarded.
   (a) Control, lightly hydrogenated—100–110 iodine value (IV) soybean oil double deodorized, with 2 ppm citric acid added after deodorization as a metal scavenger. Flavor score should be 8–9 (1–10 scale).
   (b) Buttery odor/flavor—Store control (a) 3 days at 60°C (140°F), air exposed.
   (c) Hydrogenated odor/flavor—Add freshly hydrogenated, undeodorized soybean oil (100–110 IV) to a control (a) at a level of 25%.
   (d) Bitter taste—0.1% caffeine in a good control.
   (e) Raw, grassy odor/flavor—1% undeodorized, crude soybean oil in a good control.
   (f) Corny odor/flavor—5% undeodorized corn oil in control (a).
   (g) Rancid odor/flavor—cottonseed oil aged ≥4 days at 60°C (140°F) (Schaal oven test) to a peroxide value of between 5 and 10.
   (h) Painty odor/flavor—low erucic acid rapeseed oil aged ≥8 days at 60°C (140°F) (Schaal oven test) to a peroxide value of between 15 and 20.

PREPARATION AND PRESENTATION OF OIL SAMPLE

1. Up to four 50-mL glass beakers containing 10 mL of oil each are capped with a tight-fitting ground-glass cover and are placed in hollowed-out areas of an aluminum block.
2. The aluminum blocks containing the beakers are heated in a circulating air oven or on an electrically controlled hot plate.
3. Hot plate or oven temperature is adjusted such that the oil is maintained at 50 ± 1°C.
4. Oils are heated for 30 min in the dark prior to serving and should be evaluated within 30 additional minutes.
5. If the panel size is large enough to permit using a statistical design (i.e., Cochran and Cox; see References, 1), then permutations of the order of sample presentation are balanced.
6. Samples should be coded using random numbers of at least 3 digits.

ODOR AND FLAVOR TESTING PROCEDURES

1. Odor is used in the following manner to set the order of sample evaluation if the panel size is too small for a valid balanced statistical design to have been used to establish presentation order.
   (a) Oils are evaluated for odor in the order in which samples are presented from left to right.
   (b) Covered beaker is swirled and lifted to nose.
   (c) Cover is removed as tester sniffs the volatiles.
   (d) Beaker is swirled again if necessary.
   (e) Sample with the weakest odor is evaluated first for flavor; the remaining samples are evaluated in the order of increasing odor intensity.
2. Flavor—
   (a) Unless odor has been used to establish an evaluation order, evaluate samples as presented from left to right.
   (b) 10 mL of the oil is taken into the mouth and thoroughly swished throughout the mouth, or cupped on the tongue while air is drawn over or through it.
   (c) Oil is expectorated into paper waste cups.
   (d) Carbon-filtered tap water heated to 38°C is used to rinse the mouth before testing and after testing each sample.

EVALUATIONS

1. Overall scores for quality or intensity—
   Note—Quality and intensity levels on the scale must be defined for each specific oil type prior to using the scale. Listing specific flavor characteristics, such as buttery, rancid, etc., directly next to specific scores on each scale is not recommended. However, taste testers should be trained to recognize various quality or intensity levels based on specific flavor characteristics of each oil type.
   (a) If the objective of the test is to evaluate overall oil quality, the scoring scale in Figure 1 is recommended. This scale is appropriate for oils with distinctive flavors, such as corn, peanut or olive oil. Standards for the various quality levels should be determined by the flavor panel for each type of oil.

Name_____ Date_____

Directions: Take 5 to 10 mL of warm oil into the mouth, pull air through the oil and evaluate the flavor by exhaling through the nose. Mark on the intensity scale below the overall flavor intensity for the sample.

Quality	Overall quality scores Sample numbers				Descriptions	Description intensity Sample numbers			
	___	___	___	___		___	___	___	___
10 Excellent	___	___	___	___	Nutty	___	___	___	___
9 Good	___	___	___	___	Buttery	___	___	___	___
8	___	___	___	___	Corny	___	___	___	___
7 Fair	___	___	___	___	Beany	___	___	___	___
6	___	___	___	___	Hydrogenated	___	___	___	___
5 Poor	___	___	___	___	Burned	___	___	___	___
4	___	___	___	___	Weedy	___	___	___	___
3 Very poor	___	___	___	___	Grassy	___	___	___	___
2	___	___	___	___	Rubbery	___	___	___	___
1	___	___	___	___	Melon	___	___	___	___
					Painty	___	___	___	___
					Fishy	___	___	___	___
					Other_____	___	___	___	___
					Other_____	___	___	___	___

Figure 1. Scoresheet for flavor quality evaluation.

Name_____ Date_____

Directions: Take 5 to 10 mL of warm oil into the mouth, pull air through the oil and evaluate the flavor by exhaling through the nose. From the master list of descriptions, list the one(s) appropriate for this sample and indicate the amount of flavor.

Intensity	Overall intensity scores Sample numbers				Descriptions	Description intensity Sample numbers			
	___	___	___	___		___	___	___	___
10 Bland	___	___	___	___	Nutty	___	___	___	___
9 Trace	___	___	___	___	Buttery	___	___	___	___
8 Faint	___	___	___	___	Corny	___	___	___	___
7 Slight	___	___	___	___	Beany	___	___	___	___
6 Mild	___	___	___	___	Hydrogenated	___	___	___	___
5 Moderate	___	___	___	___	Burned	___	___	___	___
4 Definite	___	___	___	___	Weedy	___	___	___	___
3 Strong	___	___	___	___	Grassy	___	___	___	___
2 Very strong	___	___	___	___	Rubbery	___	___	___	___
1 Extreme	___	___	___	___	Melon	___	___	___	___
					Painty	___	___	___	___
					Fishy	___	___	___	___
					Other_____	___	___	___	___
					Other_____	___	___	___	___

Figure 2. Scoresheet for flavor intensity evaluation.

AOCS Official Method Cd 11b-91
Reapproved 1997

Determination of Mono- and Diglycerides by Capillary Gas Chromatography

DEFINITION

This method is for the determination of mono- and diglycerides by capillary GLC. Mono- and diglycerides are converted with bis(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethylchlorsilane (TMCS) in pyridine into more volatile trimethylsilylether derivatives and determined by capillary GLC.

SCOPE

This method is applicable to mono- and diglyceride concentrates and mono- and diglycerides in fats and oils (see References, 1). Other emulsifiers and components of fats and oils, such as glycerol, fatty acids, sterols, etc., may be converted into the trimethylsilylether derivatives and analyzed by the same GLC procedure. For the identification of the components, coupled GC/MS may be advantageous.

APPARATUS

1. Gas chromatograph—with split injection or on-column injection (see Notes, 1), oven temperature programming and flame-ionization detector.
2. Recording potentiometer.
3. Electronic integrator.
4. Column—capillary, glass or fused silica, surface fully deactivated by silylation agent (see Notes, 2), 15–25 m, 0.25–0.35 mm i.d., coating SE-54 (or other phase with similar polarity), film thickness 0.1–0.2 µm (see Notes, 3).
5. Operating conditions:
   —split injection (split ratio 1:10–1:50)
   —direct injection (splitless, hold for 1 min)
   —injection port 320°C (or for on-column injection 60°C)
   —column initial 80°C (or for on-column 60°C)
   —program rate 10°C/min
   —final temperature 360°C, hold 15 min (see Notes, 3)
   —detector 350°C
   —carrier gas flow 5 mL He/min (at 80°C)
   —injection volume 1–5 µL
6. An automatic sampler is advantageous (see Notes, 4).
7. Screw-cap vials—2.5 mL; or crimp-top vials for auto sampler (e.g., 2.0 mL), with Teflon™-faced septa (see Notes, 3).
8. Heating device for vials—70°C.

REAGENTS

1. *N,N*-bis(trimethylsilyl)trifluoroacetamide (BSTFA).
2. Trimethylchlorsilane (TMCS).
3. Pyridine—analytical reagent grade, kept over KOH (see Notes, *Caution*).
4. n-Tetradecane—analytical reagent grade (minimum 99%).
5. n-Hexane—analytical reagent grade (see Notes, *Caution*).
6. Reference materials—glycerol, palmitic acid, 1-palmitoyl glycerol, 1-stearoyl glycerol, 1,2-dipalmitoyl glycerol, 1,3-dipalmitoyl glycerol, 1,2-distearoyl glycerol.
7. Internal standard solution—prepared by accurately weighing approximately 100 mg internal standard n-tetradecane into 10-mL volumetric flask and diluting to volume with pyridine.
8. Reference solution—prepared by accurately weighing approximately 100 mg of reference substance (e.g., glycerol, fatty acid, mono- and diacyl glycerol) and accurately weighing approximately 100 mg of *n*-tetradecane into the same 10-mL volumetric flask and diluting to volume with pyridine; or weigh approximately 100 mg of a mixture containing several (e.g., 5) reference materials and n-tetradecane, each component being present in about the same quantity, into a 2-mL volumetric flask and dilute to volume with pyridine (see Notes, 5).

PROCEDURE

1. Sample solution—Accurately weigh approximately 10 mg of homogenized sample of emulsifier concentrates, or 50 mg of oils and fats containing emulsifiers, into a 2.5-mL screw-cap vial with Teflon™-faced septa. Add 0.2 mL BSTFA and 0.1 mL TMCS, and then 0.1 mL of internal standard solution (Reagents, 7) containing 1 mg n-tetradecane to the sample (see Notes, 4). Moisture must be strictly excluded. Close vial and shake vigorously. Heat the reaction mixture in heating device at 70°C for approximately 20 min. Inject 1–5 µL of the reaction mixture into the gas chromatograph showing a stable base line. Avoid delay of GC analysis. The reaction is carried out two times, and two injections are made per reaction (see Notes, 1 and 2).
2. Reference solution—Add 0.10 mL of reference solution (Reagents, 8) into vials and add the silylating agents, 0.2 mL BSTFA and 0.1 mL TMCS (no internal standard solution is added). Heat the reaction mixture and inject into the gas chromatograph as described above (see Notes, 4). Use a concentration range of reference standards similar to the range of the components to be quantified in the sample solution. A plot of response factor vs. concentration of reference substances may be useful to check linearity (see Notes, 1 and 2).
3. Response factors—Check response factors periodically. Response factors should be above approximately 0.5. Lower response factors indicate some loss or decomposition. Use a concentration range of 0.5–10 mg/mL of components in both the reference and sample solutions. See Calculations, paragraph 1, for calculation of response factors.

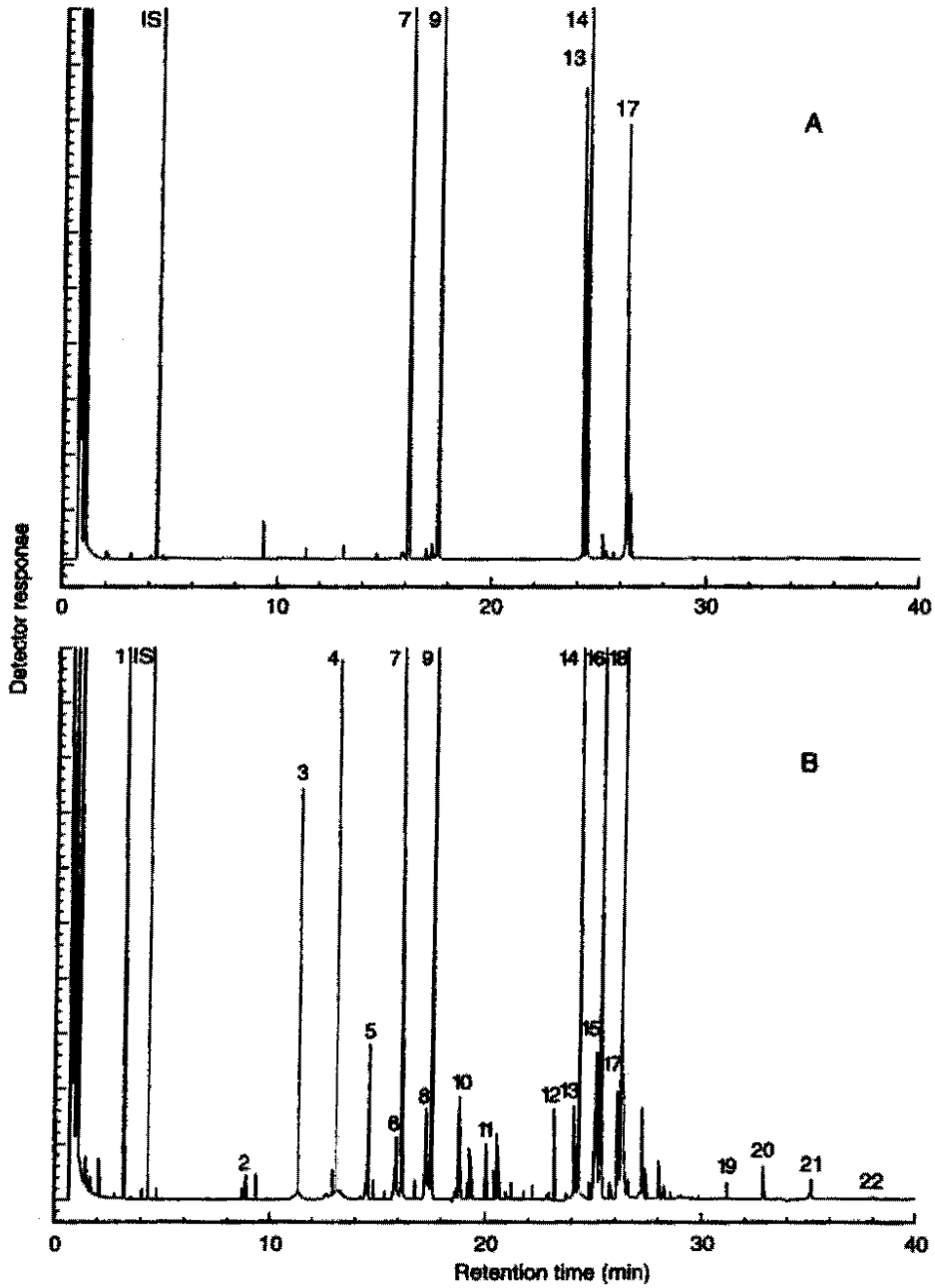


Figure 1. Typical chromatograms of trimethylsilylether derivatives of mono- and diglycerides: A, reference standards and B, mono- and diglyceride emulsifier. The silylation procedure, column specifications, operating conditions, and peak identification are as follows: (a) *Silylation*—Sample size, 10 mg; reagents, 0.1 mL pyridine containing 1.0 mg n-tetradecane, 0.2 mL BSTFA, 0.1 mL TMCS; reaction time, 30 min at 70°C. (b) *Column*—25 m × 0.31 mm i.d. fused silica; 0.17 µm film thickness (5% phenylmethyl silicon, Ultra #2, Hewlett-Packard). (c) *Operating conditions*—Injector, 320°C; hold 15 min; detector, 350°C; carrier gas, He, 5 mL/min, 80°C. (d) *Peak identification*—IS, n-tetradecane (internal standard); 1, glycerol; 2, diglycerol; 3, hexadecanoic acid; 4, octadecanoic acid; 5, glycerol 1-tetradecanoate; 6, glycerol 2-hexadecanoate; 7, glycerol 1-hexadecanoate; 8, glycerol 2-octadecanoate; 9, glycerol 1-octadecanoate; 10, glycerol 1-icosanoate; 11, glycerol 1-docosanoate; 12, glycerol 1-tetradecanoate-3-hexadecanoate; 13, glycerol 1,2-dihexadecanoate; 14, glycerol 1,3-dihexadecanoate; 15, glycerol 1-hexadecanoate-2-octadecanoate; 16, glycerol 1-hexadecanoate-3-octadecanoate; 17, glycerol 1,2-dioctadecanoate; 18, glycerol 1,3-dioctadecanoate; 19, triglyceride C48; 20, triglyceride C50; 21, triglyceride C52; and 22, triglyceride C54.

391

IDENTIFICATION

1. Analyze reference solution under the same operation conditions as sample solution. Identify peaks by comparison of retention time with known substances, or apply coupled GC/MS. See Figure 1.

CALCULATIONS AND EXPRESSION OF RESULTS

1. Response factor—Calculate response factor of the reference substance vs. internal standard using the reference solution chromatogram. The value of the response factor is given by the formula:

$$R_x = (m_{is}/m_x) \times (A_x/A_{is})$$

Where—

R_x = response factor of reference substance x
m_{is} = mass of internal standard, in mg
m_x = mass of reference substance x, in mg
A_x = peak area of reference substance x
A_{is} = peak area of internal standard

2. Calculation of sample component content—Calculate percentage of mass content of component x in the sample by the formula:

$$m'_x\,(\%) = 1/R_x \times (m'_{is}/m'_s) \times (A'_x/A'_{is}) \times 100\%$$

Where—

m'_x = percentage of mass of component x in sample
R_x = response factor of component x in sample
m'_{is} = mass of internal standard in sample, in mg
m'_s = mass of sample, in mg
A'_x = peak area of component x in sample
A'_{is} = peak area of internal standard in sample

3. Typical chromatograms—See Figure 1 for typical chromatograms of reference standards and mono- and diglycerides. The silylation procedure, column specifications, operating conditions and peak identification relating to Figure 1 are as follows:
   (a) Silylation—Sample size: 10 mg; reagents: 0.1 mL pyridine containing 1.0 mg n-tetradecane, 0.2 mL BSTFA, 0.1 mL TMCS; reaction time: 20 min at 70°C.
   (b) Column—Fused silica capillary, 25 m × 0.31 mm (i.d.); film thickness, 0.17 µm, consisting of 5% phenylmethyl silicon, Ultra #2 (Hewlett-Packard, Palo Alto, CA, USA).
   (c) Operating conditions—Injector 320°C, column initial 80°C, program 10°C/min, final 360°C, hold 15 min, detector 350°C; carrier gas, helium at 5 mL/min (at 80°C) (see Apparatus, 5).
   (d) Peak identification—IS (internal standard), tetradecane; 1, glycerol; 2, diglycerol; 3, hexadecanoic acid; 4, octadecanoate acid; 5, glycerol 1-tetradecanoate; 6, glycerol 2-hexadecanoate; 7, glycerol 1-hexadecanoate; 8, glycerol 2-octadecanoate; 9, glycerol 1-octadecanoate; 10, glycerol 1-icosanoate; 11, glycerol 1-docosanoate; 12, glycerol 1-tetradecanoate-3-hexadecanoate; 13, glycerol 1,2-dihexadecanoate; 14, glycerol 1,3-dihexadecanoate; 15, glycerol 1-hexadecanoate-2-octadecanoate; 16, glycerol 1-hexadecanoate-3-octadecanoate; 17, glycerol 1,2-dioctadecanoate; 18, glycerol 1,3-dioctadecanoate; 19, triglyceride C48; 20, triglyceride C50; 21, triglyceride C52; 22, triglyceride C54.

Table 1
Statistical analysis of results for mono- and diglyceride concentrates (expressed as percent of mass of sample).

	1-Myristate	1-Palmitate	1-Stearate	1,3-Dipalmitate	1-Palmitate-3-stearate	1,3-Distearate
No. of laboratories	8	8	8	8	8	8
No. of results	16	16	16	16	16	16
No. of laboratories retained after elimination of outliers[a]	7	8	8	7	7	8
No. of accepted results	14	16	16	14	14	16
Mean value, %	1.7	27.2	60.1	0.2	0.8	1.1
Repeatability standard deviation (S_r)	0.05	0.9	2.1	0.01	0.05	0.07
Repeatability coefficient of variation (RSD_r)	3.0	3.3	3.5	4.6	6.0	6.8
Repeatability value (r) [$2.83 \times S_r$]	0.14	2.61	5.91	0.031	0.14	0.20
Reproducibility standard deviation (S_R)	0.1	2.4	6.4	0.06	0.1	0.3
Reproducibility coefficient of variation (RSD_R)	5.7	8.9	10.7	30.0	17.8	24.8
Reproducibility value (R) [$2.83 \times S_R$]	0.3	6.8	18.1	0.2	0.4	0.8

[a]Eliminated using Dixon and Cochran test methods.

Table 2
Statistical analysis of results for mono- and diglycerides in oil (expressed as percent of mass of sample).

	1-Palmitate	1-Stearate	1,2-Dipalmitate	1,3-Dipalmitate	1,2-Distearate
No. of laboratories	8	8	8	8	8
No. of results	16	16	16	16	16
No. of laboratories retained after elimination of outliers[a]	8	8	8	8	8
No. of accepted results	16	16	16	16	16
Mean value, %	0.96	0.98	0.97	0.93	0.97
Repeatability standard deviation (S_r)	0.03	0.03	0.04	0.02	0.06
Repeatability coefficient of variation (RSD_r)	3.3	3.4	4.0	2.5	6.2
Repeatability value (r) [$2.83 \times S_r$]	0.08	0.08	0.11	0.06	0.17
Reproducibility standard deviation (S_R)	0.12	0.14	0.24	0.19	0.19
Reproducibility coefficient of variation (RSD_R)	12.0	13.8	24.4	20.2	19.8
Reproducibility value (R) [$2.83 \times S_R$]	0.34	0.40	0.68	0.54	0.54

[a]Eliminated by the Dixon and Cochran test methods.

PRECISION

1. Repeatability—When the mean of the values obtained from two single determinations, carried out in rapid succession by the same operator using the same apparatus under the same conditions for the analysis of the same test sample, lies within the range of the mean values cited in Tables 1, 2 and 3, the difference between the two values obtained should not be greater than the repeatability value (r), which can generally be deduced by linear interpolation from Tables 1, 2 and 3 (References, 2).
2. Reproducibility—When the values for the final result, obtained by operators in different laboratories, using different apparatus under different conditions for the analysis of the same laboratory sample, lie within the range of mean values cited in Tables 1, 2 and 3, the difference between the values for the final result obtained by those operators should not be greater than the reproducibility value (R), which can generally be deduced by linear interpolation from Tables 1 and 2.

NOTES

Caution

Pyridine is flammable and a dangerous fire risk. The explosive limits in air are 1.8–12.4%. It is toxic by ingestion and inhalation. The TLV is 5 ppm in air. The danger from crude pyridine is greater than from pure pyridine, the associated homologs and impurities being even more toxic than pyridine.

Hexane is flammable and a dangerous fire risk. The TLV is 50 ppm in air. The Occupational Safety and Health Administration recommends that exposure not exceed 350 ng per cubic meter for a time-weighted average. Hexane vapor causes lung irritation and produces neurotoxic effects. A fume hood should be used at all times when using hexane.

NUMBERED NOTES

1. For on-column injection, or direct injection, dilute 50 µL of reaction mixture (Procedure, 1 and 2) with 1 mL hexane and inject (1 µL). In order to lengthen lifetime of the columns, when applying on-column injections, a precolumn is useful. On-column injection gives better response factors.
2. Use length of columns required to separate mono- or diglycerides. Individual unsaturated mono- and diglycerides may not be separated from the saturated or less-unsaturated mono- or diglycerides. TLC on silica gel impregnated with boric acid, immediately prior to derivatization, can be used to resolve 2-monoglycerides from 1-monoglycerides (References, 3).
3. The final column temperature of 360°C is not compatible with the maximum operating temperature of 320°C to 325°C recommended for SE-54. The detector should be 10°C to 20°C higher than the maximum column temperature. Therefore, it is recommended to use a maximum column temperature of 325°C to

Table 3
Statistical analysis of results for mono- and diglycerides in sunflower oil (blind duplicates, expressed as percent of mass of sample).

	1-Palmitate	1-Stearate	1,2-Dipalmitate	1,3-Dipalmitate	1,2-Distearate
No. of laboratories	8	8	8	8	8
No. of results	30	30	30	30	30
No. of laboratories retained after elimination of outliers	7	8	7	7	7
No. of accepted results	26	30	26	26	30
Mean value, %	0.75	1.4	1.1	1.3	2.6
Repeatability standard deviation (S_r)	0.07	0.24	0.09	0.07	0.30
Repeatability coefficient of variation (RSD_r)	10.0	17.0	8.4	5.3	11.8
Repeatability value (r) [$2.83 \times S_r$]	0.21	0.67	0.25	0.48	0.86
Reproducibility standard deviation (S_R)	0.14	0.33	0.11	0.12	0.94
Reproducibility coefficient of variation (RSD_R)	18.5	23.4	10.4	9.2	36.3
Reproducibility value (R) [$2.83 \times S_R$]	0.39	0.92	0.32	0.28	2.6

330°C (apparently this will not affect the separation of the mono- and diglycerides), with a detector temperature of 335°C to 350°C. These proposed changes have not been validated by collaborative study.

4. For automatic samplers with 2-mL crimp-top vials, it is convenient to double the amount of sample and reagents.
5. One or more reference solutions (Reagents, 8) can also be prepared without adding n-tetradecane to the solution. Silylation of the reference solutions (Procedure, 2) is then carried out as described for the sample solution (Procedure, 1) after addition of the internal standard solution (Reagents, 7) and silylating reagents.

REFERENCES

1. *Standard Methods for the Analysis of Oils, Fats and Derivatives*, International Union of Pure and Applied Chemistry, 7th edn., Blackwell Scientific Publications, 1987, IUPAC Method 6.002 (ex. 2.326).
2. *Pure Appl. Chem. 63:*1153 (1991).
3. Christie, W.W., *Lipid Analysis*, 2nd edn., Pergamon Press, New York, 1982, pp. 101, 159.

AOCS Recommended Practice Cd 11c-93

Reapproved 1997

Quantitative Separation of Monoglycerides, Diglycerides and Triglycerides by Silica Gel Column Chromatography

DEFINITION

This method determines glyceride mixtures by solid–liquid adsorption chromatography (SLAC), using silica gel as the adsorbent. Triglycerides are eluted with 10% diethyl ether in petroleum ether, diglycerides with 25% diethyl ether in petroleum ether, and monoglycerides with 100% ether. Free fatty acids present are found predominantly in the triglyceride fraction; the remainder in the diglyceride fraction. Thin-layer chromatography (TLC) is used to verify the purity of the fractions and/or the effectiveness of the separation.

SCOPE

This method is applicable to emulsifiers and shortenings

APPARATUS

1. Chromatographic column assembly—25- × 2-cm column with Teflon™ stopcock plug and solvent reservoir, 250 mL, with glass joint, fitting the top of the column.
2. Receiving flasks—four 250 mL.
3. Variable-thickness thin-layer chromatographic spreader (see Notes, 1).
4. Glass plates—Pyrex™, 5 × 20.5 × 0.32 cm (see Notes, 1).
5. Cylindrical thin-layer developing chamber—6.5 cm diameter × 24 cm high, with cap (see Notes, 1).

REAGENTS

1. Silica gel—Davison grade 923, adjusted to 5% moisture.
2. Silica gel—GF-254 for thin-layer chromatography, E. Merck (Brinkmann Instruments, Inc., distributor).
3. Chloroform—reagent grade (see Notes, *Caution*).
4. Petroleum ether—Skelly Solve F, reagent grade (see Notes, *Caution*).
5. Diethyl ether—anhydrous, reagent grade (see Notes, *Caution*).
6. Ethyl alcohol—formula 30 (see Notes, *Caution*).
7. Acetone—reagent grade (see Notes, *Caution*).
8. Sulfuric acid—concentrated (sp. gr. 1.84), reagent grade, diluted to give a 70:30, v/v, sulfuric acid/water solution (see Notes, *Caution*).
9. Chromium trioxide—technical grade.

PROCEDURE

1. Prepare the column with 30 g silica gel (Reagents, 1) slurried in 50–60 mL petroleum ether (Reagents, 4).
2. Accurately weigh 0.7–0.9 g of sample into a 30-mL beaker. Add 3 mL chloroform (Reagents, 3) to dissolve the sample and quantitatively transfer it to the top of the column. Rinse beaker with up to 3 mL of chloroform, adding the rinsings to the column.
3. Elute the sample at a flow rate of 2 mL/min, using 250 mL of solvent for each fraction, as noted below in this section for Fractions I through IV. If the separation of methyl esters from triglycerides is desired, 200 mL of a solvent system of 5% diethyl ether (Reagents, 5) in petroleum ether (Reagents, 4), v/v, should be used prior to the elution of the triglycerides in Fraction I.

   Use the following solvent systems to elute the indicated fraction:

   (a) Fraction I (triglycerides)—250 mL 10% diethyl ether (Reagents, 5) in petroleum ether (Reagents,4).

   (b) Fraction II (diglycerides)—250 mL 25% diethyl ether (Reagents, 5) in petroleum ether (Reagents,4).

   (c) Fraction III (monoglycerides)—250 mL 100% diethyl ether (Reagents, 5).

   Note—If the elution of glycerol and/or other polar material is desired, a fourth fraction is collected:

   (d) Fraction IV (glycerol and/or polar material)—200 mL 100% ethyl alcohol (Reagents, 6).
4. Collect the effluents separately for each fraction and evaporate to dryness in tared 250-mL flasks on a steam bath under a stream of nitrogen. A boiling chip should be placed into each flask before the tare weight is determined in order to prevent bumping.
5. Weigh the fraction and repeat drying until a constant weight is obtained.

CALCULATIONS

1. If confirmatory tests by TLC indicate that the fractionation was effective, the weight percent of each fraction is determined as follows:

(a) $$\text{Fraction I (triglycerides)} = \frac{\text{mass of Fraction I, g} \times 100}{\text{mass of sample, g}}$$

(b) $$\text{Fraction II (diglycerides)} = \frac{\text{mass of Fraction II, g} \times 100}{\text{mass of sample, g}}$$

(c) $$\text{Fraction III (monoglycerides)} = \frac{\text{mass of Fraction III, g} \times 100}{\text{mass of sample, g}}$$

(d) $$\text{Fraction IV (glycerol and/or polar material)} = \frac{\text{mass of Fraction IV, g} \times 100}{\text{mass of sample, g}}$$

2. Conversion of methyl ester isolated to percent free fatty acid:

$$\text{Weight \% free fatty acid} = \frac{100 \times \text{mass of methyl ester, g} \times \text{GF}}{\text{mass of sample, g}}$$

Where—
GF = gravimetric factor
= 0.935 for free fatty acid expressed as lauric
= 0.942 for free fatty acid expressed as myristic
= 0.948 for free fatty acid expressed as palmitic
= 0.953 for free fatty acid expressed as oleic

THIN-LAYER CHROMATOGRAPHIC VERIFICATION

Note—If commercially available TLC plates (see Notes, 1) are used, omit steps 1 through 3 and proceed directly to step 4.

1. Plate preparation: Twenty-two plates (5 × 20.5 cm) are placed in a thin-layer chromatographic spreader bed. The variable-thickness spreader blade is adjusted for 0.25-mm adsorbent application thickness.
2. Adsorbent preparation: Slurry 30 g of silica gel GF-254 (Reagents, 2) with 60 mL of water. Shake vigorously for 1 min. Quickly pour slurry into the spreader and coat plates.
3. Air dry the coated plates at room temperature.
4. Activate plates by heating at 110–120°C for 30 min (or follow manufacturer's directions). Store plates in closed container over silica gel containing a moisture indicator.
5. Spot 30–60 µg of each fraction from a 30–40 mg/mL $CHCL_3$ (Reagents, 3) sample solution approximately 2 cm up from the bottom edge of the plate; also spot an appropriate standard (e.g., Dur-Em 104, Dur-Em 187, commercial 40% monoglyceride emulsifier, etc.) next to fraction samples.
6. Place TLC plate in the developing chamber after a saturated atmosphere (approximately 15 min chamber saturation time with closed chamber) is obtained with the following solvent system:
   92% petroleum ether (Skelly Solve F) (Reagents, 4),
   8% acetone (Reagents, 7).
7. Remove plate from the chamber after the solvent front has moved 16 cm from the origin of spotting and air dry plate in a suitable, operating fume hood.
8. Spray plate with a 70% aqueous sulfuric acid solution (Reagents, 8) saturated with chromium trioxide (Reagents, 9) and place on a hot plate (at 200°C) until charring is complete (white fumes cease).
9. Verify composition of fractions by comparing spots of fractions with spots of standards of known composition.

NOTES

Caution

Chloroform is a known carcinogen. It is toxic by inhalation and has anesthetic properties. Avoid contact with the skin. Prolonged inhalation or ingestion can lead to liver and kidney damage and may be fatal. It is nonflammable, but will burn on prolonged exposure to flame or high temperature. The TLV is 10 ppm in air. A fume hood should be used at all times when using chloroform.

Petroleum ether is extremely flammable. Avoid static electricity. The explosive limits in air are 1–6%. A fume hood should be used at all times when using petroleum ether.

Diethyl ether is highly flammable and is a severe fire and explosion hazard when exposed to heat or flame. It is a central nervous system depressant by inhalation and skin absorption. It will form explosive peroxides upon exposure to light. Handle empty containers, particularly those from which ether has evaporated, with extreme caution. Explosive limits in air are 1.85–48%. The TLV is 400 ppm in air. A fume hood should be used at all times when using diethyl ether.

Ethyl alcohol (ethanol) is flammable. Use a properly operating fume hood when heating or evaporating.

Acetone is highly flammable. Forms explosive peroxides with oxidizing agents. Use effective fume-removal device. Do not mix with chloroform.

Sulfuric acid is a strong acid and will cause severe burns. Protective clothing should be worn when working with this acid. It is a dehydrating agent and should not be stored in the vicinity of organic materials. Use great caution in mixing with water due to heat evolution that can cause explosive splattering. Always add the acid to water, never the reverse.

NUMBERED NOTES

1. Commercially available plates may be used.

REFERENCES

1. Distler, E., and F.J. Baur, *JAOAC 49*:812 (1966).
2. Quinlin, P., and H.J. Weiser, *JAOCS 35*:325 (1958).
3. This method was submitted by AOCS Uniform Methods Committee member Robert Manning in 1992.

396

AOCS Official Method Cd 11d-96

Reapproved 1997

Mono- and Diglycerides Determination by HPLC–ELSD

DEFINITION

This standard describes a method for the quantitative determination of monoacylglycerides and diacylglycerides. Neutral lipid classes are separated with normal-phase high-performance liquid chromatography (HPLC), and the monoglycerides and diglycerides present are determined with an evaporative light scattering detector (ELSD). Samples are dissolved in a solvent mixture consisting of n-hexane/2-propanol (90:10, v/v) and analyzed without derivatization. Because the response of the ELSD is not linear, a log(response)/log(mass) calibration curve must be established for expected analytes based on ELSD response to calibration standards identical to the sample components. The method quantitation and detection limits (S/N = 2) are approximately 0.25 mg and 0.06 mg, respectively.

SCOPE

Applicable to both oils and emulsifiers. (Phytosterols may interfere with the determination of 1,3-diacylglycerides in vegetable oils.)

APPARATUS

1. HPLC—The procedure was developed using a Hewlett-Packard 1050 (with the Hewlett-Packard ChemStation computer interfaced with the HPLC, which is not essential to the method); however, any HPLC with the following minimum requirements could be used:
   (a) Column—150 mm × 4.6 mm, with 10 µ Chromegasphere SI-60 stationary phase (ES Industries, Marlton, NJ) or equivalent.
   (b) Column temperature—maintained at 40°C with a column heater.
   (c) Dual channel gradient, programmed as noted in Table 1.
   (d) Mobile phase flow rate—2 mL/min, with an initial pressure of 27 bar (20,251 torr).
2. ELSD—The procedure was developed using a Varex ELSD II (Varex Corp., Rockville, MD); however, any suitable ELSD with the following minimum requirements could be used:
   (a) Drift tube temperature—maintained at 90°C.
   (b) Nitrogen carrier gas flow rate maintained at 30 mm (approximately 1.2 L/min) on the flow meter of the ELSD.
3. Volumetric flasks, 50 mL, 100 mL.
4. Injection syringe, capable of accurately injecting 20 µL of either standard solutions or sample solutions.

REAGENTS (see Notes, Caution)

1. HPLC-grade solvents—
   (a) n-Hexane.
   (b) 2-Propanol (isopropanol).
   (c) Ethyl acetate.
2. Reagent-grade solvents—formic acid (88%), 2-propanol, n-hexane.
3. Solvent solutions for HPLC—
   (a) Formic acid/2-propanol (10:90, v/v).
   (b) Channel A mobile phase—n-hexane [Reagents, 1(a)].
   (c) Channel B mobile phase—n-hexane [Reagents, 1(a)]/2-propanol [Reagents, 1(b)]/ethyl acetate [Reagents, 1(c)]/10% formic acid [Reagents, 3(a)] (80:10:10:1, v/v/v/v); for gradient program for mobile phase, see Table 1.
   (d) Column rinse (to remove formic acid when column is not in use)—n-hexane [Reagents, 1(a)]/2-propanol [Reagents, 1(b)]/ethyl acetate [Reagents, 1(c)] (80:10:10, v/v/v).
4. Solvent solution for sample preparation—n-hexane [Reagents, 1(a)]/2-propanol [Reagents, 1(b)] (90:10, v/v).

Table 1
The mobile phase gradient program used for neutral lipids separation on a 150 × 4.6 mm, 10 µ Chromegasphere SI-60 column. Column temperature, 40°C. Channel A, hexane; Channel B, hexane/2-propanol/ethyl acetate/10% formic acid (80:10:10:1, v/v/v/v).

Time (min)	Flow (mL/min)	% of each channel A	B
0	2	98	2
8	2	65	35
8.5	2	2	98
15	2	2	98
15.1	2	98	2
19	2	98	2

PROCEDURE

1. Sample preparation (see Notes, 1)
   Vegetable oils:
   (a) Weigh approximately 2 g (see Notes, 2) of vegetable oil, to within 0.1 mg, into a 50 mL volumetric flask [Apparatus, 3].
   (b) Dissolve sample in n-hexane/2-propanol solvent mixture (Reagents, 4) and bring to 50 mL volume with solvent mixture (Reagents, 4).
   Monoglyceride emulsifiers:
   (a) Weigh approximately 100 mg of sample, to within 0.1 mg, into a 50 mL volumetric flask [Apparatus, 3].

397

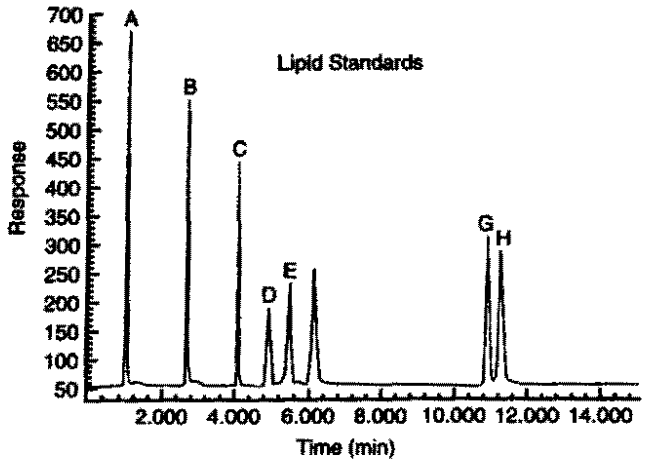


Figure 1. Chromatogram of various lipid standards: (A) cholesteryl myristate, (B) tripalmitin, (C) palmitic acid, (D) 1,3-dipalmitin, (E) cholesterol, (F) 1,2-dipalmitin, (G) 1-monopalmitin and (H) 2-monopalmitin. The concentration of each standard is in the neighborhood of 1 mg/mL. The HPLC parameters can be seen in Table 1. The Varex II ELSD parameters are: drift tube temperature, 90°C; nitrogen gas flow, 30 mm.

(b) Dissolve sample in n-hexane/2-propanol solvent mixture (Reagents, 4) and bring to 50 mL volume with the solvent mixture (Reagents, 4).

(c) Prior to HPLC analysis, dilute the 50 mL sample solution to a final volume of approximately 0.5 mg/mL using the solvent mixture (Reagents, 4).

2. Calibration

Monoacylglycerides and diacylglycerides:

The responses of the ELSD to 1,3-distearin, 1,3-dipalmitin and 1,3-dilinolein (and other diglycerides as needed) and to 1-monostearin, 1-monopalmitin and 1-monoolein (and other monoglycerides as needed) must be determined using a series of solutions prepared from individual standards. Standards should be of 99%+ purity and may be obtained from Nu-Check-Prep, Elysian, MN).

(a) Accurately weigh approximately 0.1 g (see Notes, 3) of each standard (1-monopalmitin and 1,3-dipalmitin) into a 100 mL volumetric flask [Apparatus, 3(b)], dissolve it in a portion of the n-hexane/2-propanol solvent mixture (Reagents, 4) (see Notes, 2 and 4), and dilute to 100 mL with the solvent mixture (Reagents, 4) to provide a stock standard solution.

(b) From the stock standard solution (approximately 1 mg/mL), prepare 1:2, 1:5, and 1:10 dilutions with the hexane/2-propanol solvent mixture (Reagents, 4) to obtain approximately 0.5, 0.2, and 0.1 mg/mL working standard solutions. Use the 1.0, 0.5, 0.2 and 0.1 mg/mL working standard solutions to establish the calibration curve for monoacylglycerides and diacylglycerides.

(c) Analyze the four working standard solutions according to Procedure, 3, and plot the log of the peak area (log [response]) against the log of the concentration (log [mass, µg]) of monoglyceride (1-monopalmitin) and diacylglyceride (1,3-dipalmitin) to obtain calibration curves. Use both linear and quadratic equations to calculate the results (see Notes, 5). A typical response pattern for working standards is shown in Figure 1; ELSD response curves for the working standards are shown in Figures 2 and 3.

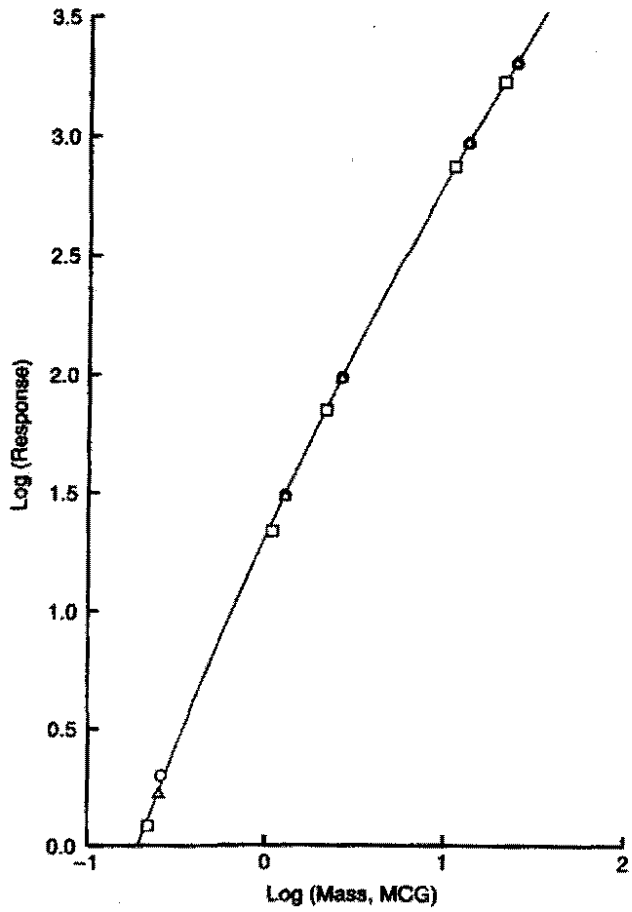


Figure 2. The response curves of 1,3-dipalmitin (○), 1,3-stearin (△) and 1,3-dilinolein (□) with a Varex ELSD using the HPLC and the ELSD parameters as described in Figure 1.

Monoglyceride emulsifiers:

Use the calibration curve of 1-monopalmitin from Procedure, 2(a) and (b) and both linear and quadratic equations to calculate the results. If a new calibration curve is needed for monoacylglyceride emulsifier samples (B series in this study), prepare the working standards from the same stock solution of Procedure 2(a). Analyze the working standard solutions according to Procedure, 3, and plot the log of the peak area against the log of the concentration of 1-monopalmitin to obtain a calibration curve.

3. Analysis procedure

(a) Inject 20 µL of solution (working standard or sample) onto the HPLC column, using the conditions noted in Apparatus, 1(a) to 1(d), with mobile phases Reagents, 3(b) and 3(c) programmed to provide the gradient noted in Table 1.

398

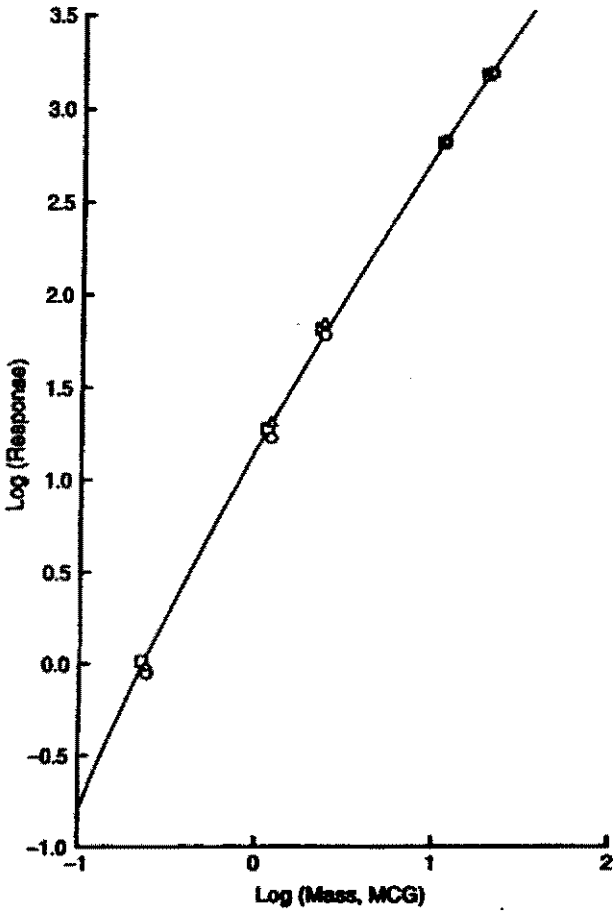


Figure 3. The response curves of 1-monopalmitin (○), 1-monostearin (△) and 1-monoolein (□) with a Varex ELSD using the HPLC and the ELSD parameters as described in Figure 1.

(b) When not in use, the column must be rinsed with the mixture of n-hexane/2-propanol/ethyl acetate [Reagents, 3(d)] to remove formic acid.

RESULTS

The 1,3-diacylglycerides are resolved from the 1,2-diacylglyceride positional isomers, although some 1,3-diacylglycerides of low molecular weight interfere with the 1,2-diacylglycerides of high molecular weight. For monoglycerides, the separation between 1-acylglycerides or 3-acylglycerides and 2-acylglycerides is optimized only between those pairs with identical fatty acyl groups.

1. The ELSD is a universal detector whose response is a function of the mass of the analyte. As shown in Figures 2 and 3, an approximate linear response may be obtained by a log/log plot of ELSD response vs. mass of the analyte, with $r^2 = 0.996$.
2. Figures 4 and 5 show results for the analysis of olive and peanut oils, which were approximately two years old. The olive and peanut oils appeared to contain free fatty acids (peak C), 1,3-diacylglycerides (peak D) and 1,2-diacylglycerides (peak F), but not detectable monoacylglycerides.
3. Figure 5 also shows the chromatogram of the peanut oil spiked with 5% (m/m) of a commercial monoacylglyceride emulsifier. Phytosterols may interfere with the determination of 1,3-diacylglycerides in vegetable oils.
4. This method may be expected to give a small relative standard deviation (<2%) between duplicates and between days. To illustrate the accuracy of this method, monoglyceride results from the analysis of a commercial monoglyceride emulsifier by HPLC–ELSD, GLC and SFC methods are compared in Table 2.

Table 2
The comparison of monoglyceride results of a commercial emulsifier as determined by the HPLC–ELSD, GC and SFC methods

	Total monoglycerides, g/100 g		
	HPLC–ELSD	GC/derivatized[a]	SFC/underivatized[a]
Lot 1840			
Mean	92.5	92.3	93.4
%RSD (n)	1.1 (4)	1.3 (4)	3.5 (11)
Lot 6022			
Mean	94.7	94.0	95.9
%RSD (n)	1.5 (4)	1.8 (4)	3.4 (12)

[a]Data are from Lee et al., *J. Assoc. Anal. Chem.* 74:533 (1991).

PRECISION

1. *Repeatability limit*—The absolute difference between two independent single test results, obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within short intervals of time, should not be greater than repeatability limit r as shown in Tables 3 through 6.

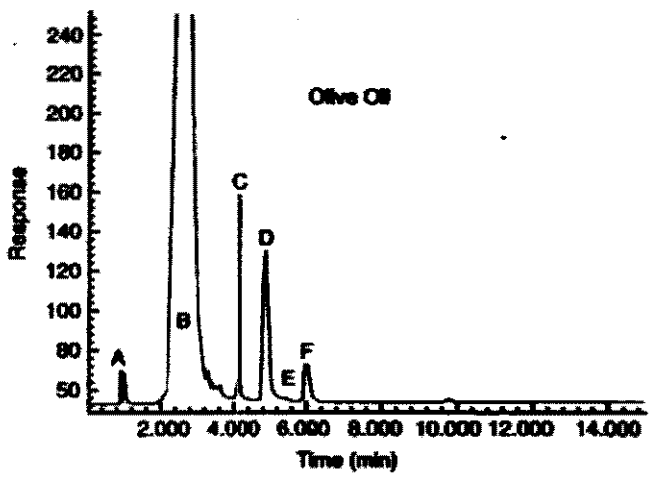


Figure 4. The chromatogram of an olive oil obtained with the HPLC and the ELSD parameters as described in Figure 1. Peaks are identified by retention time only: (A) steryl esters, (B) triglycerides, (C) free fatty acids, (D) 1,3-diacylglycerols, (E) minor 1,3-diacylglycerols and/or phytosterols and (F) 1,2-diacylglycerols.

399

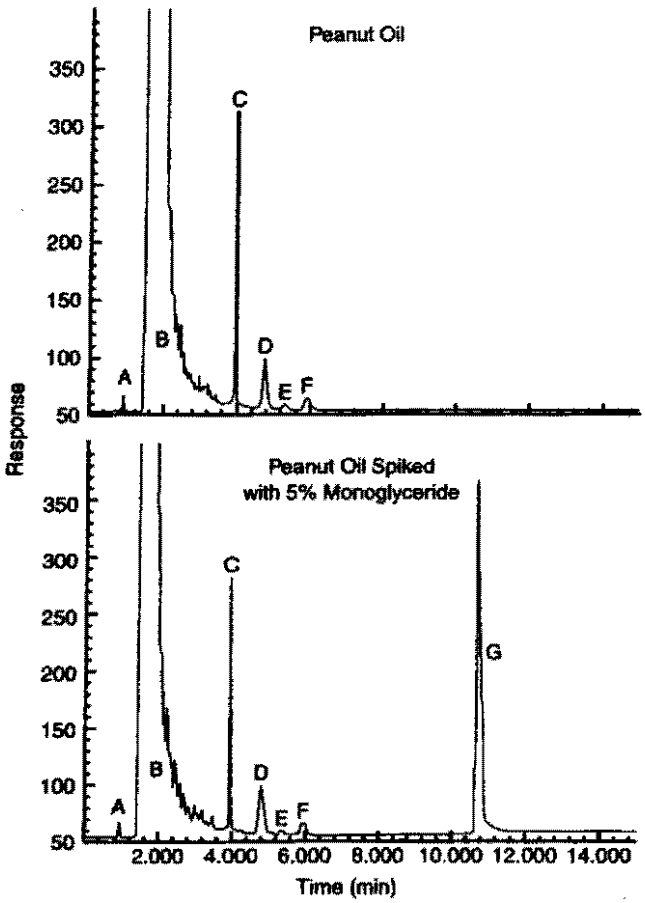


Figure 5. The HPLC–ELSD chromatogram of a peanut oil with and without the addition of a monoglyceride emulsifier. Peaks are identified by retention time only: (A) steryl esters, (B) triglycerides, (C) free fatty acids, (D) 1,3-diacylglycerols, (E) minor 1,3-diacylglycerols and/or phytosterols, (F) 1,2-diacylglycerols and (G) monoglycerides. Refer to Figure 1 for the HPLC and the ELSD parameters.

2. *Reproducibility limit*—The absolute difference between two single test results, obtained with the same method on identical test material in different laboratories with different operators using different equipment, should not be greater than the reproducibility limit R as shown in Tables 3 through 6.

NOTES

Caution

Hexane is a flammable solvent and a dangerous fire risk. All work should be performed in a fume hood, with no open flames. Always ensure that your hood is functioning before introducing volatile solvents. The TLV for hexane is 50 ppm in air. OSHA recommends that exposure not exceed 350 ng/M³ for a time-weighted average. Hexane vapor causes lung irritation and produces neurotoxic effects.

Isopropanol (isopropyl alcohol) is flammable and a dangerous fire risk. The explosive limits in air are 2–12%. It is toxic by ingestion and inhalation. The TLV in air is 400 ppm. The use of a fume hood is recommended when using isopropanol.

Table 3

For the determination of monoacylglycerides, the interlaboratory study completed by the IUPAC Commission VI.3 in 1995, in which 11 laboratories participated, each analyzing 8 samples (4 sets of blind duplicates) and obtaining two (2) values for each sample, gave the results summarized in the following table. Results were determined by linear equation.

Samples	1[a]	2[b]	3[c]	4[d]
Labs before outliers	11	11	11	11
Labs after outliers	9	8	9	9
Accepted results	34	28	34	34
Mono. (%)	0.011	0.025	0.054	0.088
Mono., known (%)	0.0102	0.0204	0.0511	0.0919
Repeatability:				
S_r	0.001	0.002	0.003	0.003
RSD_r	10.92	7.43	5.66	3.39
r	0.003	0.006	0.008	0.008
Reproducibility:				
S_R	0.001	0.008	0.010	0.017
RSD_R	12.95	32.01	18.60	19.75
R	0.003	0.023	0.028	0.049

[a]Combined results for duplicate samples A1/A7
[b]Combined results for duplicate samples A2/A5
[c]Combined results for duplicate samples A3/A8
[d]Combined results for duplicate samples A4/A6

Table 4

For the determination of monoacylglycerides, the interlaboratory study completed by the IUPAC Commission VI.3 in 1995, in which 11 laboratories participated, each analyzing 8 samples (4 sets of blind duplicates) and obtaining two (2) values for each sample, gave the results summarized in the following table. Results were determined by quadratic equation.

Samples	1[a]	2[b]	3[c]	4[d]
Labs before outliers	11	11	11	11
Labs after outliers	7	7	8	8
Accepted results	28	26	32	32
Mono. (%)	0.011	0.021	0.056	0.092
Mono., known (%)	0.0102	0.0204	0.0511	0.0919
Repeatability:				
S_r	0.001	0.001	0.003	0.003
RSD_r	8.95	2.31	5.16	3.16
r	0.003	0.001	0.008	0.007
Reproducibility:				
S_R	0.001	0.002	0.008	0.016
RSD_R	10.41	8.99	13.28	17.23
R	0.003	0.005	0.021	0.045

[a]Combined results for duplicate samples A1/A7
[b]Combined results for duplicate samples A2/A5
[c]Combined results for duplicate samples A3/A8
[d]Combined results for duplicate samples A4/A6

NUMBERED NOTES

1. In the collaborative study the sample preparation procedures were used with the different samples as follows:
   (a) Vegetable oils—P.
   (b) Monoglyceride emulsifiers—B-1 and B-2.

400

(c) Collaborative study samples—A-1, A-2, A-3, A-4, A-5, A-6, A-7 and A-8.

2. The maximum concentration of vegetable oil that may be dissolved in the solvent mixture (Reagents, 4) is 4 g/100 mL (4%, m/v).
3. Record the weight of the standards to 0.1 mg (0.0001 g).
4. Sonication may be necessary.
5. Report only the results from 1,3 diacylglycerides. Ignore the 1,2-diacylglycerides (retention time >>6 min).

REFERENCES

1. *IUPAC Commission on Oils, Fats and Derivatives* (1995). Minutes of meeting, Guildford, UK, August 4–6, 1995, pp. M31–M43.

Table 5
For the determination of diacylglycerides, the interlaboratory study completed by the IUPAC Commission VI.3 in 1995, in which 11 laboratories participated, each analyzing 8 samples (4 sets of blind duplicates) and obtaining two (2) values for each sample, gave the results summarized in the following table. Results were determined by linear equation.

Samples	1[a]	2[b]	3[c]	4[d]
Labs before outliers	11	11	11	11
Labs after outliers	6	7	6	7
Accepted results	24	24	24	26
Di. (%)	0.126	0.092	0.055	0.042
Di., known (%)	0.1270	0.0873	0.0564	0.0461
Repeatability:				
S_r	0.006	0.003	0.003	0.001
RSD_r	4.87	2.73	5.77	2.18
r	0.018	0.007	0.008	0.003
Reproducibility:				
S_R	0.019	0.013	0.006	0.006
RSD_R	14.82	14.70	10.12	14.36
R	0.053	0.038	0.016	0.017

[a]Combined results for duplicate samples A1/A7
[b]Combined results for duplicate samples A2/A5
[c]Combined results for duplicate samples A3/A8
[d]Combined results for duplicate samples A4/A6

Table 6
For the determination of diacylglycerides, the interlaboratory study completed by the IUPAC Commission VI.3 in 1995, in which 11 laboratories participated, each analyzing 8 samples (4 sets of blind duplicates) and obtaining two (2) values for each sample, gave the results summarized in the following table. Results were determined by quadratic equation.

Samples	1[a]	2[b]	3[c]	4[d]
Labs before outliers	11	11	11	11
Labs after outliers	6	7	7	7
Accepted results	24	28	28	28
Di. (%)	0.133	0.081	0.057	0.056
Di., known (%)	0.1270	0.0873	0.0564	0.0461
Repeatability:				
S_r	0.007	0.002	0.003	0.001
RSD_r	5.19	2.71	5.24	1.87
r	0.020	0.006	0.008	0.003
Reproducibility:				
S_R	0.017	0.027	0.008	0.026
RSD_R	12.93	33.87	14.51	46.54
R	0.049	0.078	0.023	0.073

[a]Combined results for duplicate samples A1/A7
[b]Combined results for duplicate samples A2/A5
[c]Combined results for duplicate samples A3/A8
[d]Combined results for duplicate samples A4/A6

401

AOCS Official Method Ce 1e-91
Reapproved 1997

Determination of Fatty Acids in Edible Oils and Fats by Capillary GLC

DEFINITION
This method is for the determination of fatty acid methyl esters by capillary gas-liquid chromatography.
SCOPE
This method is applicable to the determination of fatty acid methyl esters obtained from vegetable oils and fats according to AOCS Official Method Ce 2-66, IUPAC Method 2.301, AOAC 969.33 or other accepted standard method.

APPARATUS

The instructions given apply to normal equipment used for gas–liquid chromatography employing capillary columns and flame-ionization detection.

1. Gas–liquid chromatograph.
2. Injection system.
   Note—The injection system should be specially designed for use with capillary columns. It should be of the split type or on-column (see Notes, 1).
3. Oven.
   Note—The oven should be capable of heating the column to at least 220°C and of maintaining the desired temperature to within 0.1°C (see Notes, 2).
4. Capillary column, with following specifications—fused silica or glass; length 25–60 m with 0.20–0.35 mm i.d.
5. Stationary phase—moderate polarity, mainly of the type polyglycol (polyethylene glycol 20,000), polyester (butanediol polysuccinate) or polar polysiloxane (cyanosilicones), e.g., Carbowax, Durabond 225, FFAP, Silar 5 CP or Supelcowax. The coating should be 0.2–0.25 mm in thickness.
6. Assembly and conditioning of the column:
   (a) Observe the normal precautions for assembling capillary columns—arrangement of the column in the oven (support), choice and assembly of joints (leak tightness), positioning of the ends of the column in the injector and the detector (reduction of dead spaces). Place the column under a carrier gas flow (e.g., 0.3 bar for a 25-m column of 0.3 mm i.d.).
   (b) Condition the column by temperature programming the oven at 3°C/min from ambient temperature to a temperature 10°C below the decomposition limit of the stationary phase. Maintain at this temperature for 1 hr or until the baseline stabilizes. Return to 180°C to work under isothermal conditions.
7. Syringe—maximum capacity of 10 µL graduated in 0.1 µL.
8. Any suitable recorder, integrator or data processor may be used.

REAGENTS

1. Carrier gas—either helium or hydrogen (see Notes, 3).
2. Auxiliary gas—hydrogen 99.9% min, free from organic impurities, air or oxygen.
3. Reference standards—a mixture of methyl esters of fatty acids (see Notes, 4).

PROCEDURE

Selection of Optimum Operating Conditions

See IUPAC Method 2.304, capillary column gas–liquid chromatography of fatty acid methyl esters, section 5.1.1.

The determination of efficiency and resolution as carried out for packed columns (Ce 1-62) is no longer necessary, as commercially available capillary columns usually exhibit excellent performance. Instead, a system suitability test can be carried out by analyzing a certified reference material. In doing so, the operating conditions, the efficiency and resolution of the column can be checked at the same time.

System Suitability Test

Reference standards of known fatty acid composition (containing, e.g., C16:0, C18:0, C18:1, C18:2, C18:3 and C20:0 methyl esters) or certified reference material (e.g., CRM 162) are used to check the performance of the system (Notes, 4). If the quantitative results obtained correspond to the theoretical values or the certified values, the system can be regarded as suitable.

Sample Analysis

1. Use the syringe (Apparatus, 7) to take 0.1–1 µL of the solution of 1 to 5% of methyl esters prepared according to AOCS Official Method Ce 2-66, IUPAC Method 2.301 or AOAC 969.33 (See Notes, 1).
2. Operate the oven isothermally at a temperature of 180–210°C or operate by linear heating from 80–220°C (see Notes, 5).
3. Set injector and detector temperatures 30–50°C above the column temperature (see Notes, 7).

CALCULATION AND EXPRESSION OF RESULTS

Identification of Peaks

Analyze a reference standard mixture of known composition under the same operating conditions as those employed for the samples and determine the retention times (Notes, 4). Identify the peaks from the sample using the retention times of the reference standards.

Quantitative Analysis

(a) Apart from exceptional cases, assume that all of the components of the sample are represented on the chromatogram, so that the total of areas under the peaks

402

represents 100% of the consituents (excluding the solvent peak).

(b) For samples in which significant amounts of components below C12 are absent, calculate the content of a particular constituent (expressed as percent of methyl ester) by determining the percentage represented by the area of the corresponding peak relative to the sum of the area of all the peaks.

Area precent of the component I expressed as methyl ester =

$$\frac{A_i}{\Sigma A_i} \times 100$$

Where—

ΣA_i = sum of the areas under all the peaks

In the presence of significant amounts of components below C12, the areas obtained from the gas chromatogram have to be multiplied with correction factors in order to convert area percentages into mass percentages.

Correct the area of each peak to compensate for the flame ionization detector (FID) response for each component. The FID correction factors are calculated from the molecular weight of the FAME as follows:

$$FID_x = \frac{MW_x}{(n_x - 1)(AW_C)(FID_{16:0})}$$

Where—

FID_x = the FID factor for component x
MW_x = molecular weight of component x
n_x = the number of carbon atoms in the FAME of component x
AW_C = the atomic weight of carbon (12.01)
$FID_{16:0}$ = the FID correction factor for 16:0 (1.407).

All other FID correction factors used in the calculation are relative to $FID_{16:0}$. For example, the correction factor for 10:0 becomes 1.10. FID correction factors are listed in Table 3.

Calculate the (relative) percentage x of each component by determining the corrected area of the corresponding peak relative to the sum of the corrected areas of all the peaks, as follows:

$$x = \frac{A_x}{A_t}$$

Where—

A_x = the corrected area of the peak corresponding to component x
A_t = sum of the corrected areas under all the peaks, excluding the solvent peak

PRECISION

The results of interlaboratory studies, organized at the international level, gave the statistical results [evaluated in accordance with Internation Organization for Standardization (ISO) 5725-1986] which are summarized in Tables 1 and 2 (References, 2). See also—Tables 2.303.1 and 2.304.1 (References, 1).

Repeatability—When the mean of the values obtained from two single determinations, carried out in rapid succession by the same operator using the same apparatus under the same conditions for the analysis of the same test sample, lies within the range of the mean values cited in Tables 1 and 2, the difference between the two values obtained should not be greater than the repeatability value (r) for the level of fatty acids comparable to those cited in Tables 1 and 2.

Reproducibility—When the values for the final result, obtained by operators in different laboratories using different apparatus under different conditions from the analysis of the same laboratory sample, lie within the range of mean values cited in Tables 1 and 2, the difference between the values for the final result obtained by those operators should not be greater than the reproducibility (R) for the level of fatty acids comparable to those cited in Tables 1 and 2.

NOTES

1. On-column injection is preferred because it generally gives less discrimination with oil-column injection mode. Dilute the solution to 0.05% before injection. An automatic injection system is recommended because it improves the precision of results. If a split injector is used, adjust the split ratio to approximately 1:100.
2. Apparatus equipped with a temperature programmer is recommended.
3. Either helium or nitrogen may be suitable as a carrier gas, but these may increase helium elution times with respect to hydrogen. Hydrogen, which is used only with capillary columns, can double the speed of analysis, but it is hazardous. Safety devices are available and should be used. Oxygen must be removed from the carrier gas by suitable filters.
4. Standards of most known fatty acids are available from suppliers such as: Nu-Check-Prep, Inc., P. O. Box 172, Elysian, MN, USA; Bast of Copenhagen V, Denmark; Lanodon Fine Chemicals AB, Limhamnsgarden alle 9,

Table 3
List of FID response factors.

FAME	MW	n − 1	FID factor	Correction factor
4:0	102.13	4	2.126	1.51
6:0	130.19	6	1.807	1.28
8:0	158.24	8	1.647	1.17
9:0	172.27	9	1.594	1.13
10:0	186.30	10	1.551	1.10
11:0	200.32	11	1.516	1.08
12:0	214.35	12	1.487	1.06
13:0	228.37	13	1.463	1.04
14:0	242.40	14	1.442	1.02
15:0	156.42	15	1.423	1.01
16:0	270.46	16	1.407	1.00[a]
17:0	284.49	17	1.393	0.99
18:0	298.52	19	1.370	0.97
19:0	312.52	19	1.370	0.97
20:0	326.57	20	1.360	0.97
21:0	340.57	21	1.350	0.96
22:0	354.62	22	1.342	0.95
23:0	368.62	23	1.334	0.95
24:0	382.68	24	1.328	0.94

[a]Reference.

403

AOCS Official Method Ce 1c-89
Surplus 1997

Fatty Acid Composition by GLC *cis, cis* and *trans* Isomers

DEFINITION

This method is for the determination of fatty acid composition of hydrogenated and unhydrogenated vegetable fats and oils by capillary gas–liquid chromatography (GLC), using an SP-2340 column.

SCOPE

The method is designed to evaluate, by a single capillary GLC procedure, three properties of a vegetable oil: (a) the fatty acid composition, (b) level of *trans* unsaturation and (c) *cis, cis* methylene-interrupted unsaturation. This procedure reports the *trans* content as the area percent of all components that have one or more *trans* double bonds. The *cis, cis* value is determined by summing the results from methyl linoleate and methyl linolenate. For a discussion of the agreement between this procedure and the traditional lipoxygenase method, see References, 1. *Trans* content as determined by this procedure may not agree with *trans* content as determined by the infrared spectrophotometric method (AOCS Official Method Cd 14-61). There is a reported observation indicating that the method underestimates the *trans*-octadecenoate content in favor of the *cis* isomers in partially hydrogenated vegetable oils (Notes, 1 and References, 2).

APPARATUS

1. Gas chromatograph—with capillary injection system (split mode, operated at a split ratio of 1:100) and flame-ionization detector (FID), capable of meeting, at a minimum, the following requirements (see Notes, 1):

   Injection port, 210°C
   Detector, 210°C
   Oven temperature profile:
   - Initial temperature, 150°C
   - Initial hold time, 0 min
   - Program rate, 1.3°C/min
   - Final temperature, 200°C
   - Final hold time, 10 min

2. Capillary GLC column—60-m × 0.25-mm fused silica capillary column coated with SP-2340 (Supelco #2-4023, Supelco, Inc., Bellefonte, PA, USA). See Notes, 1 and 2.

REAGENTS

1. Carrier gas—helium, minimum purity 99.95 mol%, dried and containing a maximum of 10 mg oxygen/kg.
2. Reference standards—a mixture of *cis* and *trans* methyl esters of known composition, e.g., quantitative mixture no. K110 (#19050, Alltech Associates, Inc., Deerfield, IL, USA) or GLC-67 (Nu-Chek-Prep, Inc., Elysian, MN, USA).

PROCEDURE

1. Set up the gas chromatograph as described in the Apparatus section. Measure the average carrier gas linear velocity to 15 cm/sec, with a split ratio of 100:1. Condition a new column by purging with carrier gas overnight at room temperature and then gradually increasing the temperature (1–2°C/min) to a temperature 10–20°C higher than the normal operating temperature, but no higher than the recommended limits of the column.
2. Prepare the methyl esters from the triglycerides according to Notes, 3. Melt the samples and mix them thoroughly before sampling the vials to remove the triglycerides for methylation. Dilute the methyl esters to about 1% with hexane or heptane.
3. Inject 1 µL of the methyl esters from the reference sample into the gas chromatograph. Identify each component (see Notes, 4) and compare with Figure 1. Select conditions to provide close to baseline separation or better of *cis* $C_{18:1}$ and *trans* $C_{18:1}$ (see Notes, 5).
4. Inject 1 µL of the methyl esters from the test sample into the gas chromatograph. Calculate the amount of each component with an area normalization method with unity response factors for each component.
5. Make the proper assignments for the peaks by comparing the retention times with those of standards, using the chromatogram shown in Figure 1 as a guideline. The paper by H. Heckers, et al. (References, 4) may also be useful in making the proper assignments.
6. Calculate the composition for each sample. The reported $C_{18:1}$ level is the sum of all $C_{18:1}$ *trans* and $C_{18:1}$ *cis* components. The same is true for $C_{18:2}$ and $C_{18:3}$, which are the sum of all $C_{18:2}$ and $C_{18:3}$ components, respectively. The total *trans* level is the sum of $C_{18:1}$ *trans*, $C_{18:2}$ *trans* and $C_{18:3}$ *trans* components. The chromatogram in Figure 1 shows the elution order of these components. The $C_{18:1}$ *trans* component elutes before the $C_{18:1}$ *cis* component. The $C_{18:2}$ *trans* component consists of three peaks which elute before methyl linoleate ($C_{18:2}$ *cis, cis*). The *cis, cis* level is the sum of methyl linoleate and methyl linolenate.

DATA ANALYSIS

1. Electronic integration of peaks—Use an area normalization method with unity response factors. Report the amount for each component to the nearest hundredth of a percent. See Tables 2 and 3 for collaborative study statistics.

404

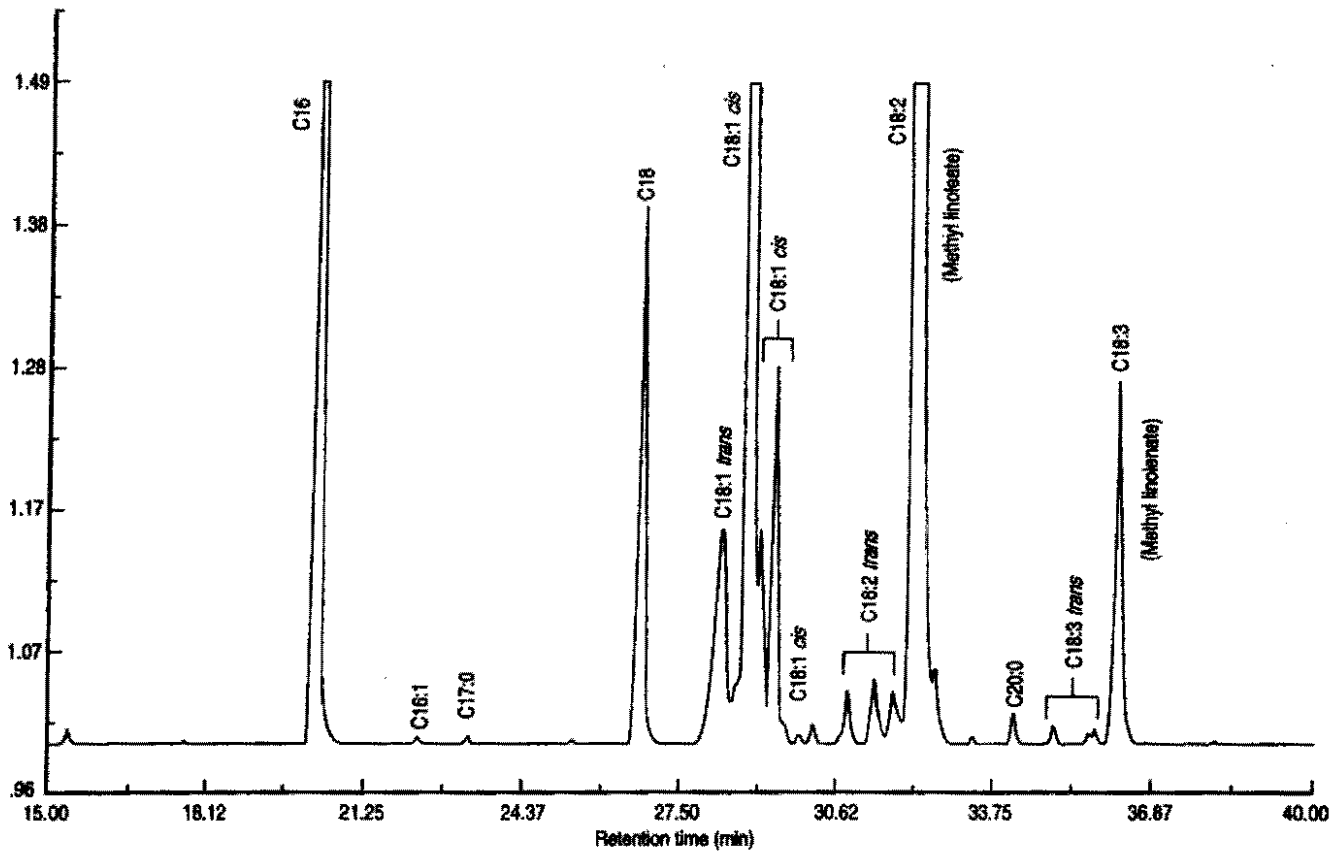


Figure 1. Chromatogram of methyl esters from collaborative study sample B1 (partially hydrogenated soybean oil).

EXAMPLE OF TYPICAL CHROMATOGRAPH

1. See Figure 1, a chromatogram of methyl esters from collaborative study sample B1 (partially hydrogenated soybean oil), and Notes, 2 and 3. By way of example, the percentages of methyl esters represented by the chromatogram in Figure 1 are as listed in Table 1.

NOTES

1. Duchateau and coworkers (References, 5) described optimum isothermal temperature conditions (verified by silver-ion exchange HPLC) for GLC separation of *cis* and *trans* isomers with three highly polar stationary phases listed below.

Stationary phase	Temperature, °C
CP Sil-88	isocratic, 175
SP 2340	isocratic, 192
BPX 70	isocratic, 198

2. Columns of medium polarity give a complicated overlapping pattern for *cis* and *trans* isomers (References, 6).
3. AOAC/International Union of Pure and Applied Chemistry (IUPAC) procedure 969.33 (References, 3) is as follows (see also AOCS Official Method Ce 2-66):

Table 1
Percentages of methyl esters corresponding to the chromatogram in Figure 1.

Methyl esters	Amount, %
$C_{14:0}$	0.1
$C_{16:0}$	11.6
$C_{18:0}$	10.8
Total $C_{18:1}$	55.1
Total $C_{18:2}$	20.0
$C_{20:0}$	0.4
Total $C_{18:3}$	0.9
$C_{22:0}$	0.4
$C_{24:0}$	0.1
Methyl oleate	28.4
Methyl linoleate	15.0
Methyl linolenate	0.6
$C_{18:1}$ *trans*	16.6
$C_{18:2}$ *trans*	3.9
Total *trans*	20.5
cis, cis	15.7

(a) Apparatus and reagents—
—Reaction flasks: 50 and 125 mL, with outer standard taper joints.
—Condenser: water-cooled, reflux type, with 20–30-cm jacket and inner standard taper joint.

405

Table 2. Collaborative study statistics, based on results from 14 laboratories.

		Sample[a]											
		A1	A2	B1	B2	C1	C2	D1	D2	E1	E2	F1	F2
$\bar{x}$, %	$C_{16:0}$	10.8	10.9	9.9	9.6	12.1	12.4	10.1	10.3	10.1	10.2	10.9	9.8
	$C_{18:0}$	1.7	1.9	4.8	4.7	10.7	10.9	8.5	8.5	8.5	8.5	7.2	6.4
	Methyl oleate	24.7	25.1	27.0	26.3	26.7	27.8	22.1	22.1	22.5	22.4	27.7	25.2
	Methyl linoleate	60.4	57.7	40.3	40.4	17.1	15.5	30.4	32.8	30.6	32.7	16.8	19.6
	Methyl linolenate	0.9	1.0	4.2	4.0	0.7	0.7	4.1	4.0	4.1	4.1	1.3	1.2
	$C_{18:1}$ *trans*	—	2.0	4.4	6.3	17.9	16.7	17.7	15.3	17.4	15.4	21.9	24.5
	Total *trans*	—	2.1	6.4	8.2	21.1	19.8	18.4	15.7	17.9	15.9	25.7	27.0
	cis, cis	61.4	58.6	44.4	44.4	17.8	16.2	34.4	36.5	34.7	36.7	18.1	20.8
RSD_R	$C_{16:0}$	7.5	3.3	3.8	4.6	6.9	4.1	3.7	6.7	4.2	5.1	6.1	5.2
	$C_{18:0}$	8.5	20.6	2.7	2.1	7.4	3.8	3.2	2.4	1.9	2.9	2.5	2.5
	Methyl oleate	1.1	2.4	3.9	3.9	7.7	6.9	10.9	9.9	10.9	11.5	9.6	9.1
	Methyl linoleate	1.2	1.6	1.7	1.8	5.2	3.0	2.4	2.2	2.0	1.7	3.2	2.5
	Methyl linolenate	17.5	16.7	3.8	4.1	15.4	15.2	4.2	9.7	3.3	3.0	9.6	9.3
	$C_{18:1}$ *trans*	—	4.0	6.5	3.5	3.8	5.1	5.2	5.4	5.7	6.6	5.7	5.0
	Total *trans*	—	7.7	8.4	4.6	4.0	4.2	5.7	6.0	5.8	6.9	5.1	4.2
	cis, cis	1.1	1.5	1.7	1.9	5.1	2.8	2.4	2.9	2.0	1.6	3.0	2.2

Table 3. Collaborative study statistics for blind duplicates.

RSD_R	Blind duplicate Pair 1 (D1, E1)	Blind duplicate Pair 2 (D2, E2)
$C_{16:0}$	1.4	3.8
$C_{18:0}$	5.3	1.0
Methyl oleate	0.8	4.0
Methyl linoleate	0.8	0.8
Methyl linolenate	2.1	1.6
$C_{18:1}$ *trans*	1.2	1.0
Total *trans*	2.4	1.9
cis, cis	0.9	0.8

[a]Sample identities are as follows:

A1, A2 = corn oil
B1, B2 = partially hydrogenated soybean oil (cooking oil)
C1, C2 = partially hydrogenated soybean oil and palm oil (shortening)
D1, D2,
E1, E2 = liquid soybean oil, partially hydrogenated soybean oil (margarine oil)
F1, F2 = partially hydrogenated soybean oil, liquid corn oil, partially hydrogenated corn oil (margarine oil)

Samples except for B1 were fortified with 2–4% of methyl elaidate and/or methyl linoleate.

—Boron trifluoride reagent: 125 g BF_3 per liter of methyl alcohol, available commercially.
—Methanolic sodium hydroxide solution: 0.5 N, prepared by dissolving 2 g NaOH in 100 mL methyl alcohol, containing ≤ 0.5% water.
—Heptane: pure as determined by GLC. If fatty acids with more than 20 carbon atoms are absent from sample, hexane may be used.
—Methyl red solution: 0.1% in methyl alcohol.
—Nitrogen: containing < 5 mg oxygen per kg.

Check new batches of reagents, particularly BF_3, by preparing and chromatographing methyl esters of pure oleic acid. If extraneous peaks appear in C_{20}–C_{22} region, reject the BF_3 preparation. Work in fume hood. Wash all glassware immediately after use. If fatty acids containing >2 double bonds are present, remove air for a few minutes. Methyl esters should be analyzed as soon as possible. If necessary, heptane solution may be kept under nitrogen in refrigerator. For prolonged storage, seal in ampule and store in freezer, or add equiva-

406

lent of 0.005% BHT. For IR analysis, solvent removal must be as complete as possible; for GLC analysis, 5–10% solution is acceptable.

Precise weighing is not required. Sample size need be known only to determine size of flask and amounts of reagents, according to the following table:

Sample, mg[a]	Flask, mL	0.5 N NaOH, mL	BF_3 Reagent, mL
100–250	50	4	5
250–500	50	6	7
500–750	100	8	9
750–1000	100	10	12

[a]Sample size of about 350 mg is preferred for GLC analysis.

(b) Procedure for fats and oils—Add sample to flask, and then add methanolic NaOH solution and boiling chip. Attach condenser and reflux until fat globules disappear (usually 5–10 min). Add BF_3 solution from bulb or automatic pipet through condenser, and continue boiling for 2 min. Add 2–5 mL heptane through condenser and boil 1 min longer. Remove heat, then condenser, and add several mL of saturated sodium chloride solution. Rotate flask gently several times. Add additional saturated sodium chloride solution to float hexane layer into neck of flask. Transfer 1 mL of upper heptane solution into glass-stoppered test tube, and add small amount of anhydrous sodium sulfate to remove water. If necessary, dilute solution to concentration of 5–10% for GLC.

To recover dry esters, transfer aqueous and heptane phases to 250-mL separator. Extract with two 50-mL portions petroleum ether (bp 30–60°C) or hexane. Wash combined extracts with 20-mL portions water until acid-free to methyl red indicator. Dry over anhydrous sodium sulfate, filter and evaporate solvent under a stream of nitrogen on steam bath. If sample is <500 mg, reduce the volume of solvents and water.

More volatile esters may be lost if evaporation is prolonged, or if stream of nitrogen is too vigorous. For IR spectroscopy, terminate evaporation as soon as solvent is removed. For GLC, method is applicable to fatty acids with ≤8 carbon atoms, if solvent is not completely removed.

(c) Procedure for fatty acids—Add fatty acid sample to flask, then add BF_3 solution and continue as in (b) Procedure for fats and oils, above, except reduce initial boiling under reflux to 2 min (see References, 3, 7–10).

4. Elution order—A discussion of the elution order of the components determined by this method is presented in the paper noted in References, 4. Each analyst should analyze a reference sample to ensure that the GLC system is operating properly.
5. Carry out the analysis of a mixture of methyl elaidate and methyl oleate. Adjust the GC conditions so that the methyl elaidate peak is recorded about 30 min after injection and 0.5–0.75 full-scale. Measure base widths in mm of methyl elaidate (W1) and methyl oleate (W2) between points of intersection, with baseline of tangents drawn to inflection points of curves. Measure the distance in mm (Y) between peak maxima for methyl elaidate and methyl oleate. Calculate resolution by the formula:

$$R = \frac{2Y}{(W1 + W2)}$$

Select conditions to obtain $R \geq 0.8$, remembering that resolution between *cis* and *trans* isomer groups increases with heating rate, while the resolution within each group decreases.

The samples used in the collaborative study of this method were commercial shortenings and oils, and margarine oils that were made from soybean, corn or palm oils.

REFERENCES

1. Athnasios, A. K., et al., *J. Assoc. Off. Anal. Chem. 69:*65 (1986). See AOCS Method Ce 1f-96.
2. *J. Am. Oil Chem. Soc. 69:*129 (1992).
3. *Official Methods of Analysis,* AOAC International, 16th ed., Vol. II, Gaithersburg, MD, 1995, Chapt. 41, p. 17, Method 969.33.
4. Heckers, H., et al., *J. Chromatogr. 136:*311 (1977).
5. Duchateau, G.S., et al. *J. Am. Oil Chem. Soc. 73:* 275 (1996).
6. *J. Chromatogr. Sci. 27:*399 (1989).
7. *Standard Methods for the Analysis of Oils, Fats and Derivatives,* International Union of Pure and Applied Chemistry, 7th edn., Blackwell Scientific Publications, 1987, IUPAC Method 2.301.
8. *J. Am. Oil Chem. Soc. 45:*103 (1968).
9. *Ibid. 62:*709 (1979).
10. *J. Assoc. Off. Anal. Chem. 68:*46 (1985).

OTHER REFERENCES

*J. Am. Oil Chem. Soc. 65:*1483 (1988).
*Ibid. 69:*96 (1992).
*Ibid.:*106 (1992).
General Referee Report on Oils and Fats, *J. Assoc. Off. Anal. Chem. 73:*105 (1990).

407

2.207 Determination of content of isolated *trans*-unsaturated compounds by infrared spectrophotometry

1 **Scope**

This Standard describes a procedure for the determination of the content of isolated (i.e. non-conjugated) *trans*-unsaturated compounds by infrared spectrophotometry.

2 **Field of application**

This Standard is applicable to natural, refiined or hydrogenated animal and vegetable oils and fats, fatty acids and fatty acid methyl esters, in which the *trans*-unsaturated compounds are present in more than 1 per cent.

The method yields spectra which can less easily be interpreted, and hence afford less accurate results, in the presence of:

– compounds with functional group absorbing in the neighbourhood of the C—H deformation vibration at 970 cm^{-1} (10.3 nm) or affecting it, e.g. ricinoleic acid in castor oil;

– compounds with conjugated unsaturated double bonds, such as tung oil.

3 **Principle**

Conversion of the oils, fats or fatty acids into fatty acid methyl esters, and measurement of the absorption of the fatty acid methyl esters at around 970 cm^{-1} (10.3 nm), absorption being caused by a deformation of the C—H bonds adjacent to the isolated *trans* double bond.

The content of isolated *trans*-unsaturated components is expressed as percentage of methyl elaidate in the methyl esters.

4 **Definition**

The content of isolated *trans*-unsaturated components in oils, fats, fatty acids or fatty acid methyl esters is given by that percentage of methyl elaidate present in a mixture of methyl elaidate and methyl stearate for which the specific absorption at 970 cm^{-1} (10.3 nm), as determined by the method prescribed below, is identical with that of the fatty acid methyl esters, either as presented, or as prepared from the oils, fats or fatty acids.

5 **Apparatus**

5.1 10-ml measuring flasks, with ground-glass stopper.

5.2 Recording, double-beam infrared spectrophotometer, suitable for quantitative measurements within a range 1100 and 910 cm^{-1} (9 and 11 nm), with an accuracy of 1 cm^{-1} (0.01 nm).

5.3 1-mm infrared cells, with windows (e.g. sodium chloride or potassium bromide) which do not absorb in the range of measurement.

The matching pairs of cells required for the calibration solutions or the test solution and for the reference solution should have the same path-length to within 1 per cent.

6 **Reagents**

6.1 Carbon disulphide, anhydrous, analytical reagent quality.

Note: carbon disulphide vapours are poisonous and flammable. Work should be carried out in a well-ventilated room, preferably in a fume cupboard.

6.2 *Standard substance:* methyl elaidate (methyl ester of 9, 10-*trans*-octadecenoic acid), at least 99 per cent pure. Failing this, a mixture with a content of at least 65 per cent isolated *trans*-unsaturated compounds, determined by means of the standard substance can be used.

6.3 *Reference substance:* methyl stearate (methyl ester of octadecanoic acid), free from *trans*-unsaturated compounds.

408

Annexe

Analytical quality control

1 Repeatability

When the mean value of two single test results obtained under *repeatability conditions* (conditions where independent test results are obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within short intervals of time) lies within the range of the values shown in the table below, the absolute difference between the two test results obtained should not be greater than the *repeatability limit* (r) deduced by linear interpolation from the data in the table.

2 Reproducibility

When the values of two single test results obtained under *reproducibility conditions* (conditions where test results are obtained with the same method on identical test material in different laboratories with different operators using different equipment) lie within the range of the values shown in the table below, the absolute difference between the two test results obtained should not be greater than the *reproducibility limit* (R) deduced by linear interpolation from the data in the table.

3 Results of the interlaboratory test

An interlaboratory test carried out at the international level in 1988 by the IUPAC Commission on Oils, Fats and Derivatives, in which 16 laboratories participated, each obtaining two test results for each sample, gave the statistical results (evaluated in accordance with *ISO 5725-1986*) summarized in Table 2.304.1.

Table 2.304.1.

	Fatty acid					
	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Number of laboratories	13	16	16	16	16	16
Number of results	13	16	16	16	16	16
Number of laboratories retained after elimination of outliers	12	14	14	14	15	14
Number of results retained after elimination of outliers	12	14	14	14	15	14
Mean value (%) (surface/surface)	0.10	10.30	3.51	22.54	53.55	6.70
Repeatability standard deviation (S_r)	0.05	0.21	0.09	0.35	0.29	0.09
Repeatability relative standard deviation	50	2.1	2.6	1.6	0.5	1.3
Repeatability limit (r) ($2.83 \times S_r$)	0.15	0.61	0.26	1.00	0.81	0.25
Reproducibility standard deviation (S_R)	0.06	0.96	0.25	0.85	1.15	0.44
Reproducibility relative standard deviation	55	9.2	7.0	3.8	2.1	6.6
Reproducibility limit (R) ($2.83 \times S_R$)	0.16	2.71	0.70	2.41	3.25	1.25

409

7 Procedure

7.1 Calibration curve

7.1.1 Preparation of the calibration solutions and the reference solution

Into a series of 10-ml measuring flasks (5.1) weigh, at the nearest 0.1 mg, respectively 0 (reference solution), 25, 50, 100 and 150 mg of the standard substance (6.2), and make up each weighting to 200 mg with methyl stearate (6.3). Dissolve each of these mixtures in carbon disulphide (6.1), and make up to the mark at 20°C.

7.1.2 Measurement of the calibration solutions

N.B. The determinations should be made with the solutions maintained at 20°C.

Rinse a clean cell (5.3) with the calibration solution containing 25 mg of methyl elaidate, and fill it with this solution. If necessary, use a syringe for filling to avoid any formation of air bubbles.

In the same way, fill the second cell (5.3) with the reference solution (containing only methyl stearate).

Place the cell containing the reference solution in one beam of IR spectrophotometer (5.2), and the cell containing the calibration solution in the other beam.

Plot the transmission (or absorption) curve between 1050 and 900 cm^{-1} (9.5–11 nm) at optimal instrumental settings.

Similarly, plot the transmission (or absorption) curves for the other calibration solutions in the order of increasing concentrations.

7.1.3 Construction of the calibration curve

Each of the four transmission (or absorption) curves of the four calibration solutions shows a minimum (or a maximum) at around 970 cm^{-1} (10.3 nm). Draw a base-line for each curve; this is the straight line between the absorption minima at *ca.* 1000 and 925 cm^{-1} (10.0 and 10.8 nm).

For a transmission curve measure the distances QR and PR (see figure) at the transmission minimum, and calculate the absorption A_i of the solution by the formula:

$$A_i = \log \frac{PR}{QR}.$$

For an absorption curve read the absorptions A_Q at Q, and A_P at P, and calculate the absorption A_i of the solution by the formula:

$$A_i = A_Q - A_P.$$

Plot the graph for the four calibration solutions, taking the amounts of the standard substance in the solutions as abscissae and the corresponding values for the absorption A_i as ordinates. Draw the best straight line through the four points plotted.

For each series of determinations, carry out a control, using one of the calibration solutions, and verify that the measured value for the absorbance lies on the calibration curve. If it does not, construct a fresh calibration curve. Do this, also, if the instrumental settings have been changed, or when parts of the apparatus, or the cells, have been replaced.

410

7.2 **Preparation of the solution to be assayed**

Prepare 250–500 mg of methyl esters from the sample of oil, fat or fatty acids according to 2.301, 'Preparation of fatty acid methyl esters'.

If necessary, melt the methyl esters by warming. Weigh, to the nearest 0.1 mg, 200 mg of the well-mixed preparation into a measuring flask (5.1). Dissolve this test portion in carbon disulphide (6.1) and make up to the mark at 20°C.

If the transmission at 970 cm^{-1} (10.3 nm) of this solution is found to be less than 10 per cent, or if enough sample is not available, weigh a smaller test portion and add sufficient methyl stearate (6.3) to make up the total amount to 200 mg.

7.3 **Measurement**

Rinse a clean cell (5.3) with the test solution maintained at 20°C, and fill it with this solution. If necessary, use a syringe for filling to avoid any formation of air bubbles.

In the same way, fill the second cell (5.3) with the reference solution, containing only methyl stearate.

Place the cell containing the test portion in one beam of the spectrophotometer (5.2) and the cell containing the reference solution in the other beam.

Plot the transmission (or absorption) curve in the range 1050–900 cm^{-1} (10.0 and 10.8 nm) at optimal instrumental settings.

If isolated *trans*-unsaturated components are present, there will be a transmission minimum (or absorption maximum) at about 970 cm^{-1}. Draw a base-line on the curve; this is the straight line between the absorption minima at around 1000 and 925 cm^{-1} (10.0 and 10.8 nm) see Fig. 2.207.1).

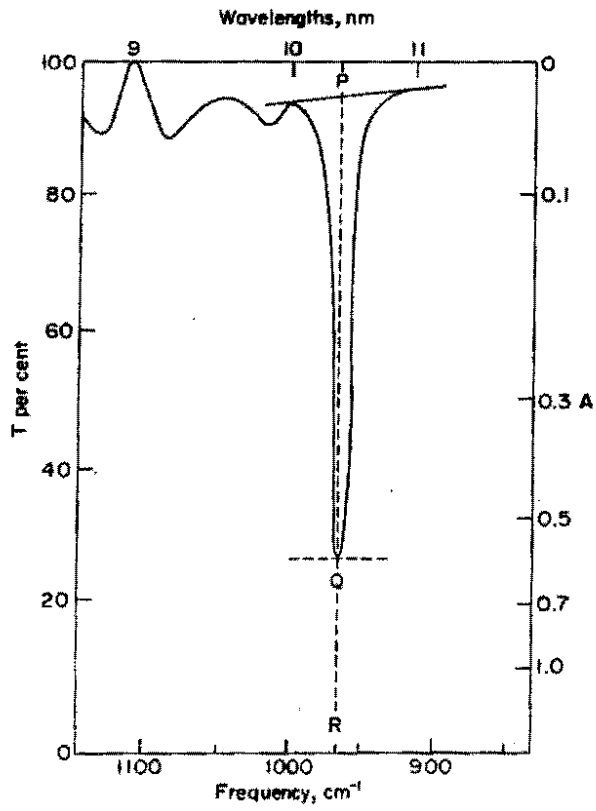


Fig. 2.207.1.

411

INT'L UNION OF PURE & APPLIED CHEMISTRY METHODS (IUPAC)

412

2.103 Spectrophotometric determination of the colour

Foreword

The subjective methods used for the estimation of the colour of oils and fats are frequently empirical and not easy to carry out; these have not been retained. A more accurate and easier method is required, viz. the determination of the transmission curve between 400 and 700 nm, or measurement of the transmission at definite wavelengths.

Crude oils and fats have transmission curves which are characteristic for each family of fats. All bleached oils and fats, on the contrary, show transmission curves which are similar and without characteristic peaks.

1 **Scope**

This Standard describes a spectrophotometric method for the determination of the colour of oils and fats by measuring their transmission in the visible spectrum (between 400 and 700 nm).

2 **Field of application**

This Standard is applicable to animal and vegetable oils and fats.

3 **Principle**

Using a spectrophotometer record the transmittance measurements between 400 and 700 nm at regular intervals of 10 nm in cells of a length such that the transmission factor relative to the reference solvent lies between 20 and 80 per cent; do not, however, use cells of less than 0.5 cm or more than 5 cm length, except for special investigations (*note 1*). Determine the position and the extent of the principal inflexions in the curve (the maxima and minima) by measurements made at 1-nm intervals. If necessary, convert the observations to a standard length of 1 cm by calculation.

4 **Apparatus**

4.1 100-ml volumetric flasks.

4.2 Spectrophotometer, provided with cells having parallel plane surfaces 0.5, 1, 2 and 5 (or 4) cm apart.

5 **Reagents**

5.1 Nickel sulphate, analytical reagent quality ($NiSO_4, 7H_2O$) (*note 2*).

5.2 Cobalt sulphate, analytical reagent quality ($CoSO_4, 7H_2O$) (*note 2*).

5.3 Carbon tetrachloride, anhydrous, for spectrophotometry.

5.4 Hydrochloric acid, aqueous solution 1 N.

5.5 Reference solution: put 11.5 ml of the aqueous solution of hydrochloric acid (5.4) in the volumetric flask (4.1); dissolve 4.7 g of nickel sulphate (5.1) (Ni = 982 mg) and 3.1 g of cobalt sulphate (5.2) (Co = 650 mg) and make up to 100 ml with distilled water.

6 **Preparation of the sample**

The samples to be examined must be clear. Filter through paper if necessary in the presence of a small quantity of a filter aid which does not affect the colour.

Solid fats should be previously melted and maintained at 10°C above their melting point long enough to ensure their clarity. They must remain clear for the duration of the experimental work. Finally, use a thermostat regulated to the minimum temperature compatible with the melting point of the fat.

413

Clarify the sample, if necessary, by drying *in vacuo* away from light.

7 Procedure

7.1 Checking the spectrophotometer

Regulate the wavelength selection device of the spectrophotometer using the following two mercury lines for calibration:

$\lambda_1 = 546.1 \pm 0.2$ nm,

$\lambda_2 = 435.8 \pm 0.4$ nm.

1 Check the transmission scale by measuring the transmittance in 1-cm cells using a minimum slitwidth for the observations of the reference solution (5.5) relative to the solvent (5.3). If the apparatus is properly adjusted the following values should be obtained:

$\lambda = 439$ nm $\quad T = 51.4 \pm 0.3$,

$\lambda = 512$ nm $\quad T = 26.9 \pm 0.5$,

$\lambda = 581$ nm $\quad T = 73.3 \pm 0.3$.

2 Two cells of the same length used alternatively for the reference solvent and the reference solution should give, at 550 nm for example, transmittances not differing by more than 0.5 per cent.

7.2 Measurement

Determine the percentage transmission of the sample in a 1-cm cell every 10 nm from 400 to 700 nm compared with that of carbon tetrachloride (5.3) as a blank and using a minimum slitwidth for each observation. At the points of maximum and minimum transmission take readings at 1 nm distance on each side as far as is necessary to secure precision. Make three observations at each wavelength and record the mean. There are two possibilities:

1 all the readings fall between 20 and 80 per cent, report the readings;

2 some of the readings fall below 20 or above 80 per cent. For readings falling below 20 per cent, repeat the observations at these wavelengths using a 0.5-cm cell. For readings falling above 80 per cent, but below 90 per cent, repeat the observations using a 2-cm cell. For readings falling above 90 per cent, repeat the observations using a 5- (or 4-) cm cell.

8 Expression of results

By the application of Lambert's law, calculate the value of the transmittance for 1 cm length using the formula:

$$\log \frac{100}{T_1} = \frac{1}{n} \log \frac{100}{T_n}$$

where T_1 is the transmission calculated for a cell of 1 cm, T_n is the transmission measured for a cell of n cm.

The corresponding values of T and $\log 100/T$ are given in the tables. Express the results thus obtained either by means of a series of transmittance values at defined wavelengths or by means of a graph plotting the transmission against the wavelength (scale: 1 cm on the abscissa = 20 nm, 1 cm on the ordinate = 5 per cent transmission).

9 **Notes**

1 In the case of special investigations, the transmission measurements below 20 per cent can be made in a 1-cm cell on a 1 in 2 volume dilution if the observed transmission is greater than 5 per cent, or in a 1 in 5 volume dilution if it is less.

The dilutions are made by weighing 9.120 g of fat into a standard 20- or 50-ml flask, depending on whether the dilution is to be 1 in 2 or 1 in 5, and then making up to volume with carbon tetrachloride (5.3) at 20°C.

The transmission of a pure fat examined in a 1-cm cell is given by the formula:

$$\log \frac{100}{T_1} = \frac{1}{d} \log \frac{100}{T_d}$$

where d is 1/2 or 1/5, T_d is the transmission measured at the dilution d.

2 The exact titration of nickel and cobalt in commercial salts may be necessary.

Warning

Carbon tetrachloride

Use of a fume hood is recommended. Carbon tetrachloride is hazardous and a cancer suspect agent.

Table 2.103.1. Table of corresponding values

$T \rightarrow \log \frac{100}{T}$

T	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
		3.000	2.699	2.523	2.398	2.301	2.222	2.155	2.097	2.046
1	2.000	1.958	1.921	1.886	1.854	1.824	1.796	1.769	1.745	1.721
2	1.699	1.678	1.657	1.638	1.620	1.602	1.585	1.569	1.553	1.538
3	1.523	1.509	1.495	1.481	1.468	1.456	1.444	1.432	1.420	1.409
4	1.398	1.387	1.376	1.365	1.357	1.347	1.337	1.328	1.319	1.309
5	1.301	1.292	1.284	1.276	1.268	1.260	1.252	1.244	1.237	1.229
6	1.222	1.215	1.208	1.201	1.194	1.187	1.180	1.174	1.167	1.161
7	1.155	1.149	1.143	1.137	1.131	1.125	1.119	1.113	1.108	1.102
8	1.097	1.092	1.086	1.081	1.076	1.071	1.065	1.060	1.055	1.051
9	1.046	1.041	1.036	1.032	1.027	1.022	1.018	1.013	1.009	1.004
10	1.000	0.996	0.991	0.987	0.983	0.979	0.975	0.971	0.967	0.963
11	0.959	0.955	0.951	0.947	0.943	0.939	0.936	0.932	0.928	0.924
12	0.921	0.917	0.914	0.910	0.907	0.903	0.900	0.896	0.893	0.889
13	0.886	0.883	0.879	0.876	0.873	0.870	0.866	0.863	0.860	0.857
14	0.854	0.851	0.848	0.845	0.842	0.839	0.836	0.833	0.830	0.827
15	0.824	0.821	0.818	0.815	0.812	0.810	0.807	0.804	0.801	0.799
16	0.796	0.793	0.790	0.788	0.785	0.782	0.780	0.777	0.775	0.772
17	0.769	0.767	0.764	0.762	0.759	0.757	0.754	0.752	0.749	0.747
18	0.745	0.742	0.740	0.738	0.735	0.733	0.730	0.728	0.726	0.724
19	0.721	0.719	0.717	0.714	0.712	0.710	0.708	0.706	0.703	0.701
20	0.699	0.697	0.695	0.692	0.690	0.688	0.686	0.684	0.682	0.680
21	0.678	0.676	0.674	0.672	0.670	0.668	0.666	0.664	0.662	0.660
22	0.658	0.656	0.654	0.652	0.650	0.648	0.646	0.644	0.642	0.640
23	0.638	0.636	0.634	0.633	0.631	0.629	0.627	0.625	0.623	0.622
24	0.620	0.618	0.616	0.614	0.613	0.611	0.609	0.607	0.606	0.604
25	0.602	0.600	0.599	0.597	0.595	0.593	0.592	0.590	0.588	0.587
26	0.585	0.583	0.582	0.580	0.578	0.577	0.575	0.573	0.572	0.570
27	0.569	0.567	0.565	0.564	0.562	0.561	0.560	0.558	0.556	0.554
28	0.553	0.551	0.550	0.548	0.547	0.545	0.544	0.542	0.541	0.539
29	0.538	0.536	0.535	0.533	0.532	0.530	0.529	0.527	0.526	0.524

415

T	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
30	0.523	0.521	0.520	0.519	0.517	0.516	0.514	0.513	0.511	0.510
31	0.509	0.507	0.506	0.504	0.503	0.502	0.500	0.499	0.497	0.496
32	0.495	0.493	0.492	0.491	0.489	0.488	0.487	0.485	0.484	0.483
33	0.481	0.480	0.479	0.478	0.476	0.475	0.474	0.472	0.471	0.470
34	0.469	0.467	0.466	0.465	0.463	0.462	0.461	0.460	0.458	0.457
35	0.456	0.455	0.454	0.452	0.451	0.450	0.448	0.447	0.446	0.445
36	0.444	0.442	0.441	0.440	0.439	0.438	0.436	0.435	0.434	0.433
37	0.432	0.431	0.430	0.428	0.427	0.426	0.425	0.424	0.422	0.421
38	0.420	0.419	0.418	0.417	0.416	0.414	0.413	0.412	0.411	0.410
39	0.409	0.408	0.407	0.406	0.404	0.403	0.402	0.401	0.400	0.399
40	0.398	0.397	0.396	0.395	0.394	0.393	0.391	0.390	0.389	0.388
41	0.387	0.386	0.385	0.384	0.383	0.382	0.381	0.380	0.379	0.378
42	0.377	0.376	0.375	0.374	0.373	0.372	0.371	0.370	0.369	0.368
43	0.367	0.366	0.365	0.364	0.363	0.362	0.361	0.360	0.359	0.358
44	0.357	0.356	0.355	0.354	0.353	0.352	0.351	0.350	0.349	0.348
45	0.347	0.346	0.345	0.344	0.343	0.342	0.341	0.340	0.339	0.338
46	0.337	0.336	0.335	0.334	0.333	0.332	0.331	0.330	0.330	0.329
47	0.328	0.327	0.326	0.325	0.324	0.323	0.322	0.321	0.320	0.320
48	0.319	0.318	0.317	0.316	0.315	0.314	0.313	0.312	0.311	0.310
49	0.310	0.309	0.308	0.307	0.306	0.305	0.304	0.303	0.303	0.302
50	0.301	0.300	0.299	0.298	0.297	0.297	0.296	0.295	0.294	0.293
51	0.292	0.291	0.290	0.290	0.289	0.288	0.287	0.286	0.285	0.285
52	0.284	0.283	0.282	0.281	0.280	0.280	0.279	0.278	0.277	0.277
53	0.276	0.275	0.274	0.273	0.272	0.271	0.271	0.270	0.269	0.268
54	0.267	0.267	0.266	0.265	0.264	0.263	0.262	0.262	0.261	0.260
55	0.260	0.259	0.258	0.257	0.256	0.255	0.255	0.254	0.253	0.252
56	0.252	0.251	0.250	0.249	0.249	0.248	0.247	0.246	0.246	0.245
57	0.244	0.243	0.242	0.242	0.241	0.240	0.239	0.239	0.238	0.237
58	0.236	0.236	0.235	0.234	0.233	0.233	0.232	0.231	0.230	0.230
59	0.229	0.228	0.228	0.227	0.226	0.225	0.224	0.224	0.223	0.222
60	0.222	0.221	0.220	0.219	0.219	0.218	0.217	0.216	0.216	0.215
61	0.214	0.214	0.213	0.212	0.211	0.211	0.210	0.209	0.209	0.208
62	0.207	0.207	0.206	0.205	0.205	0.204	0.203	0.202	0.202	0.201
63	0.201	0.200	0.199	0.198	0.198	0.197	0.196	0.195	0.195	0.194
64	0.194	0.193	0.192	0.192	0.191	0.190	0.189	0.189	0.188	0.187
65	0.187	0.186	0.185	0.185	0.184	0.183	0.183	0.182	0.181	0.181
66	0.180	0.179	0.179	0.178	0.177	0.177	0.176	0.176	0.175	0.174
67	0.174	0.173	0.172	0.172	0.171	0.170	0.170	0.169	0.168	0.168
68	0.167	0.167	0.166	0.165	0.164	0.164	0.163	0.163	0.162	0.162
69	0.161	0.160	0.160	0.159	0.158	0.158	0.157	0.156	0.156	0.155
70	0.155	0.154	0.154	0.153	0.152	0.152	0.151	0.150	0.150	0.149
71	0.149	0.148	0.147	0.147	0.146	0.145	0.145	0.144	0.144	0.143
72	0.143	0.142	0.141	0.141	0.140	0.140	0.139	0.138	0.138	0.137
73	0.136	0.136	0.135	0.135	0.134	0.134	0.133	0.132	0.132	0.131
74	0.131	0.130	0.129	0.129	0.128	0.128	0.127	0.126	0.126	0.125
75	0.125	0.124	0.124	0.123	0.123	0.122	0.121	0.121	0.120	0.120
76	0.119	0.119	0.118	0.117	0.117	0.116	0.116	0.115	0.114	0.114
77	0.113	0.113	0.112	0.112	0.111	0.111	0.110	0.109	0.109	0.108
78	0.108	0.107	0.107	0.106	0.106	0.105	0.104	0.104	0.103	0.103
79	0.102	0.102	0.101	0.101	0.100	0.100	0.099	0.099	0.098	0.097
80	0.097	0.096	0.096	0.095	0.095	0.094	0.094	0.093	0.092	0.092
81	0.091	0.091	0.090	0.090	0.089	0.089	0.088	0.088	0.087	0.087
82	0.086	0.086	0.085	0.084	0.084	0.083	0.083	0.082	0.082	0.081
83	0.081	0.080	0.080	0.079	0.079	0.078	0.078	0.077	0.077	0.076
84	0.076	0.075	0.075	0.074	0.074	0.073	0.073	0.072	0.072	0.071
85	0.071	0.070	0.070	0.069	0.069	0.068	0.068	0.067	0.066	0.066
86	0.065	0.065	0.064	0.064	0.063	0.063	0.062	0.062	0.061	0.061
87	0.060	0.060	0.059	0.059	0.058	0.058	0.057	0.057	0.056	0.056
88	0.055	0.055	0.054	0.054	0.053	0.053	0.052	0.052	0.052	0.051
89	0.051	0.050	0.050	0.049	0.049	0.048	0.048	0.047	0.047	0.046
90	0.046	0.045	0.045	0.044	0.044	0.043	0.043	0.042	0.042	0.041
91	0.041	0.040	0.040	0.039	0.039	0.038	0.038	0.038	0.037	0.037

416

2: OILS AND FATS

T	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
92	0.036	0.036	0.035	0.035	0.034	0.034	0.033	0.033	0.032	0.032
93	0.031	0.031	0.030	0.030	0.030	0.029	0.029	0.028	0.028	0.027
94	0.027	0.026	0.026	0.025	0.025	0.025	0.024	0.024	0.023	0.023
95	0.022	0.022	0.021	0.021	0.020	0.020	0.020	0.019	0.019	0.018
96	0.018	0.017	0.017	0.016	0.016	0.015	0.015	0.015	0.014	0.014
97	0.013	0.013	0.012	0.012	0.011	0.011	0.010	0.010	0.010	0.009
98	0.009	0.008	0.008	0.007	0.007	0.007	0.006	0.006	0.005	0.005
99	0.004	0.004	0.003	0.003	0.002	0.002	0.002	0.001	0.001	0.001

417

2.121 Determination of solidification point of water-insoluble fatty acids (titre)

1 **Scope**

This Standard describes a method for the preparation of the water-insoluble fatty acids of oils and fats, and for the determination of their solidification point (titre).

2 **Field of application**

This Standard is applicable to animal and vegetable oils and fats whose water-insoluble fatty acids have a solidification point of 30°C or above.

3 **Definition**

The *solidification point of the fatty acids* is the temperature determined by the method described as the maximum of a temporary temperature rise during the cooling of the melted fatty acids. If the latent heat is not sufficient to cause a rise in temperature, the temporary interruption of the cooling process is considered as the solidification point.

4 **Principle**

Saponification of the oil or fat by an ethanolic potassium hydroxide solution. After removal of ethanol, dissolution of the soap in water and decomposition of this by sulphuric acid. Washing separated fatty acids with boiling sodium chloride solution, drying and filtration. Determination of the highest temperature during the temporary rise, or the interruption of the cooling process, when the melted fatty acids are cooled.

5 **Apparatus**

5.1 500- and 1000-ml round-bottomed flasks.

5.2 Reflux condenser to fit the flasks (5.1).

5.3 100- and 1000-ml beakers.

5.4 1000-ml separating funnel.

5.5 Glass test tube, 120±5 mm in length, 27.5±0.5 mm internal diameter, 2.5±0.5 mm wall thickness. This test tube carries a mark at 55 mm from the bottom.

5.6 600-ml beaker, squat form (125 mm in height, 91 mm external diameter). This beaker should be weighted (e.g. with lead shot) to keep it steady on the bottom of the water bath (5.7).

5.7 Water bath, maintained 20–25°C below the expected solidification point.

5.8 Thermometer, accurately calibrated, graduated in 0.2°C, with a scale covering all or part of the range from 30 to 70°C, according to the sample to be tested.

5.9 Flat circular cork, having a central hole just big enough to support the test tube (5.5) and able to fit on the beaker (5.6).

5.10 Support to which the thermometer (5.8) can be suspended so that it hangs in the centre of the test tube (5.5).

5.11 Boiling chips.

6 **Reagents**

6.1 Sodium sulphate, anhydrous.

6.2 Potassium hydroxide, ethanolic solution. Dissolve 18 g potassium hydroxide in about 20 ml distilled water, and dilute with 150 ml of 95 per cent (V/V) ethanol.

6.3 Sulphuric acid, aqueous solution. Add 1 volume of sulphuric acid (ρ = 1.84 g/ml) to 4 volumes of distilled water.

6.4 Sodium chloride, 100 g/l aqueous solution.

418

7 **Preparation of the sample**

Use about 100 g of the fat, and put it in an oven maintained at about 20°C above its melting point, until totally melted.

8 **Procedure**

8.1 Preparation of the water-insoluble fatty acids

Weigh about 50 g of the prepared sample into a 500-ml flask (5.1). Add all the ethanolic potassium hydroxide solution (6.2) and some boiling chips (5.11). Fit the reflux condenser (5.2). Bring gently to the boil with occasional shaking. Maintain a gentle ebullition during 1 hour. Distil off most of the ethanol (about 125 ml); take care that the soap does not become dry. Add about 250 ml of boiling water and mix to dissolve the soap. Transfer the soap solution into a 1000-ml beaker (5.3), add 500 ml of boiling water, and boil for 45 minutes in order to remove the remainder of the ethanol. Make up the contents to about 700 ml with water.

Pour the soap solution into a 1000-ml flask (5.1) and add carefully 70 ml of the aqueous sulphuric acid solution (6.3). Fit the reflux condenser (5.2) and heat to boiling. Continue boiling until the free fatty acids float to the surface in a clear layer.

Transfer the contents to a separating funnel (5.4) and draw off the aqueous layer as completely as possible.

Wash the fatty acids twice, each time using about 500 ml of aqueous sodium chloride solution (6.4)*). After each washing draw off the aqueous layer as completely as possible.

Transfer the fatty acids to a 100-ml beaker (5.3). Add a small quantity of anhydrous sodium sulphate (6.1), stir, and filter through a dry filter paper.

Let the insoluble fatty acids crystallize in a desiccator for about 24 hours.

8.2 Determination of the solidification point of the water-insoluble fatty acids

Pour the fatty acids obtained according to 8.1 and melted at a temperature of 25°C above the expected solidification point into a test tube (5.5) up to the 55-mm mark.

Support the tube (5.5) in the cork (5.9) so that a length of about 3 cm projects above it, rest the cork laying on the rim of a beaker (5.6) and place this in a water bath (5.7), so that the water level is 10 mm above the 55-mm mark of the tube. Suspend the thermometer (5.8) in the centre of the tube so that the base of the bulb is 1 cm from the bottom of the tube.

The mercury column falls rapidly at first, and then more slowly. Simultaneously, the fatty acids crystallize, first at the bottom of the tube, and then gradually covering the base of the thermometer bulb.

When the mercury column appears to be stationary during four observations made at 5-second intervals, stir the fatty acids with a rapid, circular movement of the thermometer three times to the right and three times to the left, thus breaking up the crystals formed.

Replace the thermometer immediately in the centre of the tube and take further readings.

The mercury column, which falls sharply during the agitation, now rises again and attains a maximum or remains steady, before falling again. This maximum or standstill is the solidification point.

Carry out two determinations on the same prepared sample.

*) 'which is hot enough to maintain the fatty acids in a liquid state'.

419

9 **Expression of results**

Take as the result the arithmetic mean of the two determinations, if the requirements of repeatability are satisfied. If the difference is greater, repeat the determination until two results differing by not more than 0.2°C are obtained.

10 **Repeatability**

The difference between the results of duplicate determinations carried out simultaneously or in rapid succession by the same analyst should not exceed 0.2°C.

420

2.201 Determination of the acid value (A.V.) and the acidity

1 **Scope**

This Standard describes two methods for the determination of the free fatty acid content of animal vegetable oils and fats. This can be expressed as an acid value, or as acidity.

2 **Field of application**

This Standard is applicable to animal and vegetable oils and fats. It is not applicable to waxes.

3 **Definitions**

The acid value (A.V.) is the number of mg of potassium hydroxide required to neutralize the free fatty acids in 1 g of the fat.

The acidity is a conventional expression of the percentage of free fatty acids. According to the nature of the fat it can be expressed as in Table 2.201.1.

Table 2.201.1

Nature of fat	Expressed as	Molecular weight
Coconut, palm kernel and similar oils	Lauric acid	200
Palm oil	Palmitic acid	256
All other oils	Oleic acid	282

When the result is reported as 'acidity', but not further defined, then conventionally the acidity is always expressed as oleic acid.

If the sample contains mineral acids, by convention they are calculated as fatty acids.

4 **Acid value–indicator method**

4.1 **Principle**

Solution of a known quantity of the fat to be analysed in a mixture of ethanol and diethyl ether, followed by titration of the free fatty acids present with an ethanolic solution of potassium hydroxide.

4.2 **Apparatus**

4.2.1 250-ml conical flask.

4.2.2 25-ml burette, graduated in 0.1 ml.

4.3 **Reagents**

4.3.1 Solvent mixture 1/1 (V/V) of 95 per cent (V/V) ethanol and diethyl ether. Neutralize exactly, just before use, by means of the potassium hydroxide solution (4.3.2) in the presence of 0.3 ml of phenolphthalein solution (4.3.3) per 100 ml of the mixture (*note 1*).

4.3.2 Potassium hydroxide, 0.1 N, or if necessary 0.5 N, solution in ethanol, accurately standardized.

The exact concentration should be known, and checked immediately before use. Use a solution prepared at least 5 days previously and decanted into a bottle of brown glass, provided with a rubber stopper. The solution should be colourless or straw yellow (*note 2*).

4.3.3 Phenolphthalein, 10 g/l solution in 95 per cent (V/V) ethanol.

421

4.4 **Procedure**

Prepare the sample according to 2.001, 'Preparation of the sample'. Determine size of sample according to the following table:

Expected acid value	Mass of test portion, in g	Weighing accuracy, in g
< 1	20	0.05
1–4	10	0.02
4–15	2.5	0.01
15–75	0.5	0.001
> 75	0.1	0.0002

Weigh the test portion into a conical flask (4.2.1). Dissolve it in about 150 ml of the solvent mixture (4.3.1). Titrate, with shaking, with the solution of potassium hydroxide (4.3.2) to the end point of the indicator (pink colour of phenolphthalein persisting for at least 10 seconds) (*note 3*, *note 4*).

If the quantity of 0.1 N potassium hydroxide solution required exceeds 20 ml, a 0.5 N solution should be used (*note 5*).

4.5 **Expression of results**

The acid value (A.V.) is given by the formula:

$$\text{A.V.} = \frac{56.1 \times T \times V}{m}$$

where V is the number of ml of the standardized potassium hydroxide solution (4.3.2) used (*note 6*), T is the exact normality of the standardized potassium hydroxide solution (4.3.2), m is the mass, in g, of the test portion.

5 Acid value–potentiometric method

5.1 **Foreword**

This method should be employed instead of Method 4 for the determination of free acidity in highly coloured oils and fats.

5.2 **Principle**

The free fatty acids are titrated potentiometrically in a non-aqueous medium with a solution of potassium hydroxide in *iso*propanol.

5.3 **Apparatus**

5.3.1 150-ml tall form beakers.

5.3.2 1000-ml volumetric flasks.

5.3.3 25-ml burette, graduated in 0.1 ml.

5.3.4 pH meter equipped with glass and calomel electrodes (*note 7*).

Contact between the saturated potassium chloride solution and the test solution is made across a sintered glass or porcelain disc, at least 0.3 cm thick.

5.3.5 Stirrer, preferably magnetic stirrer.

422

5.4 **Reagents**

5.4.1 2-Propanol (*iso*propanol), analytical reagent quality.

5.4.2 Methylisobutylketone, analytical reagent quality, neutralized immediately before use by the *iso*propanolic potassium hydroxide solution (5.4.4) to a faint pink colour with phenolphthalein indicator.

5.4.3 Benzoic acid, acidimetric standard quality.

5.4.4 Potassium hydroxide, 0.1 N solution in 2-propanol (5.4.1), accurately standardized. Dissolve 7 g of potassium hydroxide pellets in 1000 ml of 2-propanol (5.4.1).

5.4.5 Potassium hydroxide, 0.5 N solution in 2-propanol, accurately standardized. Dissolve 35 g of potassium hydroxide pellets in 1000 ml of 2-propanol (5.4.1).

Standardize the solutions as follows: weigh accurately to within 0.0002 g about 0.15 g (for the 0.1 N solution) or 0.75 g (for the 0.5 N solution) of the standard benzoic acid (5.4.3). Transfer into a beaker (5.3.1) and dissolve in 50 ml of methylisobutylketone (5.4.2).

Insert the electrodes of the pH meter (5.3.4), start the stirrer (5.3.5) and titrate with the potassium hydroxide solution (5.4.4 or 5.4.5) to the equivalence point (*note 8*).

$$\text{Normality} = \frac{1000 \times m_0}{122.1 \times a_0}$$

where a_0 is the number of ml of the potassium hydroxide solution (5.4.4 or 5.4.5) used, m_0 is the mass, in g, of benzoic acid taken.

5.5 **Procedure**

Prepare the sample according to 2.001, 'Preparation of the sample'.

Weigh to within 0.01 g 5 to 10 g of prepared fat into a beaker (5.3.1). Dissolve in 50 ml of methylisobutylketone (5.4.2).

Insert the electrodes of the pH meter (5.3.4) and start the stirrer (5.3.5). Titrate with the potassium hydroxide solution (5.4.4 or 5.4.5) 0.1 or 0.5 N (according to the acidity of the sample) to the equivalence point (*note 8*).

5.6 **Expression of results**

The acid value (A.V.) is given by the formula:

$$\text{A.V.} = \frac{56.1 \times T \times N}{m}$$

where V is the number of ml of the standardized *iso*propanolic potassium hydroxide solution (5.4.4 or 5.4.5) used, T is the exact normality of the standardized *iso*propanolic potassium hydroxide solution (5.4.4 or 5.4.5) used, m is the mass, in g, of the test portion.

6 Acidity

6.1 **Principle**

The acidity is calculated from the results obtained in the determination of the acid value by the indicator method or the potentiometric method.

423

6.2 Expression of results

The acidity, in per cent, is given by the formula:

$$\frac{T \times V \times M}{10\,m}$$

where V is the number of millilitres of the standardized ethanolic (4.3.2) or *iso*propanolic (5.4.4 or 5.4.5) potassium hydroxide solution used, T is the exact normality of the standardized ethanolic (4.3.2) or *iso*propanolic (5.4.4 or 5.4.5) potassium hydroxide solution used, M is the molecular weight adopted (200, 256 or 282 according to the Table in 3), m is the mass, in g, of the test portion.

7 Notes

1 In the solvent mixture diethylether may be substituted by toluene.

2 A stable colourless solution of potassium hydroxide can be prepared in the following manner: reflux 1000 ml of ethanol with 8 g of potassium hydroxide and 5 g of aluminium pellets for 1 hour, then distil immediately. Dissolve the required amount of potassium hydroxide in the distillate. Allow the whole to stand for several days and decant the clear supernatant liquid from the deposited potassium carbonate.

The solution can also be prepared without distillation in the following manner: add 4 ml of aluminium butylate to 1000 ml ethanol and allow the mixture to stand for several days.

Decant the supernatant liquid and dissolve therein the necessary amount of potassium hydroxide. This solution is ready for use.

3 In the case of very low acid value (< 1) it is useful to blow a low stream of nitrogen into the solution to be titrated.

4 The ethanolic standardized potassium hydroxide solution (4.3.2) can be substituted by an aqueous solution when the acid value is low.

5 If the solution becomes cloudy during the titration, add a further quantity of the solvent mixture (4.3.1).

6 The normality of the ethanolic standardized potassium hydroxide solution varies with the temperature and it may be useful to correct as follows:

$$V = V_t[1-0.011(t-t_0)]$$

where V_t is the volume at the temperature t, t is the temperature, in °C, at which the determination is made, t_0 is the temperature, in °C, at which the ethanolic potassium hydroxide solution has been standardized.

7 It is advisable to store the glass electrode in water or, better still, in methylisobutylketone for 12 hours preceding the titration. Dry it very gently with a piece of filter paper before use. Immediately after the titration, rinse it with methylisobutylketone, then with 2-propanol, and finally with distilled water.

If the electrode does not function satisfactorily, it may be possible to regenerate it by immersion for 24 hours in a 1 N solution of hydrochloric acid in 2-propanol. After this treatment the electrode should be washed with distilled water, then with 2-propanol and with methylisobutylketone.

A thick sintered glass or porcelain disc between the saturated potassium chloride solution and the test solution prevents diffusion currents and parasitic potentials.

8 *Equivalence point/inflexion point.* The equivalence point usually corresponds approximately to the reading 10 on the pH scale, and can be determined graphically by observing the

424

2.201 Determination of the acid value (A.V.) and the acidity

Statistical evaluation of results of study carried out in 1989.

	Palm oil refined	Palm oil crude	Fish oil crude
Number of laboratories	5	5	5
Number of results	10	7	10
Number of laboratories retained after deletion of outliers	5	5	5
Number of accepted results	10	7	10
Mean value (% oleic acid)	**1.12**	**11.5**	**10.4**
Repeatability standard deviation*	0.008	0.03	0.09
Repeatability relative standard deviation (%)	0.79	0.27	0.84
Reproducibility standard deviation*	0.066	0.17	0.30
Reproducibility relative standard deviation (%)	5.9	1.5	2.9
Repeatability (*r*)*	**0.03**	**0.09**	**0.25**
Reproducibility (*R*)*	**0.19**	**0.49**	**0.85**

* Expressed as the percentage of oleic acid; acid value = 2 × acidity.

425

inflexion point on the neutralization curve. Alternatively, it can be calculated as the figure for which the first differential of the variation of pH as a function of the amount of *isopropanolic* potassium hydroxide solution added reaches a maximum, or the value for which the second differential becomes zero.

N.B. It is not possible to determine the inflexion point in the case of crude cottonseed oils rich in gossypol.

In this case the inflexion point may be taken arbitrarily as the pH corresponding to the equivalence point when oleic acid is neutralized by potassium hydroxide in the same solvent as that used for the titration.

Dissolve about 0.282 g of oleic acid in 50 ml of methylisobutylketone (5.4.2). Construct the curve for neutralization of the oleic acid by the potassium hydroxide solution (5.4.4 or 5.4.5) to be used. Read off on the curve the pH of the inflexion point (representing theoretically the addition of 10 ml of a 0.1 N solution of potassium hydroxide).

Apply this figure to the neutralization curve of the cottonseed oil to deduce the amount of potassium hydroxide solution required to 'neutralize' the cottonseed oil.

426

2.202 Determination of the saponification value (S.V.)

1 **Scope**

This Standard describes two methods for the determination of the saponification value of animal and vegetable oils and fats. The indicator method should be preferentially chosen to the potentiometric method when it is applicable.

2 **Field of application**

This Standard is applicable to animal and vegetable oils and fats. It is not applicable to waxes.

3 **Definition**

The saponification value (S.V.) is the number of mg of potassium hydroxide required to saponify 1 g of fat.

4 **Indicator method**

4.1 Principle

Boiling of the sample under reflux condenser with ethanolic potassium hydroxide solution, and titration of the excess potassium hydroxide with hydrochloric acid in the presence of an indicator.

4.2 Apparatus

4.2.1 250-ml round-bottomed flasks, or conical flasks, made of alkali-resistant glass.

4.2.2 1-m reflux tubes, or 30 cm minimum in length reflux condensers, to fit the round-bottomed flasks or conical flasks (4.2.1).

4.2.3 25-ml volumetric pipette.

4.2.4 50-ml burette, graduated in 0.1 ml.

4.2.5 Boiling chips.

4.2.6 Heating device (water bath, electric hot-plate or other suitable device).

4.3 Reagents

4.3.1 Potassium hydroxide, approximately 0.5 N solution in 95 per cent (V/V) ethanol.
Use a solution prepared at least 5 days previously and decanted into a bottle of brown glass, provided with a rubber stopper. The solution should be colourless or straw yellow (*note 1*).

4.3.2 Hydrochloric acid, 0.5 N aqueous solution, accurately standardized.

4.3.3 Phenolphthalein, 10 g/l solution in 95 per cent (V/V) ethanol.

4.4 Procedure

Prepare the sample according to 2.001, 'Preparation of the sample'.
Into the round-bottomed or conical flask (4.2.1) weigh to the nearest 0.005 g about 2 g of the prepared sample. With the pipette (4.2.3) add 25 ml of the ethanolic potassium hydroxide solution (4.3.1) and some boiling chips (4.2.5). Fit a reflux tube or reflux condenser (4.2.2). Boil gently with occasional shaking.
After 60 minutes, stop heating. Add to the hot solution 0.5 to 1.0 ml of phenolphthalein (4.3.3) and titrate with the hydrochloric acid solution (4.3.2) until the colour of the indicator changes.
Carry out two determinations with the same prepared sample.
Carry out a blank test in the same way.

427

4.5 **Expression of results**

The saponification value (S.V.) is given by the formula:

$$S.V. = \frac{56.1 \times T \times (V_0 \times V_1)}{m}$$

where V_0 is the number of ml of the standardized hydrochloric acid solution (4.3.2) used for the blank test, V_1 is the number of ml of the standardized hydrochloric acid solution (4.3.2) used for the test with the fat, T is the exact normality of the standardized hydrochloric acid solution (4.3.2) used, m is the mass, in g, of the test portion.

Take as the result the arithmetic mean of the two determinations, provided that the requirement of repeatability 4.6 is satisfied.

4.6 **Repeatability**

The difference between the results of two determinations carried out simultaneously or in rapid succession by the same analyst should not exceed 0.5 per cent of the mean value.

5 Potentiometer method

5.1 **Foreword**

This method should be employed instead of method 4 for the determination of the saponification value of highly coloured oils and fats.

5.2 **Principle**

Reflux boiling of the sample with an *iso*propanolic potassium hydroxide solution and potentiometric titration of the excess of potassium hydroxide acid in a non-aqueous medium.

5.3 **Apparatus**

5.3.1 100-ml flasks with ground joints, made of alkali-resistant glass.

5.3.2 1-m reflux tubes, or 30 cm minimum in length reflux condensers, to fit the flasks (5.3.1).

5.3.3 150-ml tall form beakers.

5.3.4 1000-ml volumetric flasks.

5.3.5 25-ml volumetric pipette.

5.3.6 50-ml burette, graduated in 0.1 ml.

5.3.7 pH meter equipped with glass and calomel electrodes (*note 2*). Contact between the saturated potassium chloride solution and the test solution is made across a sintered glass or porcelain disc at least 0.3 cm thick.

5.3.8 Stirrer, preferably a magnetic stirrer.

5.3.9 Boiling chips.

5.4 **Reagents**

5.4.1 2-Propanol (*iso*propanol), analytical reagent quality.

5.4.2 Ethanediol, analytical reagent quality.

5.4.3 Acetic acid, analytical reagent quality.

5.4.4 Sodium carbonate, analytical reagent quality.

5.4.5 Potassium hydroxide, approximately 0.5 N solution in 2-propanol (5.4.1): dissolve 35 g of potassium hydroxide pellets in 1000 ml of 2-propanol (5.4.1).

428

5.4.6 Hydrochloric acid, 0.5 N accurately standardized solution in 2-propanol (5.4.1).
Pour 42 ml of hydrochloric acid (ρ = 1.18) into a volumetric flask (5.3.4) and make up to the mark with 2-propanol (5.4.1).
Standardization of the solution: e.g. weigh accurately to within 0.0002 g about 0.35 g of sodium carbonate (5.4.4), which has been previously calcined to constant weight at 270-300°C. Transfer to a beaker (5.3.3). Add gradually and cautiously a slight excess of acetic acid (5.4.3) to decompose the sodium carbonate completely. When the evolution of carbon dioxide has ceased, add sufficient distillated water to dissolve the sodium acetate formed and evaporate the solution to dryness on a water bath. Dissolve the residue in 20 ml of 2-propanol (5.4.1). Add 20 ml of ethanediol (5.4.2). Titrate by whichever of the two methods described below is preferred:
1 Insert the electrodes of the pH meter (5.3.7) and start the stirrer (5.3.8).
Tritate with the *iso*propanolic solution of hydrochloric acid (5.4.6) to the equivalent point (*note 3*).
2 Add 2 drops of a 10 g/l solution of thymol blue in 2-propanol (5.4.1) and titrate with the *iso*propanolic solution of hydrochloric acid (5.4.6) until the colour changes from yellow to pink.

$$\text{Normality} = \frac{1000 \times m_0}{53 \times a_0}$$

where a_0 is the number of ml of the *iso*propanolic solution of hydrochloric acid (5.4.6) used, m_0 is the mass, in g, of sodium carbonate taken.

5.5 Procedure

Prepare the sample according to 2.001.
Into a ground-necked flask (5.3.1) weigh to within 0.002 g about 2 g of the prepared fat. Add with the pipette (5.3.5) 25 ml of the *iso*propanolic potassium hydroxide solution (5.4.5). Fit the tube or the condenser (5.3.2) and boil gently, with occasional shaking, until the solution become homogeneous.
Stop heating after 60 minutes. In order to avoid solidification of the saponified solution, transfer it quantitatively, while still warm, into a beaker (5.3.3) with the aid of three 10-ml portions of ethanediol (5.4.2). Finish rinsing with 5-ml of 2-propanol (5.4.1).
Insert the electrodes of the pH meter (5.3.7) and start the stirrer (5.3.8). Titrate with the *iso*propanolic hydrochloric acid solution (5.4.6) to the equivalence point of the neutralization curve (*note 3*).
Carry out two determinations on the same prepared sample.
Carry out a blank test in the same way.

5.6 Expression of results

The saponification value (S.V.) is given by the formula:

$$\text{S.V.} = \frac{56.1 \times T \times (V_0 - V_1)}{m}$$

where V_0 is the number of ml of the standardized *iso*propanolic hydrochloric acid solution (5.4.6) used for the blank test, V_1 is the number of ml of the standardized *iso*propanolic hydrochloric acid solution (5.4.6) used for the test with the fat, T is the exact normality of the

429

*iso*propanolic hydrochloric acid solution (5.4.6) used, *m* is the mass, in g, of the test portion.
Take as the result the arithmetic mean of the two determinations, provided that the requirement of repeatability 5.7 is satisfied.

5.7 **Repeatability**
The difference between the results of two determinations carried out simultaneously or in rapid succession by the same analyst should not exceed 0.5 per cent of the mean value.

6 Test report
The test report should show the method used.

7 Notes
1 A stable colourless solution of potassium hydroxide can be prepared in the following manner: reflux 1000 ml of ethanol with 8 g of potassium hydroxide and 5 g of aluminium pellets for 1 hour, then distil immediately. Dissolve the required amount of potassium hydroxide in the distillate. Allow the whole to stand for several days and decant the clear supernatant liquid from the deposit potassium carbonate.
The solution can also be prepared without distillation in the following manner: add 4 ml of aluminium butylate to 1000 ml ethanol and allow the mixture to stand for several days. Decant the supernatant liquid and dissolve therein the necessary amount of potassium hydroxide. The solution is ready for use.
2 It is advisable to store the glass electrode in water or, better still, in a 6/1 (V/V) mixture of ethanediol (5.4.2) and 2-propanol (5.4.1) for 12 hours preceding the titration. Dry it very gently with a piece of filter paper before use. Immediately after the titration, rinse with ethanediol, then with 2-propanol, and finally with distilled water.
If the electrode does not function satisfactorily, it may be possible to regenerate it by immersion for 24 hours in a 1 N solution of hydrochloric acid in 2-propanol. After this treatment the electrode should be washed with distilled water, then with 2-propanol and with a 6/1 (V/V) mixture of ethanediol and 2-propanol.
A thick sintered glass or porcelain disc between the saturated potassium chloride solution and the test solution prevents diffusion currents and parasitic potentials.
3 *Equivalence point/inflexion point.* The equivalence point usually corresponds approximately to the reading 10 on the pH scale, and can be determined graphically by observing the inflexion point on the neutralization curve. Alternatively, it can be calculated as the figure for which the first differential of the variation of pH as a function of the amount of *iso*propanolic hydrochloric acid solution added reaches a maximum, or the value for which the second differential becomes zero.

2.203 Determination of the ester value (E.V.)

1 **Scope**

This Standard describes a method for the characterization of esters in oils and fats.

2 **Field of application**

This Standard is applicable to the animal and vegetable oils and fats. It is not applicable to waxes.

3 **Definition**

The ester value (E.V.) is the number of mg of potassium hydroxide required to saponify the esters contained in 1 g of the fat.

4 **Procedure**

For the fat to be analysed, determination of the acid value (A.V.) according to 2.201 and the saponification value (S.V.) according to 2.202.

5 **Expression of results**

The ester value (E.V.) is given by the formula:

$$E.V. = S.V. - A.V.$$

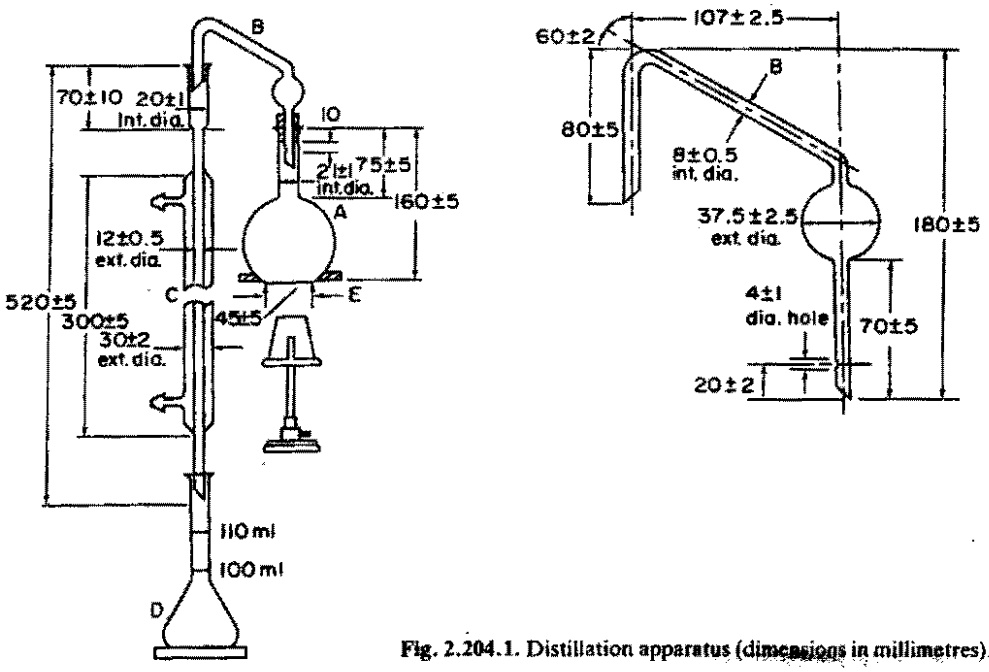


Fig. 2.204.1. Distillation apparatus (dimensions in millimetres).

431

References

Brüschweiler H, Dieffenbacher A. *Pure Appl Chem* 1991; 63(8): 1153–1163. (Report on the Collaborative Study).
D'Alonzo RP, Kozarek WJ, Wharton HW. *J Am Oil Chem Soc* 1981; 58: 215–227.
Goh EM, Timms RE. *J Am Oil Chem Soc* 1985; 62: 730–734.
Hammond EW. *J Chromatogr* 1981; 203: 397–402.
ISO 5725. *Precision of test methods. Determination of repeatability and reproducibility by inter-laboratory tests.* 1986.
IUPAC. *Standard Methods for the Analysis of Oils, Fats and Derivatives*, 7th edn.
Prevot A, Coustille J-L. *Rev Franç Corps Gras* 1982; 29: 17–24.
Riva M, Daghetta A, Galli M *Rivist Ital Sostanze Grasse* 1981; 58: 432–443.
Shasrabudhe MR. *J Am Oil Chem Soc* 1968; 45: 379–380.
Shasrabudhe MR. *J Am Oil Chem Soc* 1969; 46: 8–12.
Soe JB. *Fette Seifen Anstrichmittel* 1983; 85: 72–76.
Vaver VA, Ushakov AN. *Methods Biol Anal* 1980; 26: 327–406.

2.423 Determination of phosphorus by direct graphite furnace atomic absorption spectrometry

1 Scope and field of application

This Standard describes a method for the determination of trace amounts (mg/kg) of phosphorus in all types of crude or refined edible oils and fats.

2 Principle

Vaporization of the oil or fat in a suitable graphite furnace with or without a platform connected to an atomic absorption spectrometer, previously calibrated using standard solutions of soya lecithin, and the measurement of the phosphorus content from the observed absorption at a wavelength of 213.5 nm.

3 Apparatus

3.1 Test tubes, 10 ml.

3.2 Micropipettor, 20 μl.

3.3 Pipettor tips.

3.4 Electric oven, regulated at 60 ± 2°C.

3.5 Atomic absorption spectrometer, equipped with either 'peak height' mode and printer, or 'continuous' mode and pen recorder (full scale response in 0.2 seconds), together with the appropriate electrode-less discharge lamp (or hollow cathode lamp) and deuterium background corrector (or Zeeman atomic absorption spectrometer).

3.6 Graphite furnace atomizer, placed in the atomic absorption spectrometer (3.5), equipped with a control unit for temperature programming.

3.7 Graphite tube, normal (uncoated).

3.8 Platform: pyrolytic, in combination with uncoated or pyrolytically coated graphite tube (*note 2*).

4 Reagents

4.1 Cyclohexane, analytical grade.

4.2 Lanthanum organometallic standard: a suitable standard (Conostan, 5000 mg/kg) is available from Continental Oil Company, Ponca City, Oklahoma, USA.

4.3 Matrix modifier (*note 1*): 0.05 per cent (m/V) Lanthanum solution is prepared by dissolving 5 g of lanthanum standard (4.2) in 50 ml of cyclohexane (4.1).

4.4 Blank oil: refined liquid edible oil with a phosphorus content not greater than 1 mg/kg (*note 3*).

4.5 Lecithin: a well-defined lecithin containing 2 per cent phosphorus, e.g. soya-lecithin (*note 3*).

4.6 Standard stock solution: a stock solution of 400 mg phosphorus/kg is prepared by dissolving 1 g of lecithin (4.5) in 4 g of cyclohexane (4.1) and 45 g of blank oil (4.4).

4.7 Standard working solutions: 10, 20 and 40 mg phosphorus/kg are prepared by diluting the 400 mg/kg stock solution (4.6) with blank oil (4.4).

4.8 Argon, purity 99.99 per cent minimum.

5 Procedure

5.1 Treatment of samples, blank and standards

5.1.1 Place all samples, blank oil and standard working solutions in the oven (3.4), regulated at 60 ± 2°C.

5.1.2 Shake samples vigorously.

2.325 Determination of the individual triglycerides in oils and fats by high performance liquid chromatography

1 **Scope**

This Standard describes a method for the separation, identification and determination of individual triglycerides in edible oils and fats. This Standard is complementary to method 2.323 (Determination of triglycerides [according to their carbon number] by gas–liquid chromatography) and method 2.324 (Determination of triglycerides according to their partition number by high performance liquid chromatography).

2 **Field of application**

This Standard is applicable to vegetable and animal oils and fats which contain only triglycerides of long-chain fatty acids (particularly of 16–22 carbon atoms) (*note 1*).

3 **Principle**

Separation of the individual triglycerides by direct high performance liquid chromatography (reversed phase). Identification and quantitation by reference to external standards and the determined fatty acid composition of the sample.

4 **Apparatus**

4.1 High performance liquid chromatograph, as required for method 2.324, equipped as described under 2.324 paras 4.1–4.5.

5 **Reagents**

5.1 Chloroform, suitable for HPLC.

5.2 Acetone, suitable for HPLC.

5.3 Acetonitrile, suitable for HPLC.

5.4 HPLC mobile phase: prepare a mixture of acetonitrile (5.3) and acetone (5.2), initially in the proportion of 25:75 (V/V) (see para. 6.1 below). The mobile phase should be de-gassed before use—it may be recycled several times without effect on the separation of the triglycerides.

5.5 Solubilization solvent: acetone (5.2) alone, or acetone–chloroform (5.2, 5.1) 1:1 (V/V) (*note 2*).

5.6 Reference triglycerides: commercial triglycerides such as tripalmitin, tristearin, triolein, trilinolenin etc. Alternatively, a vegetable oil of known triglyceride composition, e.g. soya oil.

5.7 Reference triglyceride solution 5 per cent (W/V): transfer 0.5 ± 0.01 g of each triglyceride (5.6) to a 10-ml graduated flask and make up to volume with the solubilization solvent (5.5).

6 **Procedure**

6.1 Optimization of working parameters

Pump the mobile phase (5.4) through the column at a flow rate of about 1.5 ml/min for at least 30 minutes or until a stable baseline is obtained. Adjustment of the relative proportions of the solvents in the mobile phase and the flow rate should be made as required in order to achieve the desired separation of the individual triglycerides (see Fig. 2.325.1).

434

7 **Repeatability**

The difference between the results of two determinations carried out simultaneously or in rapid succession by the same analyst should not exceed 0.4 to 1 unit according to the expected value.

Warning

Use of a fume hood is recommended. Wijs solution causes severe burns and vapours can cause lung and eye damage. Carbon tetrachloride is also hazardous causing irritation, headaches, and nausea upon mild exposure.

More severe exposure can lead to liver and kidney damage. Carbon tetrachloride is a cancer suspect agent.

with the amount (expressed as a percentage (m/m)) of this fatty acid shown to be present in the sample when its fatty acid composition is determined using method 2.302, 2.303 or 2.304. For example, the balance sheet applicable in the case of oleic acid is given by the formula:

$$T_O = T_{O\text{-}tri}$$

where:

T_O is the amount, expressed as a percentage (m/m), of the oleic acid in the sample's fatty acid composition

$T_{O\text{-}tri}$ is the sum of the amounts, expressed as a percentage (m/m), of the oleic acid occurring in the sample's triglycerides.

Confirmation that all the individual triglycerides have been identified correctly is indicated when the sum of the amounts (expressed as a percentage (m/m)) of each fatty acid present in the sample's triglycerides corresponds, respectively, to the amount (expressed as a percentage (m/m)) of each fatty acid represented in the fatty acid composition of the sample.

8 Quality assurance

8.1 For *general principles* of analytical quality control, see the section on *Quality Assurance* in the introductory part of the *Compendium of Standard Methods*.

8.2 For *specific applications* of analytical quality control, see the Annexe to this standard method.

9 Notes

1 Triglycerides of hydrogenated fats are generally not very well resolved under the working conditions described, resulting very often in broad triglyceride peaks. The presence of hydrogenated fats in blends may thus be indicated in the chromatogram by a more or less pronounced shoulder on the side of a triglyceride peak, indicating the presence of a partially superimposed peak of another triglyceride. The method does not allow separation of all the individual triglycerides in lauric oils (e.g. coconut and palm kernel oils) because of the complex nature of their glyceride composition.

2 Usually oils and fats are readily soluble in acetone, with the exception of those containing appreciable amounts of saturated triglycerides, such as high melting fats and hydrogenated oils and blends thereof. In such cases the proportion of chloroform in the solubilization solvent (5.5) can be increased to advantage.

3 The elution order of the triglycerides can be determined by calculating their partition numbers or equivalent carbon numbers (ECNs) as described in method 2.324, *note 4* of para. 9.

4 For ease of reference, the abbreviations below are used for those fatty acids that occur in the triglycerides most commonly encountered:

La, lauric acid
My, myristic acid
P, palmitic acid
S, stearic acid
Ar, arachidic acid
Po, palmitoleic acid

436

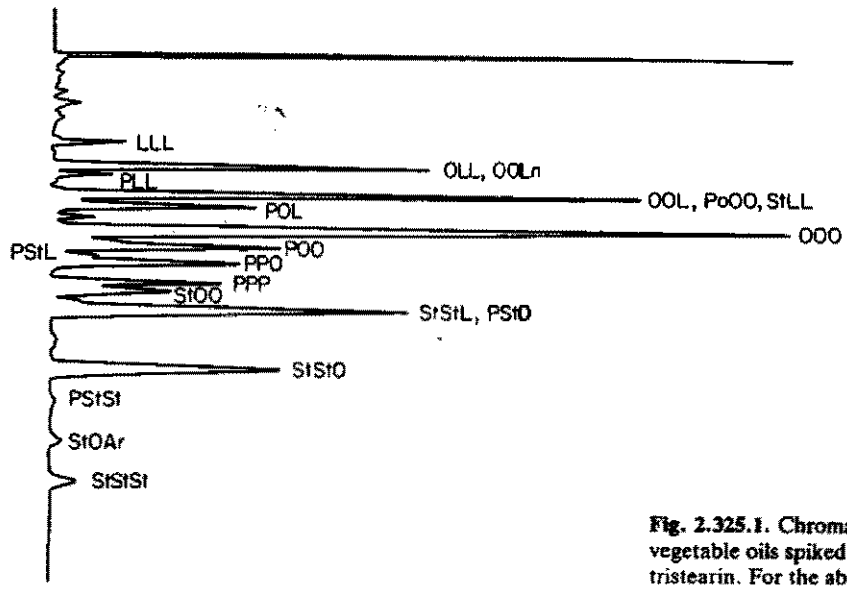


Fig. 2.325.1. Chromatogram of a blend of vegetable oils spiked with tripalmitin and tristearin. For the abbreviations used for the designation of triglycerides see *note 4*.

6.2 Preparation of test sample solution

Prepare a 5 per cent (W/V) solution by transferring 0.5 ± 0.01-g sample to a 10-ml graduated flask and making up to volume with the solubilization solvent (5.5). Inject 10 μl of this solution.

Identify the triglyceride peaks (see 6.3 below). Record the retention times of the solvent and triglyceride peaks; also record the peak area of each triglyceride.

6.3 Identification of triglyceride peaks

Analyse the reference triglyceride solution (5.7) by injecting 10 μl of the solution under the same conditions as the test sample solution (6.2), recording retention times and peak areas as in the case of the chromatographed sample (6.2). Proceed with the identification of the individual triglyceride peaks as described in para. 6.3 of method 2.324.

7 **Calculation and expression of results**

7.1 Using the internal normalization method, the total sum of the peak areas corresponding to all the chromatographed individual triglycerides is assumed to be 100 per cent. The relative percentage (T_i), of each triglyceride, i, is then given by the formula:

$$T_i = \frac{\text{Area of peak i}}{\text{Sum of peak areas}} \times 100$$

Express the results to one decimal place.

7.2 Verification of the identification of triglycerides

7.2.1 Determine the fatty acid composition according to methods 2.301 and 2.302 or 2.303 and 2.304.

7.2.2 To verify that the correct identification of triglycerides has been made use a 'balance sheet': for a given fatty acid, compare the sum of the amounts (expressed as a percentage (m/m)) of this fatty acid wherever it occurs in the individual sample triglycerides,

Table 2.325.1. Triglycerides* in soyabean oil (Collaborative Study 1990).

	LLnLn	OLL	OOL	OOO
Number of laboratories	13	13	12	13
Number of results	26	25	26	26
Number of laboratories retained after eliminating outliers	13	12	12	13
Number of accepted results	26	24	24	26
Mean (percentage of total triglycerides)	1.1	17.3	8.7	3.5
Repeatability limit (r)	0.25	1.57	0.79	0.69
Reproducibility limit (R)	0.45	2.13	0.79	1.26

Table 2.325.2. Triglycerides* in palm oil (Collaborative Study 1990).

	PPL	POO	StOO
Number of laboratories	12	13	13
Number of results	24	26	26
Number of laboratories retained after eliminating outliers	12	13	11
Number of accepted results	24	26	22
Mean (percentage of total triglycerides)	10.8	21.7	2.4
Repeatability limit (r)	0.74	0.61	0.33
Reproducibility limit (R)	1.05	2.58	0.58

Table 2.325.3. Triglycerides* in beef tallow (Collaborative Study 1990).

	POO	PPP	StStSt
Number of laboratories	13	13	9
Number of results	25	25	17
Number of laboratories retained after eliminating outliers	12	12	7
Number of accepted results	24	24	14
Mean (percentage of total triglycerides)	20.2	4.1	1.2
Repeatability limit (r)	1.33	0.94	0.32
Reproducibility limit (R)	5.24	2.03	0.61

* Abbreviations used for the component fatty acids of triglycerides: L, linoleic acid; Ln, linolenic acid; O, oleic acid; P, palmitic acid; St, stearic acid.
Results for r and R are expressed as a percentage of total triglycerides.

O, oleic acid
L, linoleic acid
Ln, linolenic acid

The triglycerides can then be designated as shown in the following examples:

PPP, tripalmitin
OOO, triolein
POP, palmito-oleopalmitin
POS, palmito-oleostearin
PPO, palmitopalmito-olein
PSO, palmitostearo-olein

References

Litchfield C. *Analysis of Triglycerides*. New York: Academic Press, 1972.
Pei PTS, Henly RS, Ramachandran S. *Lipids* 1975: **10**: 152.
Perrin JL, Naudet M. *Rev Franç Corps Gras* 1983: **30**: 279.
Podlaha O, Toregard B. *J High Res Chrom* 1982; 5: 553.

Annexe

Analytical quality control

1 Repeatability
When the mean value of two single test results obtained under *repeatability conditions* (conditions where independent test results are obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within short intervals of time) lies within the range of the values shown in the tables below, the absolute difference between the two test results obtained should not be greater than the *repeatability limit* (r) deduced by linear interpolation from the data in the tables.

2 Reproducibility
When the values of two single test results obtained under *reproducibility conditions* (conditions where test results are obtained with the same method on identical test material in different laboratories with different operators using different equipment) lie within the range of the values shown in the tables below, the absolute difference between the two test results obtained should not be greater than the *reproducibility limit* (R) deduced by linear interpolation from the data in the tables below.

3 Result of the interlaboratory tests
An interlaboratory test carried out at the international level in 1990 by the IUPAC Commission on Oils, Fats and Derivatives, in which 13 laboratories participated, each obtaining two test results for each sample, gave the statistical results (evaluated in accordance with *ISO 5725-1986*) summarized in the following tables.

2.326 Determination of mono- and diglycerides by capillary gas chromatography

1 **Scope**

This Standard describes a method for the determination of mono- and diglycerides.

2 **Field of application**

The Standard is applicable to mono- and diglyceride concentrates and mono- and diglycerides in fats and oils.

3 **Principle**

Conversion of mono- and diglycerides with *N,N*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) in pyridine into more volatile trimethylsilyl ether derivatives and quantitative determination by capillary gas chromatography using an internal standard (*n*-tetradecane).

4 **Apparatus**

4.1 Gas chromatograph, with split injection or on-column injection (*note 1*), oven temperature programming and flame-ionization detector. Recorder and integrator.

4.2 Column, capillary, glass or fused silica, surface fully deactivated by silylation agent (*note 2*), 15–25 m in length, 0.25–0.35 mm internal diameter, coating SE-54 (or phase with similar polarity), film thickness 0.1–0.2 μm.

4.3 Operating conditions, split injection (split ratio 1:10–1:50); direct injection (splitless, hold for 1 minute); temperatures: injection port 320°C, or on-column injection 60°C, column initial 80°C (or 60°C on-column), programme 10°C/min, final 360°C, hold for 15 minutes, detector 350°C, carrier gas flow 5 ml helium/min (at 80°C), injection volume 1–5 μl. An automatic sampler is advantageous.

4.4 Screw-cap vials (2.5 ml) or crimp-top vials for autosampler (e.g. 2.0 ml), with Teflon-faced septa.

4.5 Heating device for vials, 70°C.

5 **Reagents**

5.1 *N,N*-bis(trimethylsilyl)trifluoroacetamide (BSTFA).

5.2 Trimethylchlorosilane (TMCS).

5.3 Pyridine, analytical grade, kept over potassium hydroxide.

5.4 *n*-Tetradecane, analytical grade (internal standard).

5.5 *n*-Hexane, analytical grade.

5.6 Reference standards: glycerol, palmitic acid, 1-*O*-palmitoylglycerol, 1-*O*-stearoylglycerol, 1,2-di-*O*-palmitoylglycerol, 1,3-di-*O*-palmitoylglycerol, 1,2-di-*O*-stearoylglycerol.

5.7 Internal standard solution: accurately weigh about 100 mg *n*-tetradecane (5.4) into a 10-ml volumetric flask and dilute to volume with pyridine (5.3).

5.8 Reference solution: accurately weigh about 100 mg of reference standard (e.g. glycerol, fatty acid, mono- and di-*O*-acetylglycerol) and about 100 mg of *n*-tetradecane into a 10-ml volumetric flask. Dilute to volume with pyridine. Alternatively, weigh about 100 mg of a mixture containing several (e.g. five) reference standards and *n*-tetradecane, each component being present in about the same quantities, into a 2-ml volumetric flask and dilute to volume with pyridine.

6 Procedure

6.1 Sample solution

Accurately weigh about 10 mg of homogenized sample of emulsifier concentrates or 50 mg of oils and fats containing emulsifiers into a 2.5 ml screw-cap vial with Teflon-faced septa. Add 0.1 ml of internal standard solution (5.7) containing 1 mg of *n*-tetradecane, 0.2 ml of BSTFA and 0.1 ml of TMCS to the sample (*note 3*).

Humidity is strictly excluded. Close vial and shake vigorously. Heat the reaction mixture in heating device at 70°C for about 20 minutes. Inject 1–5 µl of the reaction mixture into the gas chromatograph showing a stable baseline (*note 1*).

Avoid delay of gas chromatographic analysis. The reaction is carried out twice and duplicate injections are made per reaction.

6.2 Reference solution

Transfer 0.10 ml of reference solution (5.8) to a vial and add the silylating agents, 0.2 ml of BSTFA and 0.1 ml of TMCS (no internal standard solution is added), and inject as described above (*notes 1, 3*).

Use a concentration range of reference standards similar to that of the substances to be quantified in sample solution. A plot of response factor versus concentration of reference standards may be useful to check linearity.

Check response factors periodically. Response factors should be above about 0.5. Lower response factors indicate some loss or decomposition. Use concentration range of 0.5–10 mg/ml of components in reference and sample solutions.

6.3 Identification

Analyse the reference solution under the same operating conditions as the sample solution. Identify peaks by comparison of retention time with known substances or apply coupled GC/MS (gas liquid chromatography/mass spectrometry).

7 Calculation and expression of results

7.1 Response factor

Calculate the response factors of the reference substances versus internal standard using the reference standard chromatogram. The value of the response factor is given by the formula:

$$R_x = (m_{is}/m_x) \times (A_x/A_{is})$$

where:

R_x is the response factor of reference standard x
m_{is} is the mass, in mg, of internal standard
m_x is the mass, in mg, of reference standard x
A_x is the peak area of reference substance x
A_{is} is the peak area of internal standard.

7.2 Calculation of sample component content

Calculate the percentage of mass content of component x in the sample by the formula:

$$m'_x(\%) = 1/R_x \times (m'_{is}/m'_s) \times (A'_x/A'_{is}) \times 100$$

where:
m'_x is the percentage (m/m) of mass of component x in the sample
R_x is the response factor of component x in the sample
m'_{is} is the mass, in mg, of internal standard in the sample
m'_s is the mass, in mg, of the sample
A'_x is the peak area of the component x in the sample
A'_{is} is the peak area of the internal standard in the sample.

8 Quality assurance

8.1 For *general principles* of analytical quality control, see the section on *Quality Assurance* in the introductory part of the *Compendium of Standard Methods*.

8.2 For *specific applications* of analytical quality control, see the Annexe to this standard method.

9 Notes

1 For on-column injection, or direct injection, dilute 50 µl of reaction mixture (6.1; 6.2) with 1 ml of hexane and inject (1 µl).
In order to lengthen the life-time of the columns when applying on-column injections, a pre-column is useful. On-column injection gives better response factors.
2 Use a length of column required to separate 1,2- from 1,3-diglycerides.
3 For automatic samplers with 2-ml crimp-top vials, it is convenient to double the amount of sample and reagents.
4 Beside mono- and diglycerides, other components such as glycerol, fatty acids, sterols etc. can be analysed by the same method.

Annexe

Analytical quality control

1 Repeatability
When the mean of the values obtained from two single determinations carried out in rapid succession by the same operator, using the same apparatus under the same conditions for the analysis of the same test sample, lies within the range of the mean values cited in the tables below, the difference between the two values obtained should not be greater than the repeatability limit (r), which can generally be deduced by linear interpolation from the values in the tables below.

2 Reproducibility
When the values for the final result, obtained by operators in different laboratories using different apparatus under different conditions from the analysis of the same laboratory sample, lie within the range of mean values cited in the tables below, the difference between the values for the final result obtained by those operators should not be greater than the reproducibility limit (R), which can generally be deduced by linear interpolation from the values in the tables below.

3 Results of the interlaboratory test
An interlaboratory test carried out at an international level in 1985 by the IUPAC Commission on Oils, Fats and Derivatives, in which eight laboratories participated, each

442

obtaining two test results for each sample, gave the statistical results (evaluated in accordance with *ISO 5725-1986*) summarized in the following tables for mono- and diglyceride concentrates (Table 2.326.1) and for mono- and diglycerides in oils (Table 2.326.2).

Table 2.326.1. Statistical analysis of results for mono- and diglyceride concentrates (expressed as percentage of mass of sample).

	1-Myristate	1-Palmitate	1-Stearate	1,3-Dipalmitate	1-Palmitate-3-stearate	1,3-Distearate
Number of laboratories	8	8	8	8	8	8
Number of results	16	16	16	16	16	16
Number of laboratories retained after elimination of outliers	7	8	8	7	7	8
Number of accepted results	14	16	16	14	14	16
Mean value (g/100 g sample)	1.7	27.2	60.1	0.2	0.8	1.1
Repeatability standard deviation (S_r)	0.05	0.9	2.1	0.01	0.05	0.07
Repeatability relative standard deviation	3.0	3.3	3.5	4.6	6.0	6.8
Repeatability limit (r) ($2.83 \times S_r$)	0.14	2.61	5.91	0.31	0.14	0.20
Reproducibility standard deviation (S_R)	0.1	2.4	6.4	0.06	0.1	0.3
Reproducibility relative standard deviation	5.7	8.9	10.7	30.0	17.8	24.8
Reproducibility limit (R) ($2.83 \times S_R$)	0.3	6.8	18.1	0.2	0.4	0.8

Table 2.326.2. Statistical analysis of results for mono- and diglycerides in oils (expressed as percentage of mass of sample).

	1-Palmitate	1-Stearate	1,2-Dipalmitate	1,3-Dipalmitate	1,2-Distearate
Number of laboratories	8	8	8	8	8
Number of results	16	16	16	16	16
Number of laboratories retained after elimination of outliers	8	8	8	8	8
Number of accepted results	16	16	16	16	16
Mean value (g/100 g sample)	0.96	0.98	0.97	0.93	0.97
Repeatability standard deviation (S_r)	0.03	0.03	0.04	0.02	0.06
Repeatability relative standard deviation	3.3	3.4	4.0	2.5	6.2
Repeatability limit (r) ($2.83 \times S_r$)	0.08	0.08	0.11	0.06	0.17
Reproducibility standard deviation (S_R)	0.12	0.14	0.24	0.19	0.19
Reproducibility relative standard deviation	12.0	13.8	24.4	20.2	19.8
Reproducibility limit (R) ($2.83 \times S_R$)	0.34	0.40	0.68	0.54	0.54

2.205 Determination of the iodine value: Wijs' method

Statistical evaluation of results of studies carried out in 1989/90.

Table 2.205.1.

Solvent:*	Mean value CTC	Mean value CHX	r CTC	r CHX	R CTC	R CHX	No. of accepted results (CTC, CHX)
Sample							
Sunflower seed oil	133.6	132.9	3.0	3.6	7.2	4.8	(20, 20)
Palm oil, refined	53.1	53.0	0.65	0.82	1.43	1.90	(20, 20)
Fish oil, crude	109.1	108.5	1.67	1.40	4.35	2.80	(18, 14)
Tung oil	164.5	163.1	3.47	2.39	5.27	4.32	(14, 12)
Tallow (beef fat)	47.2	46.9	1.37	1.33	2.75	3.10	(20, 20)
Soyabean oil, hydrogenated	63.3	65.3	1.86	13.8	17.2	14.9	(12, 16)
Palm oil, crude	52.5	52.6	1.29	2.15	2.19	2.89	(18, 20)
Frying oil, used	37.7	37.7	0.97	1.38	1.18	2.48	(14, 14)
Fish oil, hydrogenated	63.3	68.6	3.61	1.69	17.2	11.0	(12, 12)
Palm kernel oil	18.2	18.3	0.17	0.14	0.51	0.64	(16, 14)
Olive oil	82.3	82.2	0.75	1.75	1.86	2.62	(14, 10)

Table 2.205.2.

Sample:† Solvent:*	SBO1 CTC	SBO1 CHX	SBO2 CTC	SBO2 CHX
Number of laboratories	17	17	17	17
Number of results	34	34	34	34
Number of laboratories retained after elimination of outliers	16	17	17	17
Number of accepted results	32	34	34	34
Mean value	102.6	102.3	74.7	74.8
Repeatability limit (r)	1.4	2.2	1.7	1.5
Reproducibility limit (R)	4.8	5.1	3.7	2.1

Table 2.205.3.

Sample:† Solvent:*	HFO CCT	HFO CHX
Number of laboratories	17	17
Number of results	34	34
Number of laboratories retained after elimination of outliers	16	17
Number of accepted results	32	34
Mean value	73.0	72.8
Repeatability limit (r)	1.7	1.6
Reproducibility limit (R)	2.5	2.3

* CTC, carbon tetrachloride; CHX, cyclohexane + glacial acetic acid (1:1).
† SBO, hydrogenated soyabean oil; HFO, hydrogenated fish oil.
Statistical analysis according to *ISO 5725-1986*.

Reference

Pocklington WD. *Pure Appl Chem* 1990; **62**(12): 2339–2343.

444

8 **Calculation**

For a transmission curve:

$$\text{Absorption } A = \log \frac{PR}{QR}$$

where PR and QR are the lengths measured on the graph.

For an absorption curve:

$$\text{Absorption } A = A_Q - A_P$$

where A_Q is the absorption at Q, A_P is the absorption at P.

Using the calibration curve constructed according to 7.1.3, determine the amount m', in mg, of methyl elaidate corresponding to the value found for the absorption A.

The per cent amount of isolated *trans*-unsaturated compounds, expressed as methyl elaidate, is:

$$\frac{100m'}{m}$$

where m is the mass, in mg, of the test portion.

Warning

Carbon disulphide

Carbon disulphide is an insidious poison, being harmful either by vapour inhalation, prolonged or repeated skin contact or by oral intake.

Chronic poisoning may ensue from repeated exposure to vapour. Extreme precautions should be taken when using this solvent.

445

2.501 Determination of the peroxide value (P.V.)

1 **Scope**

This Standard describes a method for the determination of the peroxide value of oils and fats.

2 **Field of application**

This Standard is applicable to animal and vegetable oils and fats.

3 **Definition**

The peroxide value is the quantity of those substances in the sample, expressed in terms of milliequivalents of active oxygen per kg, which oxidize potassium iodide under the operating conditions described.

Note. This definition does not agree with the definition given in Method II.D.13 (Fifth Edition).

4 **Principle**

Treatment of the test portion, in solution in acetic acid and chloroform, by a solution of potassium iodide. Titration of the liberated iodine with standardized sodium thiosulphate solution.

5 **Apparatus**

All the equipment used shall be free from reducing or oxidizing substances.

Note. Do not grease ground surfaces.

5.1 3-ml glass scoop.

5.2 Flasks, with ground necks and stoppers, of about 250 ml capacity, dried beforehand and filled with a pure, dry inert gas (nitrogen or, preferably, carbon dioxide).

5.3 25- or 50-ml burette, graduated in 0.1 ml.

6 **Reagents**

6.1 Chloroform, analytical reagent quality, freed from oxygen by bubbling a current of pure, dry inert gas through it.

6.2 Glacial acetic acid, analytical reagent quality, freed from oxygen by bubbling a current of pure, dry gas through it.

6.3 Potassium iodide, saturated aqueous solution, recently prepared, free from iodine and iodates.

6.4 Sodium thiosulphate, 0.01 or 0.002 N accurately standardized aqueous solution, standardized just before use.

6.5 Starch solution, 10 g/l aqueous dispersion, recently prepared from natural soluble starch.

7 **Sample**

Take care that the sample is taken and stored away from the light, kept cold and contained in completely filled glass containers, hermetically sealed with ground-glass or cork stoppers.

8 **Procedure**

The test shall be carried out in diffuse daylight or in artificial light. Weigh in a glass scoop (5.1) or, failing this, in a flask (5.2), to the nearest 0.001 g, a mass of the sample in accordance with Table 2.501.1, according to the expected peroxide value:

446

Table 2.501.1

Expected peroxide value (m.e.q.)	Mass of test portion (g)
0 to 12	5.0 to 2.0
12 to 20	2.0 to 1.2
20 to 30	1.2 to 0.8
30 to 50	0.8 to 0.5
50 to 90	0.5 to 0.3

Unstopper a flask (5.2) and introduce the glass scoop containing the test portion. Add 10 ml of chloroform (6.1). Dissolve the test portion rapidly by stirring. Add 15 ml of acetic acid (6.2), then 1 ml of potassium iodide solution (6.3). Insert the stopper quickly, shake for 1 minute, and leave for exactly 5 minutes away from the light at a temperature from 15 to 25°C.

Add about 75 ml of distilled water. Titrate the liberated iodine with the sodium thiosulphate solution (6.4) (0.002 N solution for expected values less than 12, and 0.01 N solution for expected values above 12) shaking vigorously, using starch solution (6.5) as indicator.

Carry out two determinations on the same test sample.

Carry out simultaneously a blank test. If the result of the blank exceeds 0.05 ml of 0.01 N sodium thiosulphate solution (6.4), replace the impure reagents.

9 **Expression of results**

The peroxide value (P.V.), expressed in milliequivalents of active oxygen per kg, is given by the formula:

$$\text{P.V.} = \frac{V \times T}{m} \times 1000$$

where V is the number of ml of the standardized sodium thiosulphate solution (6.4) used for the test, corrected to take into account the blank test, T is the exact normality of the sodium thiosulphate solution (6.4) used, m is the mass, in g, of the test portion.

Take as the result the arithmetic mean of the two determinations carried out.

Warning

Chloroform

Even at moderate concentrations chloroform vapour tends to produce gradual fatigue and headaches.

Following exposure to excessive concentrations or repeated exposure to lower concentrations jaundice may ensue after an interval. Continued inhalation of sub-narcotic doses may lead to chronic deterioration of organs.

The strong degreasing action of liquid chloroform on the skin produces burns and cracking.

447

where V_3 is the number of ml of the standardized sodium thiosulphate solution (4.3.7) used for the blank test, V_4 is the number of ml of the standardized sodium thiosulphate solution (4.3.7) used for the test portion, T is the exact normality of the sodium thiosulphate solution (4.3.7) used, m is the mass, in g, of the test portion.

5 The Hanus method

5.1 Principle

Addition to the test portion of an iodine monobromide solution in an acetic acid and carbon tetrachloride mixture. After a standardized time of reaction, determination of the excess halogen by addition of potassium iodide aqueous solution and titration of the liberated iodine with a standardized sodium thiosulphate solution.

5.2 Apparatus

See 4.2.

5.3 Reagents

5.3.1 Glacial acetic acid, free from ethanol.

5.3.2 Carbon tetrachloride, analytical reagent quality.

The two reagents must be free from oxidizable matter and are tested as in 4.3.

5.3.3 Iodine monobromide (IBr), pure.

5.3.4 Potassium iodide, 100 g/l aqueous solution.

This must contain no free iodine or iodate.

5.3.5 Sodium thiosulphate, 0.1 N accurately standardized aqueous solution.

5.3.6 Starch solution, 10 g/l aqueous dispersion, recently prepared from natural soluble starch.

5.4 Preparation of the Hanus reagent

Into a brown glass bottle weigh 10 g of iodine monobromide (5.3.3) and dissolve it in 500 ml of acetic acid (5.3.1).

5.5 Procedure

The weight of fat to be taken, in g, is about 25/I.V. (expected iodine value).

Into a glass scoop (4.2.1) weigh the appropriate quantity accurately to within 0.001 g. Insert the scoop with contents into a ground-necked bottle (4.2.2). Add 10 ml of carbon tetrachloride (5.3.2) and dissolve.

Then add exactly 25 ml of the reagent and insert the stopper. Shake gently, and place in the dark for 1 hour.

Add 20 ml of the potassium iodide solution (5.3.4) and 100 ml of distilled water. Titrate with the aqueous thiosulphate solution (5.3.5), using starch solution (5.3.6) as indicator, continuing the titration until the blue colour just disappears after very vigorous shaking.

5.6 Expression of results

See 4.6.

448

6 The Hübl method

6.1 Principle

To a carbon tetrachloride solution of the test portion, addition of an excess of an ethanolic solution of iodine and mercuric chloride. After a standardized time of reaction, determination of the excess halogen by addition of potassium iodide aqueous solution and titration of the liberated iodine with a standardized sodium thiosulphate solution.

6.2 Apparatus

See 4.2.

6.3 Reagents

6.3.1 Ethanol, 95 per cent (V/V).

6.3.2 Carbon tetrachloride, free from oxidizable matter (see 4.3).

6.3.3 Iodine, pure, re-sublimed.

6.3.4 Mercuric chloride, analytical reagent quality.

6.3.5 Potassium iodide, 300 g/l aqueous solution.
This must contain no free iodine or iodate.

6.3.6 Sodium thiosulphate, 0.1 N accurately standardized aqueous solution.

6.3.7 Starch solution, 10 g/l aqueous dispersion, recently prepared from natural soluble starch.

6.4 Preparation of the Hübl reagent

Into a brown glass bottle dissolve 25 g iodine (6.3.3) in 500 ml of ethanol (6.3.1).
Into another bottle weigh 20 g mercuric chloride (6.3.4) and dissolve in 500 ml of ethanol (6.3.1). Filter this solution, if necessary.
24 hours before it is required for use, mix equal volumes of these two solutions. This mixture does not keep for more than 48 hours.

6.5 Procedure

The weight of fat to be taken varies according to its expected iodine value according to the table given in 4.5.
In a small glass scoop (4.2.1) weigh the appropriate quantity of fat accurately to within 0.001. Insert into a 500-ml ground-necked bottle.
Dissolve in 10 ml of carbon tetrachloride (6.3.2). Add exactly 25 ml of the reagent. Insert the stopper, shake gently and place the bottle in the dark.
For oils having an iodine value below 150, leave for 12 hours; for those with iodine value above 150 and for polymerized or oxidized oils, leave for 24 hours.
Then add 20 ml of the potassium iodide solution (6.3.5). If a mercuric iodide precipitate appears, add more potassium iodide until the precipitate redissolves. Then add 300 ml of distillated water.
Titrate with the aqueous sodium thiosulphate solution (6.3.6) in the presence of starch solution (6.3.7) as indicator, continuing the titration until the blue colour just disappears after very vigorous shaking.
Carry out a blank test without the fat under the same conditions.

6.6 Expression of results

See 4.6.

449

2.304 Capillary column gas–liquid chromatography of fatty acid methyl esters

1 **Scope**

This Standard describes a general method for the application of capillary column gas–liquid chromatography to determine the qualitative and quantitative composition of a mixture of fatty acid methyl esters.

2 **Field of application**

This Standard is applicable to the fatty acid methyl esters prepared from oils and fats (*note 1*) according to method 2.301.

3 **Apparatus**

The instructions given apply to normal equipment used for gas–liquid chromatography employing capillary columns and flame-ionization detection.

3.1 Gas–liquid chromatography

3.1.1 **Injection system**

The injection system should be specially designed for use with capillary columns. It should be either of the split type or on-column (splitless) type (*note 2*).

3.1.2 **Oven**

The oven should be capable of heating the column to at least 220°C and of maintaining the desired temperature to within 0.1°C (*note 3*).

3.1.3 **Capillary column**

3.1.3.1 *Column*

Fused silica or glass, length 25–60 m, internal diameter 0.20–0.35 mm, with a stationary phase coating thickness of 0.1–0.2 μm (*note 4*).

3.1.3.2 *Stationary phase*

This should be of moderate polarity, such as the polyglycol type (e.g. polyethylene glycol 20 000), polyester type (e.g. butanediol succinate) or polar polysiloxane type (e.g. cyanosilicones) (*note 5*).

3.1.3.3 *Assembly and conditioning of the column*

Observe the normal precautions for assembling capillary columns, i.e. ensuring adequate support of the column in the oven, checking joints to injector and detector for leaks, positioning of the ends of the column at the injector and detector such that the dead-space in these areas is reduced to a minimum.

Subject the column to a suitable carrier gas flow and condition the column by heating it to about 10–50°C above the maximum oven temperature to be used for the analysis, but not exceeding the maximum temperature guaranteed for the stationary phase or recommended conditioning temperature for the column. Maintain the column at this temperature for at least 1 hour to achieve stabilization of the baseline. Reduce the oven temperature to 180°C for analysis under isothermal conditions.

3.2 Syringe, maximum capacity 10 μl, graduated in 0.1 μl.

450

3.3 Recorder, according to method 2.302, and compatible with the apparatus used.

3.4 Integrator, electronic, as specified in method 2.302, or data processor.

4 Reagents

4.1 Carrier gas: helium or hydrogen (*note 6*).

4.2 Auxiliary gases: hydrogen 99.9 per cent minimum, free from organic impurities; air or oxygen, free from organic impurities.

4.3 Reference standards or natural mixtures of lipids of known fatty acid composition corresponding to that of the sample. Otherwise, reference standards of individual fatty acids, or fatty acid methyl esters, or mixtures thereof with a fatty acid composition corresponding to that of the sample (*note 7*).

5 Procedure

5.1 Test conditions

5.1.1 **Selection of optimum operating conditions**

The efficiency of the column and permeability properties of the phase mean that the separation between constituents and duration of the analysis is largely dependent on the type of carrier gas, column dimensions, temperature and type of fatty acid. It will be necessary to optimize the operating conditions of temperature and flow rate according to whether one wishes to improve the separations or to make a rapid analysis. For general purposes a linear gas velocity of 40 cm per minute for hydrogen and 30 cm per minute for helium should be satisfactory (*note 6*).

5.1.2 **Determination of the efficiency and resolution**

Use correspondingly the formula given in method 2.302. The operating conditions selected (5.2) should be those that afford the number of theoretical plates for methyl stearate to be at least 2000 per metre (*note 8*) and the resolution at least 1.25.

5.2 Operating conditions

Operate the oven either isothermally at a temperature of 180–210°C, or operate by temperature-programming from 80–220°C (*note 9*).

Set the injector and detector temperatures about 30–50°C above the column temperature (*note 10*).

5.3 Analysis

Using the syringe (3.2), inject 0.1–0.2 μl of the solution of 1–5 per cent fatty acid methyl esters prepared from the selected reference standard (4.3) according to method 2.301. With on-column injection mode, dilute the solution to 0.05 per cent before injection.

Similarly inject 0.1–0.2 μl of a solution of fatty acid methyl esters prepared from the test sample in exactly the same way.

6 Calculation and expression of results

6.1 Qualitative analysis

See method 2.302 (para. 6.1).

When identifying the peaks of the sample fatty acids by reference to the peak retention times of known fatty acids, it should be realized that critical pairs or triplets of fatty acid peaks may have been formed, depending on the stationary phase (3.1.3.2) used for the analysis, e.g. $C_{20:0}$ and $C_{18:3}$ (*n*-6); $C_{22:0}$ and $C_{20:3}$ (*n*-6); $C_{20:3}$ (*n*-3); $C_{22:1}$ (*n*-9); and $C_{20:4}$ (*n*-3) (*note 11*).

6.2 **Quantitative analysis**

Use the formula given in method 2.302 (para. 6.2).

6.2.1 **Use of internal standards**

In the assay of fatty acids $C_{4:0}$ and $C_{6:0}$ fatty acids in dairy fats (or blends containing dairy fats), $C_{5:0}$ should be used as the internal standard. In cases where not all fatty acid methyl esters are eluted, fatty acid $C_{15:0}$ or $C_{17:0}$ should be used as the internal standard. The content (P_i) of component i, expressed as a percentage (m/m), is given by the formula:

$$P_i = \frac{m_s \times K'_i \times A_i}{m \times K'_s \times A_s}$$

where:

m_s is the mass, in mg, of the internal standard added to the test portion

m is the mass, in mg, of the test portion

K'_i is the correction factor for the component

K'_s is the correction factor for the internal standard

A_i is the area of the peak corresponding to the component i

A_s is the area of the peak corresponding to the internal standard.

For the calculation of the correction factors K'_i and K'_s use the formula given in method 2.302 (para. 6.2.1) (*note 12*).

7 Quality assurance

7.1 For *general principles* of analytical quality control see the section on *Quality Assurance* in the introductory part of the *Compendium of Standard Methods*.

7.2 For *specific applications* of analytical quality control see the *Annexe* to this standard method.

8 Notes

1 The precision of the method when applied to butyric and lauric oils and fats has not been determined.

2 On-column injection is preferred as it generally prevents sample injection discrimination involving losses of highly volatile short-chain fatty acid methyl esters. In this case the theoretical response factors can be used instead of the empirical calibration factors. An automatic injection system is recommended as it improves the repeatability of results.

3 Apparatus equipped with a temperature programmer is recommended.

4 For cold on-column injection, a retention gap column must be used to re-concentrate the fatty acid methyl esters at the beginning of the separation column. The retention gap

452

must be 0.5–1 m in length with an internal diameter of 0.53 mm to allow insertion of the needle into the column.

5 Carbowax, Durabond 225, FFAP, Silar 5 CP, Supelcowax, or CP Sil 88 are suitable stationary phases. The selection of a particular phase depends on the intended separation of fatty acid methyl esters. For separations of unsaturated fatty acid methyl esters, cyanosilicones are preferred to Carbowax columns. If the coating is too thin, this will increase difficulties due to active sites in the column. For this reason a coating of 0.2 μm is to be preferred.

6 Either helium or nitrogen may be suitable as a carrier gas but they will increase elution times. Hydrogen is preferred for the analysis of fatty acid methyl esters derived from fats containing short-chain fatty acids. It is also preferred for the analysis of fats with long-chain fatty acids since the elution time is some two times shorter than with helium—consequently peak broadening is less. Oxygen traces in the carrier gas may damage the column coatings and considerably reduce the life of capillary columns.

7 Standards of most known fatty acids are available from suppliers such as Nuspet: Nu-Check-Prep. Inc., PO Box 172, Elysian, Minnesota 56028 USA; for Europe: Bast of Copenhagen V, Denmark.

Otherwise, natural oils and fats of known fatty acid composition (available from the European Community of Reference (BCR), rue de la Loi 200, B-1049 Brussels, Belgium) or those with a specific fatty acid, such as blackcurrant seed oil for $C_{18:3}$ (*n*-6) (gamma-linolenic acid) and for $C_{18:4}$ (*n*-3) (stearidonic acid), can be used.

8 The separation power in terms of theoretical plate number depends on the length and inside diameter of the column. The longer the column and the thinner the coating of stationary phase, the higher is the theoretical plate number.

9 Operating the oven by temperature programming increases the speed of fatty acid elution but does not improve or reduce the resolution of the peaks. A suitable heating programme is: Start at an oven temperature of 80°C. Hold for 2 minutes, then heat up at a rate of 20°C per minute until 125°C is reached. Hold it there for 1 minute, then continue to heat up at a rate of 3°C per minute until 220°C is reached. Hold at this temperature for at least 5 minutes, until all high carbon number fatty acid methyl esters are eluted.

10 For on-column injection, set the injector and oven 10°C beneath the boiling point of the solvent and start with heating immediately after injection, e.g. 50°C can be used in the case of pentane.

11 In the abbreviated symbols $C_{4:0}$, $C_{18:2}$, the figures 4 and 18 stand for the number of carbon atoms in the fatty acid chain and the figures immediately following the colon (:) refer to the number of unsaturated bonds in the fatty acid chain; *n*-3 and *n*-6 refer to the place of the first double bond in the fatty acid chain counted from the methyl group end of the chain.

12 If a correction factor of 1.000 is taken for $C_{16:0}$, most fatty acid methyl esters will be found to have correction factors close to 1.00, except in the case of the short-chain fatty acids $C_{4:0}$, $C_{6:0}$ and $C_{8:0}$. The correction factors should correspond with the theoretical response factors within certain margins. This is especially important in the case of the analysis of short-chain fatty acids because incorrectly determined correction factors have been found to be a common factor in the obtaining of erroneous results by many laboratories.

5.1.3 Weigh 1.00 g of sample in a 10-ml test tube, together with 1.00 g of matrix modifier (4.3) and mix thoroughly (*note 4*).

5.1.4 Carry out instructions 5.1.2–5.1.3 also for the three standard working solutions (4.7) and the blank oil (4.4).

5.2 Preparation of apparatus

5.2.1 Switch on the atomic absorption spectrometer and the background correction (Deuterium *or* Zeeman).

5.2.2 In accordance with the manufacturer's instructions supplied with the spectrometer, adjust: lamp current, slit, wavelength and amplification. The required wavelength is 213.5 nm.

5.2.3 Optimize the position of the graphite furnace atomizer (3.6) in the atomic absorption spectrometer (3.5), and set the required programme on the control unit of the furnace. If available, place platform in graphite tube.

5.2.4 Pretreat before each injection, the pipettor tip (3.3) by pipetting and then discarding 20 µl of cyclohexane.

5.2.5 Inject 20 µl of the standard working solution with a content of 40 mg phosphorus/kg (4.7) with the micropipettor (3.2) into the graphite furnace, initiate the temperature programme and record the absorption.

5.2.6 Repeat instruction 5.2.5 until the absorption is constant (*note 5*).

Programme for the graphite furnace atomizer (see *note 6*):

Table 2.423.1.

Step no.	Temp. (°C)	Ramp-time (s)	Hold-time (s)	Int. Gas-flow (ml/min)
1	600	40	20	300
2	1600	50	40	300
3	2800	0	5	0
4	2800	1	3	50

5.3 Determination

5.3.1 Measurement of the graphite tube blank: record the absorption, if any, of the graphite tube as such and autozero this absorption.

5.3.2 Measurement of the blank: inject 20 µl of the blank solution prepared according to 5.1.4 into the graphite furnace, initiate the temperature programme and record the absorption.

5.3.3 Measurement of the working standards: inject 20 µl of the three standard solutions prepared according to 5.1.4 into the graphite furnace and record the absorptions.

5.3.4 Measurement of sample solutions: inject 20 µl of the sample solution prepared according to 5.1.3 into the graphite furnace, initiate the temperature programme and record the absorption.

6 Calculation and expression of results

6.1 Calculation

6.1.1 Measure the peak height on the recorder-chart or take the reading of the display or printer.

6.1.2 Draw a calibration curve by plotting the absorption of the three standards (5.3.3), corrected for the blank (5.3.2), against their respective phosphorus content (*note 3*).

6.1.3 Measure the absorption of the sample and correct for the blank.

6.1.4 Read the phosphorus content of the sample from the calibration curve.

6.2 Expression of results

Express the results as mg/kg (to two significant figures).

7 Quality assurance

7.1 For *general principles* of analytical quality control, see the section on *Quality Assurance* in the introductory part of the *Compendium of Standard Methods*.

7.2 For *specific applications* of analytical quality control, see the Annexe to this standard method.

8 Notes

1 The amount of phosphorus found depends on the types of phosphatide present in the oil. The addition of lanthanum proved to be essential to find the total amount of phosphorus. Instead of lanthanum, calcium may be used.

2 Both atomization off the wall and atomization off the platform can be used. It is not necessary to change the temperature programme for the graphite furnace.

3 The concentration of phosphorus in the blank oil and in the lecithin is determined with method 2.421.

4 If the expected or found concentration of phosphorus is higher than 40 mg/kg, dilute the sample with blank oil (4.4). In that case multiply the observed absorption (5.3.4) with the dilution factor.

5 With a new graphite tube, three to four 'determinations' with a particular working standard have to be carried out in order to obtain an acceptable 'state of equilibrium'.

6 For those with a Varian apparatus, the following temperature programme should be used:

Table 2.423.2.

Step no.	Temp. (°C)	Time (s)	Gas-flow (l/min)
1	120	20	3.0
2	120	20	3.0
3	500	30	3.0
4	500	10	3.0
5	1600	50	3.0
6	1600	20	3.0
7	1600	2	0.0
8	2800	1	0.0
9	2800	2	0.0
10	2800	5	3.0
11	40	20	3.0

Annexe

1 Repeatability limit

The absolute difference between two independent single test results, obtained with the same method on identical test material in the same laboratory by the same operator

using the same equipment within short intervals of time, should not be greater than the repeatability limit (r) as calculated from the formulae in Table 2.423.3.

Table 2.423.3. Repeatability (r) and reproducibility (R) limits.

P in edible oil	$r = 0.19m$	$R = 0.30M$

M, corresponding mean concentration value.

2 Reproducibility limit

The absolute difference between two single test results, obtained with the same method on identical test material in different laboratories with different operators using different equipment, should not be greater than the reproducibility limit (R) as calculated from the formulae in Table 2.423.3.

3 Trueness (bias)

The bias of the method was demonstrated in the collaborative study of the method (see Table 2.423.4) to be negligible when used for the determination of concentration levels of phosphorus in the range of 10–30 mg/kg.

4 Sensitivity

The sensitivity of the method is demonstrated by the low values for r and R at the low concentration levels studied (see Table 2.423.4), the limit of detection is 0.1 mg/kg and the limit of determination is 1 mg/kg.

Interference by other elements is not to be expected, provided the measurements are carried out at the wavelength specific for phosphorus (213.5 nm).

5 Statistical and other data derived from the results of the interlaboratory test

The interlaboratory test carried out at the international level in 1989 by the IUPAC Commission on Oils, Fats and Derivatives, in which 21 laboratories participated, each obtaining two test results for each sample, gave the statistical results (evaluated in accordance with *ISO 5725-1986*) summarized in Table 2.423.4.

Table 2.423.4.

Sample:	Sunflower oil		
Batch:	A (high)	B (medium)	C (low)
Number of laboratories retained after eliminating outliers	17	17	17
Number of outliers (laboratories)	2	1	1
Number of accepted results	32	34	34
Mean value (mg/kg sample)	29.55	20.00	10.18
True or accepted value (mg/kg)	28.50	19.00	9.00
Repeatability standard deviation (S_r in mg/kg)	2.30	1.68	0.91
Repeatability relative standard deviation (CV_r in %)	7.8	8.4	9.0
Repeatability limit (r) ($2.8 \times S_r$)	6.45	4.71	2.56
Reproducibility standard deviation (S_R in mg/kg)	3.51	2.33	1.24
Reproducibility relative standard deviation (CV_R in %)	11.9	11.7	12.2
Reproducibility limit (R) ($2.8 \times S_R$)	9.82	6.63	3.48

456

References

Hendrikse PW, Dieffenbacher A. *Pure Appl Chem* 1991; 63(8): 1193–1200 (Report on the Collaborative Study).

IUPAC. *Standard Methods for the Analysis of Oils, Fats and Derivatives*, 7th edn. Oxford: Blackwell Scientific Publications, 1989.

Slikkerveer FJ, Braad AA, Hendrikse PW. *At Spectr* 1980; 1: 30.

AOCS Official Method Ca 13-56
Reapproved 1997

Total Gossypol in Oils

DEFINITION
Total gossypol defines gossypol and gossypol derivatives, both free and bound, in cottonseed products that are capable of reacting with 3-amino-1-propanol in dimethylformamide solution to form a diaminopropanol complex, which then reacts with aniline to form dianilinogossypol under the conditions of the test method. Gossypol, gossypol analogs and gossypol derivatives having an available aldehyde moiety are measured by the method.

SCOPE
Applicable to crude cottonseed oil and cottonseed soap stock.

APPARATUS

1. Water bath—for operation at 95–100°C, with clamps for supporting 50- and 25-mL volumetric flasks. Metal washers may be placed over the necks of the flasks for support.
2. Spectrophotometer—for operation at 440 nm with 1-cm light path cuvettes.
3. Volumetric flasks—25, 50 and 100 mL.
4. Pipets—class A volumetric, 1, 2, 4, 5 and 10 mL.
5. Filter paper—medium retentivity, 11 cm in diameter.

REFER TO AOCS OFFICIAL METHOD BA 8-78 FOR THE FOLLOWING SECTIONS:
Reagents (including standards), Preparation of Sample, Sample Size, Procedure, Calibration, Calculations, Precision, Notes.

2.501 Determination of peroxide value* (P.V.)

Statistical evaluation of results of study carried out in 1971

	Palm oil	Rapeseed oil	Soya oil 1	Soya oil 2	Coconut oil
Number of laboratories	7	7	7	7	7
Number of results	21	21	21	21	21
Number of laboratories retained after deletion of outliers	7	7	7	7	7
Number of accepted results	21	21	21	21	21
Mean value (mEq/kg)	8.56	1.03	3.30	0.80	0.11
Repeatability standard deviation†	0.16	0.22	0.12	0.08	0.08
Repeatability relative standard deviation (%)	1.87	21.6	3.62	9.8	73.3
Reproducibility standard deviation†	0.79	0.25	0.31	0.18	0.14
Reproducibility relative standard deviation (%)	9.21	25.2	9.5	22.2	123.1
Repeatability (r)†	0.45	0.63	0.34	0.22	0.24
Reproducibility (R)†	2.23	0.73	0.89	0.50	0.40

† Expressed as mEq/kg.

* *Note:* Method 2.501 requires the use of chloroform, which is now discouraged in analytical laboratories. Recent collaborative studies organized by the AOCS in which the peroxide values of lard, groundnut, maize oil, linseed oil, rapeseed oil, soybean oil and sunflower seed oil were determined using both chloroform and iso-octane have demonstrated that 'there is good one-to-one correlation between the results for the two solvents'. Subsequently the AOCS published (1990) its method Cd 8b-90 which uses iso-octane in place of chloroform. However, the precision of the method is not stated and it should be noted that the procedure is not technically equivalent to that outlined in the IUPAC method 2.501. A Caution note in the text of the AOCS method advises that iso-octane is toxic by ingestion and inhalation.

2.504 Determination of the *p*-anisidine value (*p*-A.V.)

1 **Scope**

This Standard describes a method for the determination of the amount of aldehydes (principally 2-alkenals) in the oils and fats.

2 **Field of application**

This Standard is applicable to animal and vegetable oils and fats.

3 **Definition**

The *p*-anisidine value is defined by convention as 100 times the optical density measured in a 1-cm cell of a solution containing 1.00 g of the oil in 100 ml of a mixture of solvent and reagent according to the method described.

4 **Principle**

Reaction in an acetic solution of the aldehydic compounds in an oil and the *p*-anisidine (*note 1*), and then determination of the absorbance at 350 nm.

5 **Apparatus**

5.1 10-ml minimum test tubes, with ground-glass stoppers.

5.2 25-ml graduated flasks.

5.3 Automatic pipette or automatic burette.

5.4 Spectrophotometer suitable for observation at 350 nm.

5.5 1.00 (±0.01)-cm glass cells, the two cells of each pair must be identical.

6 **Reagents**

6.1 *Iso*-octane (2,2,4-trimethylpentane), optically clear (*note 2*).

6.2 Glacial acetic acid, analytical quality (*note 3*).

6.3 *p*-Anisidine, analytical reagent quality (*note 4*) 2.5 g/l solution in glacial acetic acid (6.2) (*note 5*).

7 **Procedure**

N.B. The sample should be perfectly clear and dry (*note 3*).

Weigh 0.5–4.0 g of the sample, to the nearest 0.001 g, into a volumetric flask (5.2). Dissolve and dilute to volume with *iso*-octane (6.1).

Measure the absorbance (A_b) of the solution at 350 nm in a cell (5.5) with the spectrophotometer (5.4), using the reference cell filled with solvent as a blank.

Pipette exactly 5 ml of the fat solution into one test tube (5.1) and exactly 5 ml of the solvent into a second test tube. By means of an automatic pipette (5.3) add exactly 1 ml of the *p*-anisidine reagent (6.3) to each tube, and shake.

After exactly 10 minutes measure the absorbance (A_s) of the solution in the first test tube in a cell (5.5) at 350 nm, using the solution from the second test tube as a blank in the reference cell.

8 **Expression of results**

The *p*-anisidine value (*p*-A.V.) is given by the formula:

$$p\text{-A.V.} = \frac{25 \times (1.2A_s - A_b)}{m}$$

460

where A_s is the absorbance of the fat solution after reaction with the *p*-anisidine reagent (6.3), A_b is the absorbance of the solution of the fat, m is the mass, in g, of the test portion.

9 **Notes**

1 In the presence of acetic acid, *p*-anisidine reacts with aldehydic compounds in oils or fats. The intensity of colour of the yellowish reaction products formed depends not only on the amount of aldehydic compounds present but also on their structure. It has been found that a double bond in the carbon chain conjugated with the carbonyl double bond increases the molar absorbance four or five times. This means that 2-alkenals, especially, will contribute substantially to the value found.

2 In most cases *n*-hexane can be substituted for *iso*-octane as solvent. However, oils containing high amounts of oxidized fatty acids will not dissolve completely in *n*-hexane. For such oils *iso*-octane should be used as the solvent.

The absorbance of the solvent used (*iso*-octane or *n*-hexane), measured in a 1.00-cm cell between 300 and 380 nm, must be nil or nearly nil. The commercial product can be freed from absorptive material by percolating it through a glass column (3–5 cm internal diameter, and 100 cm long) filled with silica gel.

3 The reaction between *p*-anisidine and aldehydes involves the formation of water.

Hence, the presence of moisture in any of the reagents or in the sample leads to incomplete reaction and, consequently, low values.

Since glacial acetic acid is highly hygroscopic, it is essential to check its moisture content by a Karl Fischer determination. If the content exceeds 0.1 per cent, the acetic acid must be discarded.

4 In storage, *p*-anisidine tends to darken as a result of oxidation. A discoloured reagent can be reduced and decolorized in the following way:

Dissolve 40 g of *p*-anisidine in 1 l of water at 75°C. Add 2 g of sodium sulphite and 20 g of active carbon and stir for 5 minutes. Then filter through a double filter paper. If carbon passes through, repeat the filtration.

Cool the filtered solution to about 0°C. Allow to stand at this temperature for at least 4 hours, or, preferably, overnight. Filter off the crystallized *p*-anisidine and wash with a small amount of water at a temperature of about 0°C.

After drying in a vacuum desiccator, transfer the crystals into a brown glass bottle. If stored in the dark and at low temperature, the crystals obtained should not darken appreciably during 1 year.

5 Reagent solutions having an absorbance greater than 0.200 when measured in a 1.00-cm cell at 350 nm against *iso*-octane or *n*-hexane as a blank should be discarded.

2.506 Determination of stability (the modified 'Swift test')

1 **Scope**

This Standard describes a method giving an indication of the stability to oxidation of oils and fats during storage.

The test measures the time in hours required for a sample of oil or fat aerated under carefully controlled conditions (at 98.0±0.2°C) to reach a peroxide value of 100 (in milliequivalents of active oxygen per kg of fat).

This figure for the time is regarded as an index of the resistance of the fat to autoxidation.

2 **Field of application**

This Standard is applicable to all oils and fats of low acidity, of animal or vegetable origin, whether or not they contain antioxidants. It is not applicable to free fatty acids.

3 **Principle**

Bubbling of air at a controlled flow in the fat or oil maintained at 98.0±0.2°C, then determination of the peroxide value at carefully determined times. Estimation of the time, in hours, for the obtainment of a peroxide value of 100.

4 **Apparatus**

4.1 Bath or heating apparatus capable of maintaining a constant temperature of 98.0±0.2°C. This must be controlled by a daily check of the temperature of a sample of oil or fat in a test tube, placed successively in the various holes in the lid under the conditions of test (air-bubbling). During the determination, the temperature must be measured in a test tube containing the prescribed amount of a mineral oil which is stable under the test conditions.

4.2 Air-distributing manifold constructed of stainless steel, nickel, aluminium or glass. The capillaries must be calibrated to permit the same flow (to within ±10 per cent) through each outlet when the total flow is adjusted to 2.33 ml per tube per second.

4.3 Air-purification train (see Fig. 2.506.1) comprising:

(A) Air inlet tube from a source of compressed air, equipped with a stainless steel needle valve.

(B) Cylindrical wash bottle, 375 mm tall and 50 mm external diameter, containing distilled water.

(C) Cylindrical wash bottle of the same size as (B), filled to a depth of about 25 cm with a solution of 2 per cent of potassium dichromate in 1 per cent sulphuric acid. The solution must be replaced after 72 hours of continuous use.

(D) Cylindrical wash bottle of the same size as (B), filled to a depth of about 25 cm with distilled water. This must be replaced with fresh distilled water as soon as any yellow tint appears.

(E) Water-cooled Allihn condenser with five bulbs, 300-mm jacket.

(F) Trap comprising a wide-mouthed 500-ml flask, filled with glass wool.

(G and H) Pressure regulating bottles, comprising bottles of the same size as (B) and containing distilled water. The pressure may also be regulated by means of a pressure-regulating valve.

(J) Air-distributing manifold (4.2).

4.4 Source of low pressure, clean and oil-free compressed air. An individual air compressor of the pistonless, diaphragm type is preferable to the use of industrial compressed air.

4.5 Thermometer, capable of measuring temperature to within 0.1°C between 95 and 105°C.

4.6 Gas flow meter, preferably of the calibrated cone type.

462

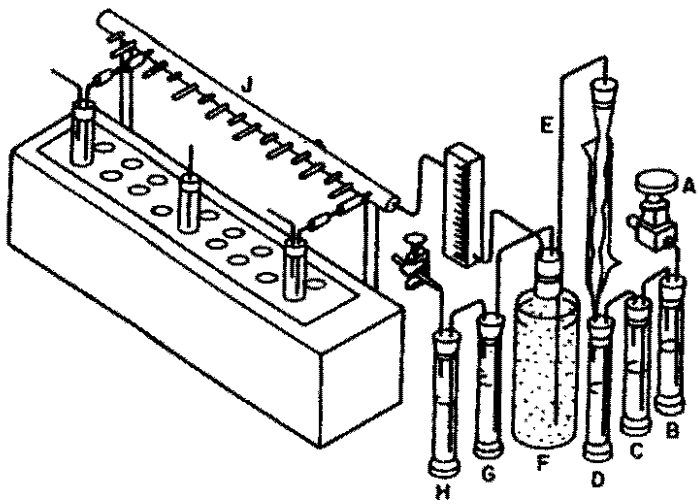


Fig. 2.506.1. Air-purification train.

4.7 25×200-mm test tubes of Pyrex glass. It is advantageous to calibrate these with a mark at the 20-ml level. Each tube is fitted with a neoprene stopper with two holes and an aeration tube (see Fig. 2.506.2). The tube used for temperature control must, additionally, be able to take the thermometer (4.5).

4.8 Oven, regulated at 103±2°C.

4.9 Equipment for the determination of the peroxide value (see 2.501).

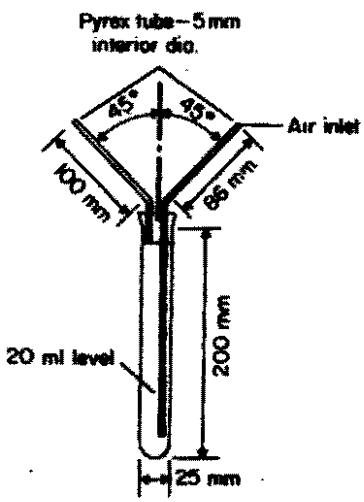


Fig. 2.506.2.

5 Reagents

5.1 Light petroleum, analytical reagent quality, b.p. 40–60°C.

5.2 Acetone, analytical reagent quality.

5.3 Detergent suitable for cleaning the apparatus, which must not leave any contaminating residues.

5.4 Reagents for the determination of the peroxide value (see 2.501).

6 Procedure

6.1 Cleaning of the test tubes and aeration tubes

It is essential that the test tubes and the aeration tubes (4.7) should be most thoroughly cleaned and free from any traces of organic and inorganic matter (especially traces of metals).

After cleaning with light petroleum (5.1) and acetone (5.2), it is advisable to wash all the tubes with near boiling detergent solution (5.3), and to rinse very thoroughly.

Dry in an oven (4.8) at 103 ± 2°C, and store in a dust-free location.

6.2 Determination

Prepare the sample according to 2.001, 'Preparation of the sample'.

Introduce 20 ml of the prepared sample into each of two perfectly clean test tubes (4.7). Do this in such a way that none of the fat comes into contact with the upper part of the tube and consequently the stopper.

Fit the aeration tube and adjust it so that the tip of the air-delivery tube is 5 cm below the surface of the test portion.

Place the assembled tube containing the test portion in a vessel containing boiling water for 5 minutes. Remove the tube, wipe dry and place immediately in the constant-temperature heater (4.1) maintained at 98.0°C. Having previously adjusted the air-flow rate, connect the aeration tube to the capillary on the manifold (4.2) and record the starting time.

A short time before the expected end-point is reached (*note 1*), determine the peroxide value according to 2.501, using a test portion of 0.3 g.

If the peroxide value so determined is above 175 m.e.q., the test portion must be rejected, and another determination started (*note 2*).

If the peroxide value obtained is below 75 m.e.q., estimate the time at which a figure of 75 m.e.q. would be expected, and at that time do a peroxide determination on a fresh test portion of 0.3 g. This will afford the first figure to be recorded.

Determine the peroxide value again on another test portion of 0.3 g taken from the same test tube exactly 1 hour after the first one. This affords the second value to be recorded. The two figures so obtained for the peroxide value must fall between 75 and 175 m.e.q.

7 Expression of results

Taking the reaction times as abscissae, plot the two values of the peroxide value as ordinates on 2-coordinate graph paper.

Join the two points by a straight line. The stability value is then the time (in hours) at which this line cuts the ordinate for 100.

Repeat the whole operation with the second test portion and calculate the average of the results in hours (to the nearest whole number).

8 Notes

1 After some experience, the approach of the end-point can be estimated from the odour of the effluent air from the tubes.

2 It is desirable to heat and aerate the test portions continuously until the end-point. When this is not practicable, remove the tube from the heating bath, chill it immediately, and store it at below 10°C, until the test tube is carried on again, starting as from 6.2, 'Place the assembled tube containing the test portion in a vessel containing boiling water for 5 minutes . . .'.

464

2.507 Determination of polar compounds in frying fats

1 **Scope**

This Standard describes a method for the determination of polar compounds in frying fats. Polar compounds are formed during the heating of fats.

2 **Field of application**

This Standard is applicable to animal and vegetable oils and fats. The method serves to assess the deterioration of used frying fats.

3 **Definition**

Polar compounds are those compounds in fats which are determined by column chromatography under the conditions specified (*note 1*).

4 **Principle**

Separation of frying fats by column chromatography into non-polar and polar compounds. Elution of the non-polar compounds. Determination of polar compounds by calculating the difference between weight of the sample added to the column and that of the non-polar fraction eluted.

5 **Apparatus**

5.1 250- and 500-ml round bottom flasks with ground necks.

5.2 100-ml beakers.

5.3 Ground-glass stoppers to fit the 500-ml flasks (5.1).

5.4 Chromatographic glass column, 21 mm internal diameter, 450 mm in length, with stopcock (preferably in polytetrafluorethylene), and ground-glass joint.

5.5 250-ml dropping funnel, with ground-glass joint to fit the column (5.4).

5.6 Glass funnel, about 8 cm in diameter.

5.7 Glass rod, about 60 cm in length.

5.8 50-ml volumetric flask.

5.9 20-ml volumetric pipette.

5.10 2-μl capillary pipettes for thin-layer chromatography.

5.11 Glass plates for thin-layer chromatography, 20 × 20 cm, coated with silica gel (without fluorescence indicator), 0.25 mm layer thickness.

5.12 Glass developing tank for thin-layer chromatography, with ground-glass lid.

5.13 Spray for thin-layer chromatography.

5.14 Porcelain dish, about 20 cm in diameter.

5.15 Oven regulated at 103 ± 2°C.

5.16 Drying oven controllable between 120 and 160°C.

5.17 Water bath.

5.18 Desiccator, containing a suitable desiccant such as silica gel with moisture indicator (blue gel).

5.19 Apparatus for removing solvent under vacuum, e.g. rotary evaporator.

5.20 Shaking machine.

6 **Reagents**

6.1 Light petroleum (b.p. 40–60°C), chromatographic quality, redistilled.

6.2 Ethanol, 95 per cent (V/V).

6.3 Chloroform, pure.

465

6.4 Diethyl ether, free from peroxides and residue.

6.5 Acetic acid, 100 per cent, analytical reagent quality.

6.6 Elution solvent: mixture of light petroleum (6.1) and diethyl ether (6.4), 87/13, (V/V).

6.7 Developing solvent: mixture of light petroleum (6.1), diethylether (6.4) and acetic acid (6.5), 70/30/2, (V/V/V).

6.8 Silica gel, particle size 0.063–0.200 mm (70–230 mesh), Merck N° 7734 or equivalent, adjusted to a water content of 5 per cent (m/m) (*note 2*).

6.9 Phosphomolybdic acid, analytical reagent quality, 100 g/l solution in ethanol (6.2).

6.10 Sea sand, purified by acid calcined.

6.11 Cotton wool, surgical quality.

6.12 Nitrogen, 99.0–99.8 per cent.

7 Procedure

7.1 Preparation of the sample

Remove visible impurities by filtration after homogenization. If water is present, use a hydrophobic filter paper.

For semi-liquid and solid samples, warm to a temperature slightly above the melting point and homogenize carefully, avoid overheating.

7.2 Preparation of the column

Fill the column (5.4) with about 30 ml of the elution solvent (6.6). Introduce a wad of cotton wool (6.11) into the lower part of the column with the aid of a glass rod (5.7) and remove air by pressing the wool.

Prepare in a beaker (5.2) a slurry of 25 g silica gel (6.8) in about 80 ml of the elution solvent and pour this slurry into the column with the aid of the funnel (5.6). To ensure complete transfer of the silica gel into the column, rinse with the elution solvent.

Open the stopcock and drain off the elution solvent into a second beaker (5.2) until the level of the elution solvent is 10 cm above the silica gel. Level the silica gel by tapping against the column.

Add about 4 g of sea sand (6.10) with the aid of the funnel. Drain off the supernatant elution solvent as far as the sand layer (*note 3*).

7.3 Column chromatography

Note. For the determination of polar compounds, the non-polar fraction is used only. However, if the efficiency of the fractionation is assessed by thin-layer chromatography or by recovery of the sample, the polar and non-polar fractions are required.

Weigh, to the nearest 0.001 g, 2.5±0.1 g of the sample prepared as (7.1) into a volumetric flask (5.8). Dissolve in about 20 ml of the elution solvent (6.6) while warming slightly. Allow to cool to room temperature, and fill up to the mark with the elution solvent.

Introduce with a volumetric pipette (5.9) 20 ml of this solution onto the column prepared as (7.2). Avoid disturbing of the surface (*note 4*).

Dry two 250 ml flasks (5.1) in the oven (5.15) at a temperature of 103±2°C. Allow to cool to room temperature and weigh accurately to within 0.001 g. Place one of them under the outlet of the column.

Open the stopcock and let the sample solution drain off to the level of the sand layer.

Elute non-polar compounds with 150 ml of the elution solvent (6.6) using a dropping funnel

(5.5). Adjust the flow rate so that 150 ml passes through the column within 60–70 min.
After completion of the elution, wash any substance adhering to the outlet of the column into the flask with the elution solvent using a pipette (5.9).
If the polar compounds are required, elute them into a second 250 ml dry flask with 150 ml diethyl ether (6.4) as described before.
After completing the elution, the silica gel is discarded.
Remove the solvent from the flask(s) with the aid of a rotary evaporator (5.19) using a water bath (5.17) at a temperature no higher than 60°C. Avoid losses due to foaming (*note 5*).
Shortly before the end of the distillation, introduce nitrogen (6.12) into the system. Weigh the flask(s).

8 **Expression of results**

The content of polar compounds, in per cent (m/m), is given by the formula:

$$\frac{m-m_1}{m} \times 100$$

where: m_1 is the mass, in g, of the non-polar fraction, m is the mass, in g, of the sample contained in 20 ml of the solution added to the column.

9 **Thin-layer chromatographic assessment of column efficiency**

The efficiency of the fractionation can be assessed by thin-layer chromatography (*note 6*).
For the thin-layer chromatographic investigation prepare 10 per cent solutions of the substances in chloroform (6.3), and apply 2 μl spots onto a plate (5.11) using a capillary pipette (5.10).
Line the developing tank (5.12) with filter paper to achieve saturation. Place the plate in the developing tank and carry out the development with the developing solvent (6.7). Normally, after 35 min, the solvent front ascends to a height of about 17 cm. Remove the plate and allow the solvent to evaporate.
Spray the plate with the phosphomolybdic acid solution (6.9). After evaporation of ethanol, heat the plate in the drying oven (5.16) at 120–130°C. As an example see Fig. 2.507.1 showing a chromatogram obtained after fractionation of a frying fat into individual fractions.

10 **Notes**

1 The polar compounds include polar substances such as monoglycerides, diglycerides, free fatty acids which occur in unused fats as well as polar transformation products formed during frying of foodstuffs and/or during heating. Non-polar compounds are mostly unaltered triglycerides.

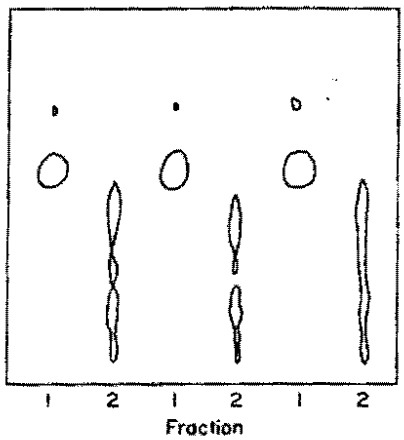
2 Place the silica gel in the porcelain dish (5.14), dry in an oven (5.16) at 160°C for at least 4 h and cool in a desiccator (5.18) to room temperature. Adjust the silica gel to a water content of 5 per cent: e.g. weigh 152 g of silica gel and 8 g of water in a 500 ml flask (5.1). Close the flask with a stopper (5.3) and shake mechanically with the aid of the shaking machine (5.20).

3 The excess solvent mixture drained off should not be used for elution.

4 For fats containing low amounts of polar components the amount of sample added to the column can be raised from 1 up to 2 g.

467

5 If a rotary evaporator is not available the elution solvent can be evaporated on a steam plate under a stream of nitrogen.
6 The efficiency of the fractionation can also be assessed by checking the recovery of the sample. For samples containing greater amounts of polar material, recovery of the sample may be incomplete. This is due to small amounts of highly polar material, generally not more than 1–2 per cent, which is not eluted under the conditions specified.



1: Non-polar compounds
2: Polar compounds

Fig. 2.507.1.

Warning

Chloroform

Even at moderate concentrations chloroform vapour tends to produce gradual fatigue and headaches.
Following exposure to excessive concentrations or repeated exposure to lower concentrations jaundice may ensue after an interval. Continued inhalation of sub-narcotic doses may lead to chronic deterioration of organs.
The strong degreasing action of liquid chloroform on the skin produces burns and cracking.

468

2.508 Determination of polymerized triglycerides in oils and fats by high performance liquid chromatography

1 **Scope**

This Standard describes a method to determine the polymerized triglycerides content in oils and fats which contain not less than 3 per cent (m/m) of these polymers.

2 **Field of application**

This method is applicable to any kind of fats and oils, heated or not. It can also be applied to the determination of polymers in animal-feed fats.

3 **Principle**

Dissolution of the sample in a suitable solvent and separation of the polymerized triglycerides by gel permeation chromatography according to molecular size.

4 **Apparatus**

4.1 Solvent reservoir of about 250 ml of capacity with a mobile-phase liner filter (pore size 1 μm).

4.2 HPLC pump, pulseless, with a flow of 0.7–1.5 ml/min.

4.3 Injection valve with a 10-μl loop and a suitable syringe with a volume of 50–100 μl (Rheodyne or Valco).

4.4 Stainless steel column: 300 mm in length 7.7 mm internal diameter, packed with a high performance spherical gel made of styrene–divinylbenzene co-polymer; diameter of the particles: 5 μm; pore size: 100 Å (0.1 nm) of the equivalent in terms of exclusion power and resolution.

The storage of such column must be achieved in toluene.

4.5 Detector: refractive index detector with a sensitivity at full scale at least 1×10^{-4} of refractive index.

4.6 Recorder and/or integrator: to allow display and accurate quantification of the peak areas.

5 **Reagents**

5.1 Tetrahydrofuran, analytical grade.

5.2 Toluene, analytical grade.

6 **Procedure**

6.1 Starting up HPLC equipment

It is advisable to follow carefully the manufacturer's recommendations. Switch on the system and pump tetrahydrofuran at a rate of 1 ml/min to purge the whole system up to the injection valve. Connect the column to the injection valve and wash it with about 30 ml of tetrahydrofuran. Connect the column to the sample cell of the detector. Fill the reference cell with the tetrahydrofuran. Adjust the mobile phase flow to 0.8–1.0 ml/min. Wait until a convenient stabilization of the system (no appreciable deviation of the baseline) is obtained (*note 1*).

6.2 Preparation of the samples and analysis

As the samples may contain suspended particles, resulting in the blockage of the porous fritted filter at the top of the column, it is advisable to filter them before injection. Filtration can be quickly and effectively realized with a 1-μm pore size filter (*note 2*).

The samples must be anhydrous. If not, they must first be dried (*note 3*).

Weigh about 50 mg of fat and add 1 ml of tetrahydrofuran. Homogenize. Take with the

469

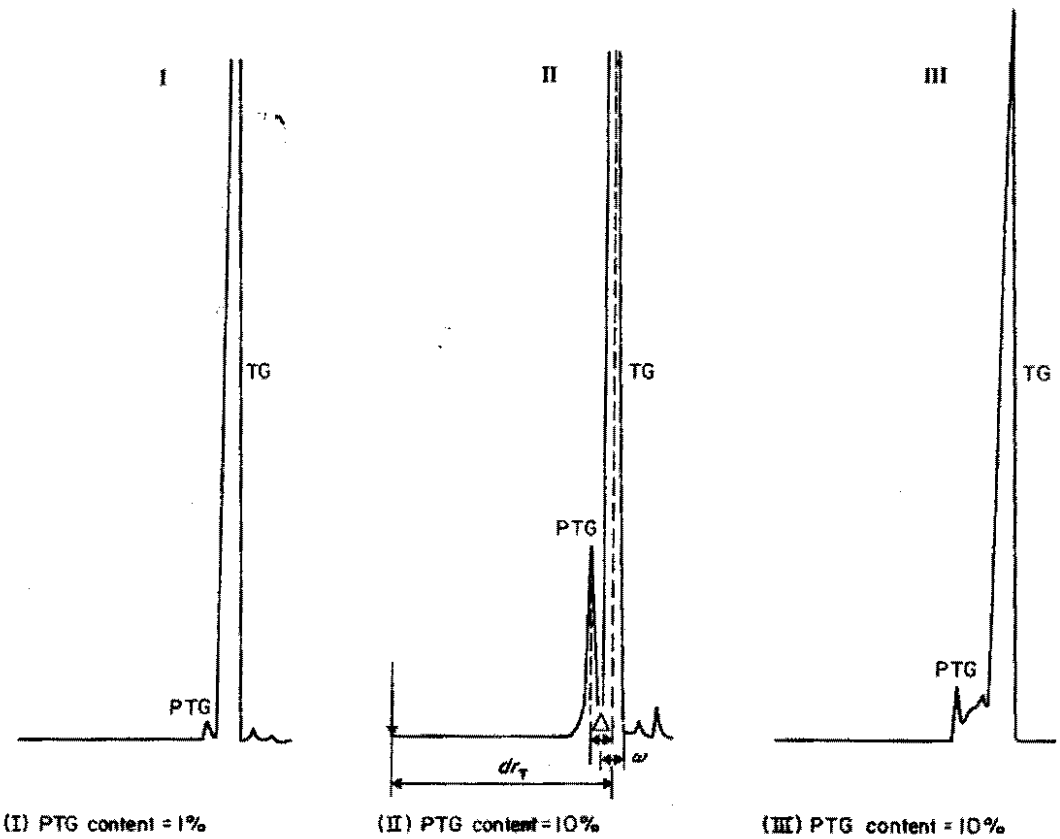


Fig. 2.508.1. HPLC of triglycerides (TG) and polymerized triglycerides (PTG).

syringe 50–100 µl of that solution. Fill the injection loop. Inject and switch on the integrator.
With a mobile phase flow of 1 ml/min, the analysis time is about 10 minutes.

7 Results

7.1 Qualitative analysis

The chromatographic pattern of the determination may show a main peak representative of monomeric triglycerides (mol. wt. about 900) and one or several smaller peaks with a shorter pattern retention distance than triglycerides, representative of polymerized triglycerides (dimers and upper oligomers).
Reference should be made to the three typical chromatograms presented in Fig. 2.508.1. Under suitable conditions, triglycerides and polymerized triglycerides can be separated with good resolution (I and II) even at low levels of polymerized triglycerides (I). However, in some cases, which seem to be connected to the complex degradation phenomena (hydrolysis), the peak pattern preceding the triglycerides peaks may be less clear (III), with consequent difficulties in calculation.

7.2 Quantitative analysis

The calculation is achieved by the internal standard method, assuming that all components of the sample are eluted.

The polymerized triglycerides content can be calculated using the formula:

$$\%PTG = \frac{A_{PTG}}{\Sigma A} \times 100$$

where:
%PTG is the percentage of polymerized triglycerides
A_{PTG} is the sum of areas of the polymerized triglycerides peaks
ΣA is the sum of areas of all peaks.
For calculating A_{PTG}, two cases are possible:
(a) *Good resolution between peaks* ($R \geqslant 1$) (similar to I and II). The general methods of integration (manual and electronic) can be used to calculate individual and total areas.
(b) *Poor resolution between peaks* ($R < 1$) (similar to III).
It is assumed that all components eluted before dr_T (see below) are polymerized triglycerides.
The resolution is calculated from:

$$R = \frac{\Delta}{\omega}$$

where:
Δ is the distance, in mm, between the peak maxima of the non-polymerized triglycerides peak (TG) and the adjacent polymerized triglycerides peak
ω is the width, in mm, of the triglycerides peak at the baseline, measured between the points of the intersection between tangents and baseline
dr_T is the retention distance, in mm, from the beginning of the chromatogram to the peak maximum for triglycerides.
Using electronic integration, the integrator has to be carefully adjusted (backward horizontal integration) to integrate all the surfaces included between the curve and the baseline. If a manual technique is used, it is necessary to determine the triglycerides peak area by triangulation.
Express the results to one decimal place.

8 Quality assurance

8.1 For *general principles* of analytical quality control, see the section on *Quality Assurance* in the introductory part of the *Compendium of Standard Methods*.

8.2 For *specific applications* of analytical quality control, see the Annexe to this standard method.

9 Notes

1 If the composition of the column is as indicated, an acceptable stabilization of the system should be obtained in about 15 minutes. With other column packings, the stabilization of the system may be more difficult: for example, changing the mobile phase should be done stepwise from toluene to tetrahydrofuran, with different mixtures, each time 25 per cent higher in tetrahydrofuran. Acceptable stabilization is normally obtained in about 12 hours.

2 The sample can be filtered with a 1-μm pore size filter made of polytetrafluoroethylene or cellulose esters. Syringe disposable filter units are available from manufacturers of HPLC equipment.

3 Samples that may contain water must first be dried: dissolve 50–100 mg of the sample in 1 ml of tetrahydrofuran. Add 50 mg of anhydrous sodium sulphate, shake, and leave for about 2 minutes. Filter upper layer through a 1-μm pore size filter.

Annexe

1 Repeatability

When the mean of the values obtained from two single determinations carried out in rapid succession by the same operator, using the same apparatus under the same conditions for the analysis of the same laboratory sample, lies within the range of the mean values cited in the table below, the difference between the two values obtained should not be greater than the repeatability limit (r), which can generally be deduced by linear interpolation from the values in the table below.

2 Reproducibility

When the values for the final result, obtained by operators in different laboratories using different apparatus under the same conditions for the analysis of the same laboratory sample, lie within the range of the mean values cited in the table below, the difference between the values for the final result obtained by those operators should not be greater than the reproducibility limit (R), which can generally be deduced by linear interpolation from the values in the table below.

3 Results of the interlaboratory tests

Two interlaboratory tests carried out at an international level in 1986–87 by the IUPAC Commission on Oils, Fats and Derivatives, in which 10 and 17 laboratories participated, each obtaining two test results for each sample, gave the statistical results (evaluated in accordance with *ISO 5725-1986*) summarized in Table 2.508.1.

Table 2.508.1.

	Sample							
	A	B	C	D	E	F	G	H
Number of laboratories	17	10	10	10	17	16	10	10
Number of laboratories retained after eliminating outliers	15	9	10	16	10	16	15	10
Number of accepted results	30	18	20	32	20	32	30	20
Mean value (percentage of total triglycerides)	1.8	2.4	3.6	5.2	7.2	9.7	10.0	22.0
Repeatability standard deviation (S_r)*	0.1	0.07	0.12	0.1	0.37	0.3	0.2	0.18
Repeatability relative standard deviation†	0.1	2.8	3.3	2.3	5.2	3.2	2.2	0.8
Repeatability limit (r)* ($2.8 \times S_r$)	0.3	0.19	0.34	0.3	1.06	0.9	0.6	0.51
Reproducibility standard deviation (S_R)*	0.4	0.61	0.45	0.3	2.14	0.4	1.2	1.48
Reproducibility relative standard deviation†	23.7	25.9	12.5	6.7	29.8	4.6	12.2	6.8
Reproducibility limit (R)* ($2.8 \times S_R$)	1.2	1.7	1.25	1.0	6.05	1.3	3.5	4.2

* Expressed as percentage of total triglycerides; † coefficient of variation.

472

2.603 Determination of small quantities of water (modified Karl Fischer method)

1 **Scope**

This Standard describes a method for the determination of small quantities of water in oils and fats by a modified Karl Fischer method.

2 **Field of application**

This Standard is applicable to animal and vegetable oils and fats when the amount of water present is so small that the methods 2.601 and 2.602 are not sufficiently precise.

3 **Principle**

Reaction between water, iodine and sulphur dioxide in the presence of an alcoholic hydroxyl group and pyridine:

$$H_2O+I_2+SO_2+3C_5H_5N+ROH \rightarrow 3C_5H_6N^++2I^-+RSO_4^-.$$

4 **Apparatus**

4.1 150-ml conical flasks.

4.2 1-l brown glass bottles, with ground-glass stoppers.

4.3 100-ml graduated measuring cylinders, with ground-glass stoppers.

4.4 25-ml automatic burette, protected from atmosphere moisture by a suitable dehydrant.

4.5 25-ml graduated pipette.

4.6 Rubber stoppers, without holes, to fit the flasks (4.1).

4.7 Rubber stoppers, to fit the flasks (4.1): pierce one hole in each stopper in such a manner that the delivery tip of the burette (4.4) can be inserted to reach to below the stopper; a desiccant is fitted in a second hole to dry any entering air.

4.8 Rubber bulb to fit the pipette (4.5).

5 **Reagents**

5.1 Methanol, anhydrous.

5.2 Chloroform, pure.

5.3 Pyridine, anhydrous.

5.4 Sulphur dioxide, anhydrous, liquid.

5.5 Iodine, pure.

5.6 Solvent mixture: mixture of chloroform (5.2) and methanol (5.1), 3/1 (V/V), stored in a bottle (4.2).

5.7 *Solution A*: dissolve 90 g of iodine (5.5) in methanol (5.1); make up to 1 l with methanol; store the solution in a bottle (4.2) at a temperature not exceeding 4°C.

5.8 *Solution B (note 1)*: mix 450 ml of pyridine (5.3) and 450 ml of methanol (5.1) in a bottle (4.2); cool to about 4°C and, with the aid of a glass bubbling-tube, dissolve in it, a little at a time, a total of 90 g of sulphur dioxide (5.4). Protect the flask from the entry of atmospheric moisture during the operation and as far as possible maintain it at below 4°C by arresting the entry of the gas whenever the temperature shows a tendency to rise.

As soon as the addition of the sulphur dioxide is complete remove the bubbler, stopper the flask, mix well, and keep in a refrigerator at about 4°C.

5.9 *Solution C* (reagent): immediately before use pour off the desired volume of solution A into a graduated measuring cyclinder (4.3) and stopper it.

Pour into another graduated measuring cylinder (4.3) an equal solution of solution B. Stopper it.

473

Place both cylinders in a bath of melting ice for about 15 minutes. With the aid of filter paper, dry the stoppers and upper parts of the measuring cylinders so as to eliminate all traces of water which may have condensed thereon during their stay in the ice bath. In one operation pour solution A into solution B and immediately stopper this measuring cylinder B. Shake well.
Allow to reach room temperature.

6 Procedure

Throughout the determination great care must be taken to avoid the entry of atmospheric moisture.

6.1 Calibration of the reagent (5.9)

With the aid of a rubber bulb (4.8) pipette out exactly 25 ml of the solvent mixture (5.6) into a flask (4.1). Fit a stopper (4.7) and titrate whilst stirring (a magnetic stirrer is recommended) with the solution C (5.9) until the colour changes from yellow to orange-red.
In another flask (4.1), accurately tared to within 0.0001 g, put a drop of water (*note 2*). Note, in g, to within 0.0001 g, the rise of mass. Immediately pipette exactly 25 ml of the solvent mixture (5.6). Titrate whilst shaking as described above.
The correspondence A, in g of water by ml of reagent C, is given by the formula:

$$A = \frac{m_0}{V_1 - V_0}$$

where V_0 is the number of ml of the solution C used with the solvent alone, V_1 is the number of ml of the solution C used with the solvent and the water added, m_0 is the mass, in g, of the water added.

6.2 Determination of the water content of fats

Into a flask (4.1), tared to within 0.005 g, weigh accurately, to within 0.005 g, 5 to 20 g of fat. With the aid of the rubber bulk (4.8) and the pipette (4.5) pipette into the flask exactly 25 ml of the solvent mixture (5.6). Dissolve by shaking and titrate with the solution C (5.9) while shaking continuously as described in (6.1).

7 Expression of results

The percentage (m/m) of water present in the fat is given by the formula:

$$\frac{100 \times (V - V_0) \times A}{m}$$

where V is the number of ml of the solution C used in (6.2), V_0 is the number of ml of the solution C used in (6.1) for the titration of 25 ml of solvent alone, m is the mass, in g, of the test portion.

8 Notes

1 The preparation of solution B is rather difficult because the sulphur dioxide gas has to be dissolved in the liquid. Operators are advised therefore to employ commercial preparations of A and B when these are separately available: these must be stored in a refrigerator at about 4°C. The mixture, solution C, should be prepared as indicated above immediately before use.

474

A single reagent solution, with a somewhat different composition, is also available commercially and this may be utilized instead of the mixed reagents.

2 For the calibration of the reagent a hydrated crystalline salt (sodium tartrate, sodium acetate) may also be used instead of a drop of water.

Warning

Chloroform

Even at moderate concentrations chloroform vapour tends to produce gradual fatigue and headaches.

Following exposure to excessive concentrations or repeated exposures to lower concentrations jaundice may ensue after an interval. Continued inhalation of sub-narcotic doses may lead to chronic deterioration of organs.

The strong degreasing action of liquid chloroform on the skin produces burns and cracking.

Pyridine

The danger from crude pyridine is greater than that from pure, the associated homologues and impurities being generally more toxic than pyridine itself. Odour and irritation furnish clear warning of vapour concentrations likely to be dangerous.

475

2.631 Determination of copper, iron and nickel by direct graphite furnace atomic absorption spectrometry

1 **Scope and field of application**

This Standard describes a method for the determination of trace amounts (μg/kg) of copper, iron and nickel in all types of crude or refined oils and fats, including contaminants from bleaching earth and/or catalysts.

2 **Principle**

Vaporization of the oil/fat in a suitable graphite furnace connected to an atomic absorption spectrometer, previously calibrated using standard solutions of organocompounds of copper, iron and nickel. Measurement of the metal content from the observed absorptions at selected wavelengths. The elements are analysed sequentially.

3 **Apparatus**

3.1 Polyethylene or polypropylene-capped bottles, 20 and 50 ml, metal-free (*note 1*).

3.2 Micropipettors, 20 and 50 μl.

3.3 Pipettor tips.

3.4 Atomic absorption spectrometer, equipped with either 'peak height' mode and printer, or 'continuous' mode and pen-recorder (full-scale response in 0.2 seconds), together with appropriate hollow cathode tubes and deuterium background corrector. The spectrometer should be located in a dust-free atmosphere.

3.5 Graphite furnace atomizer (equipped with facilities).

3.6 Graphite tubes for use in a furnace atomizer (3.5).

3.7 Chromatographic columns (diameter/height ratio 1/10) (*note 2*).

3.8 Electric oven, regulated at 60 ± 2°C and suitable for heating at 150°C.

3.9 Water bath.

4 **Reagents**

4.1 *n*-Heptane, analytical grade.

4.2 Light petroleum, b.p. 40–60°C, analytical grade.

4.3 Sunflower oil, refined, or a similar, stable liquid oil with a low metal content (*note 2*).

4.4 Aluminium oxide, chromatographic grade (*note 3*).

4.5 Nitric acid, 2 M, free from traces of iron, nickel and copper.

4.6 Niobium nitrate ($Nb(NO_3)_5$), aqueous solution 1000 mg/l (*note 4*).

4.7 Standard stock solutions: Fe 10 mg/kg, Ni 10 mg/kg and Cu 2 mg/kg, prepared by appropriate dilution of organometallic standards with sunflower oil (4.3) (*note 5*).

4.8 Standard working solutions: prepare daily the following working solutions by diluting the stock solutions (4.7) with oil with a low metal content (4.3):

Copper	0.05,	0.1,	0.2 mg/kg
Iron	0.25,	0.5,	1.0 mg/kg
Nickel	0.25,	0.5,	1.0 mg/kg

4.9 Argon, purity 99.99 per cent minimum (*note 6*).

5 **Procedure**

5.1 Treatment of samples and solutions

Place all samples and working solutions in the oven (3.8) during the period of determination.

476

Shake samples vigorously before analysis.
If the metal content of a crude oil is known to be outside the range specified for the standards in 4.8, dilute the sample with low metal content oil (4.3) to bring the metal content within that range.

5.2 Preparation of apparatus

Switch on the atomic absorption spectrometer (3.4) and the D_2 corrector. In accordance with the instructions of the manufacturer, adjust: lamp current, slit, wavelength and amplification. The required wavelengths are (nm):

Copper	324.7
Iron	302.1
Nickel	232.0

Optimize the position of the graphite furnace atomizer (3.5) in the atomic absorption spectrometer (3.4) and set the required programme for the furnace as follows:

Table 2.631.1.

	Step no.	Temp. (°C)	Ramp-time (s)	Hold-time (s)	Int. gas-flow (ml/min)
For Cu	1	900	50	30	300
	2	2700	1	5	50
For Fe, Ni	1	1200	50	30	300
	2	2700	1	5	50

If it is not possible to programme the graphite furnace exactly as above, use a comparative programme suitable for the equipment (*note 7*).
Use a normal graphite tube (*note 8*).
Pretreat the pipettor tip (3.3) by pipetting (using a micropipettor, 3.2) and then discarding 20 µl of heptane. The film of heptane remaining on the wall of the tip facilitates a reproducible transfer of the oil sample. The tip has to be pretreated before each injection of an oil sample.

5.3 Determination

5.3.1 Graphite tube blank

Record the absorption, if any, of the graphite tube (3.6) as such and autozero this absorption.

5.3.2 Liquid oil blank

By means of a micropipettor (3.2), inject 20 µl of low-metal oil (4.3) into the graphite furnace (3.5), initiate the temperature programme and record the absorption.

5.3.3 Standardization of apparatus

By means of a micropipettor (3.2), inject 20 µl of the three standard working solutions of the metal under investigation (4.8) into the graphite furnace (3.5) and record the absorption.

5.3.4 Sample for analysis.

5.3.4.1 **Oil (liquid) samples**

By means of a micropipettor (3.2), inject 20 µl of the oil sample into the graphite furnace (3.5), initiate the temperature programme and record the absorption.

5.3.4.2 **Fat samples (m.p. 40°C and higher)**

By means of a micropipettor (3.2), introduce an extra temperature programming step: hold-time 20 seconds, temperature 60°C, internal gas flow 0 ml/min. Initiate the temperature programme. Within the first programme step, introduce by means of a micropipettor (3.2) 20 µl of the melted fat into the graphite furnace (3.5), allow the tip to remain in the injection opening to liquefy the fat and then inject. Record the absorption (*note 9*).

6 Calculation and expression of results

6.1 Calculation

Measure the peak height on the recorder chart or read from the display or printer.

Draw a calibration curve by plotting the absorption of the three standards (5.3.3), corrected for the blank (5.3.2), against their respective metal contents (*note 10*).

Read the metal content of the sample from the relevant calibration curve.

6.2 Repeatability value

The differences between the values obtained from two single determinations, carried out in rapid succession by the same operator using the same apparatus for the analysis of the same test sample, should not be greater than the repeatability value (r) as calculated from the formulae in Table 2.631.2, which express the precision in relation to the determined mean value.

Table 2.631.2. Repeatability (r) and reproducibility values (R).

Metal	Substrate	r	R
Cu	Oil	$0.0102 + 0.140\,M$	$0.0085 + 0.358\,M$
	Fat	$0.0076 + 0.109\,M$	$0.0028 + 0.492\,M$
Fe	Oil	$0.077 + 0.081\,M$	$0.040 + 0.480\,M$
	Fat	$0.026 + 0.196\,M$	$0.031 + 0.543\,M$
Ni	Oil	$0.056 + 0.127\,M$	$0.027 + 0.442\,M$

M = corresponding mean concentration value.

6.3 Reproducibility value

The difference between the values for the final result, obtained by two (or more) laboratories using this standard method for the analysis of the same laboratory sample, should not be greater than the reproducibility value (R) as calculated from the formulae in Table 2.631.2, which express the precision in relation to the determined mean value.

7 **Notes**

1 The polyethylene- or polypropylene-capped bottles are made metal-free in the following way: clean the bottles thoroughly with warm nitric acid (4.5), rinse with distilled water and dry the bottles in a drying oven (3.8) at about 80°C.

2 A sample of a low metal content oil is obtained by the following procedure: dissolve 1 part of oil (4.3) (m) in 3 parts (V) of light petroleum (4.2). Prepare an aluminium oxide column (3.7) using twice the mass of aluminium oxide (4.4), activated by heating in an oven (3.8) at 150°C for 14 hours, as the mass of the oil to be purified. Add the oil solution to the column and elute with 5 parts (V) of light petroleum. Evaporate the light petroleum of the eluate on a heated water bath (3.9) using a gentle stream of nitrogen (2–5 l/min) (4.9). Remove final traces of light petroleum under vacuum.

3 Merck product no. 1077 is suitable.

4 Solution (code 88083) is available from Alfa Division, 152 Andover Street, Danvers, MA 01923, USA.

5 Suitable standards are available from, for example, Continental Oil Company, Ponca City, Oklahoma, USA (Conostan, 5000 mg/kg) or Merck, D-6100 Darmstadt, Germany (metal in standard oil, 1000 mg/kg).

6 If argon is not available, nitrogen may be used as purge gas. At temperatures above 2300°C, nitrogen forms toxic cyanogen gas; therefore, continuous ventilation in the furnace area should be provided.

7 If in this case the background correction fails, dilute blank, standards and samples with an organic fat solvent, e.g. heptane (4.1) to a maximum of 1:2 (m/m) and work at ambient temperature.

8 The graphite tube for the determination of iron has to be coated with niobium to ensure that the total amount of iron is determined. With an uncoated tube the result will vary according to the type of iron compound present in the oil. A suitable coating procedure is: by means of a micropipettor (3.2), inject 100 µl of niobium nitrate solution (4.6) into the furnace. Start the temperature programme to dry at 100°C for 60 seconds and then atomize at 2700°C for 5 seconds. Repeat this procedure until 300 µl of niobium nitrate solution has been injected. Atomize at 2700°C until constant absorbance (to remove any iron contamination).

9 The normal, minimum limit of detection can be improved by either a greater scale expansion or by repeated injections of the sample at the end of the ashing operation, then allowing the programme to proceed to completion. If the metal content is too high (i.e. exceeds the calibration curve), measure the absorption after a further dilution of the sample with sunflower oil.

10 With the use of sophisticated equipment, autocalibration can be applied.

References

Hendrikse PW, Sikkerveer FJ, Zaalberg J, Hautfenne A. *Pure Appl Chem* 1988; 60(6): 893–900 (Report on the Collaborative Study).
King AE, Roschen HL, Irwin WM. *Oil Soap* 1933; 10: 204.
List GR, Evans CD, Kwolek WF. *J Am Oil Chem Soc* 1971; 48: 438.
List GR, Evans CD, Moser HA. *J Am Oil Chem Soc* 1972; 49: 287.
List GR, Evans CD, Black LT, Mounts TL. *J Am Oil Chem Soc* 1978; 55: 275.
List GR, Mounts TL, Heakin AJ. *J Am Oil Chem Soc* 1978; 55: 280.
Marcuse R, Fredriksson PO. *J Am Oil Chem Soc* 1971; 48: 448.
Mertens WG, Swindells CF, Teasdale BF. *J Am Oil Chem Soc* 1971; 48: 544.
Notevarp O, Chamine MH. *J Am Oil Chem Soc* 1972; 49: 247.

479