PN ABG-987
69741

Flavor Research at the Post Harvest Technology Centers at IIHR, IARI, CIHNP & NRCC

December 14, 1990
New Delhi

Terry E. Acree

Winrock International
Petit Jean Mountain
Morrilton, AR 72110 USA

USAID
Project No. 386-0470

Table of Contents

Title Page	i
Table of Contents	ii
Definition of Abbreviations	iii
Introduction	1
Terms of reference	2
Executive Summary	3
Methods of Accomplishing Assignment	5
Present Situation	6
Accomplishments	6
Flavor Technology	7
Flavor Research Workshop	8
Sensory Analysis	8
Scientific Working Groups	8
Summary for IIHR	8a
Miscellaneous	9
Summary for IARI	9a
Summary for CIHNP & NRCC	9b
Schedule of Major Activities	Appendix A
Flavor Workshop Handout	Appendix B
HP 5985A Reference List	Appendix C
CAS search of tropical Flavor Literature	Appendix D

Definition of Abbreviations

ARP	Agricultural Research Project
CIHNP	Central Institute of Horticultural Research for Northern Plains
GC	Gas Chromatography
GCO	Gas Chromatography Olfactometry
HRGC	High Resolution Gas Chromatography
IARI	Indian Agricultural Research Institute
ICAR	Indian Council of Agricultural Research
MS	Mass Spectroscopy
NRCC	National Research Center for Citrus
PHT	Post Harvest Technology
USAID	United States Agency for International Development

Introduction

Initiated in 1985, the project entitled "Research and Development Requirements for Post harvest Technology of Fruits and Vegetables" (No. 386-0470) had as its central goal the application of research to minimize post harvest losses of important horticultural crops in India. This goal was implemented through the establishment of PHT divisions at each center, hiring and training personnel, installing research equipment, conducting workshops and sending consultants to each center. The ICAR research centers chosen for this were IIHR, IARI, CIHNP, and CMRS.

By December 1990 many of the scientist in the centers had received training in the USA, some but not all of the needed research equipment had been delivered, and all of the planed consultants had visited the Centers. It was disappointing that the research equipment was not delivered before the scientists went for training, however, because of these delays some of the equipment that will be installed in 1991 will be far superior and more reliable than what was anticipated in 1986. Thus, it is now possible to incorporate flavor analysis into the research activities of some of the PHT Centers.

This report addresses flavor analysis at IIHR and its transfer to the other three PHT centers.

Terms of Reference

1.Isolation and identification of aroma components characteristic of a particular variety of fruit. As we don't have standards and columns for identification for major fruits like mango, citrus, banana, pineapple, guava, etc. If possible, he may bring these for demonstration purpose at least.

2. To study changes in aroma components during ripening, processing and storage of above mentioned major fruits.

3.To standardize methods for aroma recovery from the horticultural crop wastes both from orchard and the processing industry.

4. To study differences if any, between natural and synthetic aroma principals with special reference to their sensory properties and their relative stability in the products at different storage temperatures.

5. To identify specific equipment or accessories of the available equipment considered essential for extraction as well as analysis of substances responsible for both aroma and taste.

6. Visit the Institutes and laboratories like BARC and CFTIR working on these aspects(subject to availability of time).

7. Deliver lecture/seminar at each center on the recent advances in flavor technology with special reference to horticultural crops.

8. To arrange for supply of relevant literature on this subject to concerned scientist s at all the Centers.

9. To submit consultancy report to the Council at the end of the visit.(i.e. before leaving India).

Executive Summary

Accomplishments

1. A Workshop on Flavor Technology was conducted at IIHR with approximately 15 participants, including one from IARI and one from CHINP. The principles of flavor analysis were discussed and demonstrations in flavor extraction, volatile analysis and GC sniffing (Gas Chromatography Olfactometry) were conducted.
2. An extract of Mango pulp, CV Alfonso, was prepared by Dr. Krishna Murty and Sri Sreedhara. I will take these extracts back for GCO and GCMS analysis in my lab. We will prepare standards of the key compounds identified and send them to each PHT center along with an appropriate column, GC operating conditions and a typical chromatogram.
3. The HP5840 GC in Dr. Murti's plant hormone laboratory was converted from packed columns operation to High Resolution Gas Chromatography with columns I brought from my Lab. Dr. Murti and Dr. Upreti are now using HRGC to analyze several plant hormone simultaneously.
3. An investigation of the use of stochastic sensory analysis at the PHT-Centers was conducted and the following agreement was reached: *central to all PHT research programs is Sensory Analysis*. Furthermore, the very competent Sensory group at CFTRI headed by Dr. Rajalakshmi seemed willing to help a sensory working group at IIHR establish a modern sensory laboratory there.

Recommendations for Immediate Implementation

1. Order Accessory 19251A "Injector for split/splitless operation" for the 4 HP 5890A GCs being delivered to the 4 centers. This is required for the analysis of flavor volatiles.
2. Have standards and columns sent from my laboratory to each center.
3. Order 3 or 4 reference texts on sensory data collection and analysis for each center. I will provide titles after I discuss it with my colleagues.
4. Hire a sensory analyst trained at CFTRI at both IIHR and IARI. and build a sensory lab at each center.

Recommendations for Future Development

1. Using the Sensory working groups at IIHR, IARI, and CFTRI develop a sensory lab, analytical protocols and statistical procedures at NRCC and CHINP for the analysis of flavor. Provide the same spread sheet and statistical software to all 4 labs so that they can standardize their procedures and exchange data.
2. Purchase a GCMS and install it at IIHR. It is needed for advanced chemical analysis by all groups at the Center including the PHT and Plant Biochemistry groups. Although GCMS capabilities exists at CFTRI, their research is mostly concerned with spices and processed food flavor The PHT research centers will ultimately be the best places to study fresh fruit and vegetable flavor chemistry. However, cooperation between these two centers via Spectroscopy Working Groups could enhance the productivity of both.
3. Encourage the frequent interaction of scientists in scientific working groups in order to maintain quality research at all four centers. I see the need for an association of sensory scientist in India that can disseminate information about the latest research in sensory analysis. Up-to-date abstracts of all of the literature in sensory science are presently in the library at CFTRI where a news letter could be prepared and sent to all the interested parties in India including the PHT centers. An Indian Sensory Science Association (ISSA) would be welcomed by many.
4. Once the personnel and basic infrastructure needed for sensory analysis are in place in at least one of the centers should seek funding to have an expert consultant in sensory analysis run workshops and conduct experiments in flavor analysis at that center. Begin the assignment with a visit to CFTRI.

Methods of Accomplishing Assignment

The primary thrust in the Terms of Reference was to train selected personal in the use of gas chromatography for flavor analysis. However, none of the gas chromatographs needed for this training was to be delivered by the time this consultant was to arrive in India . Thus the following was the means used to accomplish the assignment:

1. A Flavor Technology Workshop organized by Dr. Krishna Murty was conducted at IIHR Hessaraghatta. Borrowed equipment from Dr. G.S.R. Murti's lab was modified for HRGC and GCO analysis of flavor volatiles using columns, standards and supplies brought from the consultants lab. Lectures, demonstrations and one-on-one discussions were used to impart a general understanding of the techniques used in flavor analysis and to answer specific questions.

2. Before leaving the US the consultant conducted a search of the Chemical Abstract data base and brought a list of 370 references (see Appendix D). Full text copies of four articles and two reference texts typical of the techniques used in flavor research were also brought.

3. In order to asses future developments in flavor analysis at the PHT Centers the consultant visited CFTRI, IARI, Kissan Products Ltd., and two retail markets.

Present Situation

Accomplishments

It is easy to be disappointed by the slow construction of new buildings. This made it impossible to install new laboratory equipment before the scientist went for technical and scientific training abroad, but the human resources that have been focused, encouraged and developed by this project must be congratulated. I am sure that a realization of the accomplishments of many of the scientists involved will be evident soon after the labs are fully functional. Two examples, at IIHR impressed me very much.

1.Good quality vinegar was produced by the fermentation of mango processing wastes. The idea was developed under the PHT project and brought to the demonstration stage by scientists trained under the PHT program. This type of technical development is exactly what the original mandate intended. I heard of numerous similar examples, from remote village mango past production to the very impressive zero energy storage systems. Once the research buildings are built I am sure the work of the PHT scientists will become more advanced and produce many benefits for the Indian food system.

2. Heat treat ing mangos by dipping them in 52C water produced more uniform ripening and less product decay. A seemingly simple notion but it was the logical and scientific test of this idea by trained IIHR scientists that convinced Kisson Products Ltd. to do a trial in one of their plants last year. They were so impressed with the reduction in fruit loss during ripening (reduced from 15% to below 4%) that they have constructed an automated process line to use during the next mango season. There is no stronger recommendation for an idea than that a private industry decided to invest money to implement it!

My concern was determine how flavor analysis fit into this program.

Flavor Technology

For the purposes of this report I will describe three levels of flavor research based on the technology and talent required:

1. *Sensory Analysis* - This is the cornerstone or foundation of flavor research. Indeed sensory analysis is the central measurement in all food quality assessments and specifications. All of the scientists in PHT are aware of its importance to their research and they seem to agree that it should be a priority for immediate development in all the programs.
2. *Chromatographic Analysis of Flavor-Active Compounds* - With the installation of the liquid and gas chromatographic equipment (including the split/splitless capillary accessory -19251A) compounds responsible for taste and aroma in the fruits and vegetables will be usefully analyzed by the PHT staff Research using chromatographic analysis will depend on the use of standards defined in publications by researchers outside the PHT Centers e.g. CFTRI and BARC. However, some compounds can be identified from their chromatographic retention properties combined with their flavor-activity assessed by sensory analysis, e.g. GCO. These special sensory techniques could be established at all the Centers eventually, however, it is likely that the scientists at IIHR and CFTRI will be the first to implement them. Through workshops they should transfer chromatoraphic analysis of flavors to the other Centers.
3. *Spectroscopic Analysis*- The identification of new and previously unidentified compounds in fruits and vegetables is highly unlikely until a modern gas chromatography-mass spectrometer (GSMS) is installed at the center. However, there is already a need in several of the other laboratories at the center for such equipment. Furthermore, one laboratory at IIHR contains equipment equal in sophistication to a GCMS. It is operating in pristine condition and has done so for 13 years producing first rate scientific publications. With the help of these scientists a GCMS center could be developed at IIHR for use by all the scientists in the 4 horticulture centers. With a GCMS it is very likely that new and important flavor active compounds specific to the unique fruits of India could be identified by the chemists already at IIHR. Ultimately, they could help in

the establishment of a similar center in IARI. However, it will be several years before NRCC and CIHNP have the infrastructure necessary to support a GCMS system.

Flavor Research Workshop A workshop was conducted on November 22 to the 28 to convey the basic principles of flavor analysis. A copy of the handout developed from the workshop is included in Appendix C. Since I have made many changes and additions since the completion of the workshop copies should be distributed to all participants and to anyone at the four centers interested in the subject including those outside the PHT group e.g. Dr. Y. Selvaraj and Dr. G.S.R. Murti.

Sensory Analysis - The block diagrams on the following page shows an idealized set of research areas for the PHT groups. Listed below each block are the corresponding human resources now in place at IIHR. This diagram was created to show the lack of personal with primary training in sensory and flavor analysis. A sensory scientist at IIHR could work with the other members of the PHT group to obtain better quantative data on the effect post harvest technology on flavor. A sensory scientist could also work with the chemists and biochemists at IIHR to do modern flavor chemistry. Both of these efforts would lead to standard methods of flavor assessment and indices of quality usable at all PHT Centers.

Scientific Working Groups In order facilitate a common understanding of a technology or science that is rapidly changing or unfamiliar to those who must use it I have propose that scientific working groups be established to meet ever month or so. At these meetings a member who uses the technology will give an example from an actual or anticipated experiment. This will demonstrate to the members the many ways a technology can be used. These groups can also provide personal and material for training the other PHT Centers where similar groups should be formed. The people listed below the names of the three proposed working groups are just a guess. Such

Primary Research Areas for Post Harvest Technology and the manpower at the Indian Institute of Horticultural Research

Physiology Chemistry	Pathology Microbiology	Sensory Flavor	Processing	Economics Engineering
Krishna Murthy Rao Sreedhara	Ethiraj Suresh Jayaram Ullasa	?	Gowda Narayan Sarojini	Mandhar Madan

1. Need a Sensory- Flavor Scientist
2. Need a proper sensory analysis room.

Scientific Working Groups

The formation of these working groups on a voluntary and temporary basis would facilitate the development of common technical expertise with in each PHT Center, would provide groups able to share expertise between PHT Centers, and could form a nucleus for the establishment of national scientific associations, e.g. an Indian Sensory Science Association

Sensory-Flavor	Chromatography	Spectroscopy
"?" Selvaraj Krishna Murthy Sreedhara Rao Suresh Gowda Narayan	Murthy Upreti Selvaraj Krishna Murthy Sreedhara Rao Gowda Suresh Nararyan	Murthy Upreti Selveraj Sabastian
Should work w/ CFTRI's Sensory Scientists to develop a sensory lab.	Should install Acessory 19251A on HP 5890 learn capillary column chromatography.	Should prepare justification for the installation of a GC-MS-DS in the Plant Hormone Lab for use by all groups at IIHR.

Training and Education

1. Send a member of the Sensory Flavor Working Group to Terry Acree's lab for training in Flavor Analysis.

2. Send several members of the Chromatography Working Group to an HP gas chromatography training program AFTER THE GC IS INSTALLED AT IIHR.

3. Hire a new scientist trained at CFTRI in Sensory Analysis or send one for training.

groups should be voluntary and based on the interest and needs of the scientist

The Sensory-Flavor Working Group would have as one of its tasks the establishment of standard methods of analysis for all the Centers to use. Clearly as the center developmore expertise in sensory analysis,annual meetings of groups from all the centers including representatives from CFTRI should be held to discuss common procedures and problems. It is likely that the Chromatography working groups will be strongest at IARI and IIHR and they should assist the scientists at CIHNP and NRCC set up the procedures for respiratory gas analysis. The spectroscope working group try meet with chemists from CFTRI until a system is installed at IIHR them they should encourage participation by interested scientists at IARI.

On the following two pages are similar lists prepared for IARI, CIHNP, and NRCC. Again we see an even distribution of human resources at IARI across all but one of the primary research categories: Sensory Flavor. Not only is their a need for a sensory lab but there is a large group of people who need both the facility and the assistance. It would seem that a sensory scientist would fit in well here. The situations at CIHNP and NRCC are less clear since I did not visit these centers. In discussions with Dr. Tandon we made an attempt to predict the composition of the working groups that might be formed there.

It is my feeling that a working group can have 2 people or 20. Ideally it should have no money and no power other than the power of convincing argument and clear thinking. It can be sustained by one interested person and it should disappear when it becomes useless. If it gets too large it will become a society.

Miscellaneous - Two simple ideas were discussed with the staff at IIHR:
1. The work by Dr. Suresh and Dr. Ethiraj on wine fermentations is an example of the application of objective sensory analysis. Using the Davis 14

Acree's Views

Primary Research Areas for Post Harvest Technology and the manpower at the Indian Agricultural Research Institute (PUSA)

Physiology Chemistry	Pathology Microbiology	Sensory Flavor	Processing	Economics Engineering
Chakrabarti Pal Srivastava Meena	Murti Sethi Bhagwan Sing	?	Khurdiya Maini Kumar	Atteri Sharma

1. Need a Sensory- Flavor Scientist
2. Need a proper sensory analysis room.

Scientific Working Groups

The formation of scientific working groups on a voluntary and temporary basis would facilitate the development of common technical expertise with in each PHT Center, would provide groups able to share expertise between PHT Centers, and could form a nucleus for the establishment of national scientific associations, e.g. an Indian Sensory Science Association .

Sensory-Flavor

"?"
Khurdiya
Pal
Maini
Sethi
Srivastava
Kumar
Bhagwan

Should work w/ CFTRI's Sensory Scientists to develop a sensory lab.

Chromatography

Pal
Maini
Srivastava
etc.

Should install Accessory 19251A on HP 5890 learn capillary column chromatography.

Training and Education

1. Send a member of the Sensory Flavor Working Group to Terry Acree's lab for training in Flavor Analysis.

2. Send several members of the Chromatography Working Group to an HP gas chromatography training program AFTER THE GC IS INSTALLED AT IIHR.

3. Hire a new scientist trained at CFTRI in Sensory Analysis or send one for training.

Acree's Views

Scientific Working Groups
at
Central Institute of Horticulture for the Northern Plains
&
National Research Center for Citrus

The formation of scientific working groups on a voluntary and temporary basis would facilitate the development of common technical expertise with in each PHT Center, would provide groups able to share expertise between PHT Centers, and could form a nucleus for the establishment of national scientific associations, e.g. an Indian Sensory Science Association .

CIHNP:

Sensory-Flavor

Tandon
Ajay Verma
Neelirva Gasg
Rajan
Sinha
Pandey
Rekha Chaurasia

Should work w/ CFTRI's Sensory Scientists to develop a sensory lab.

Chromatography

Kalsa
Pavdan
Khader
Dixit
Kumar
Singh
Tandon

Should install Accessory 19251A on HP 5890 learn capillary column chromatography.

NRCC:

Similar working groups should be encouraged as appropriate personnel are hired. As soon as the personnel are hired that will use the gas analysis technology they should meet with the other chromatography working groups.

Training and Education

1. Send several members of the Chromatography Working Group to an HP gas chromatography training program AFTER THE GC IS INSTALLED AT IIHR.

3. Hire a new scientist trained at CFTRI in Sensory Analysis or send one for training.

point system and a trained sensory panel they were able to produce wine from the cultivars developed at IIHR that had excellent flavor, meeting the standards of any European or American wine research center. I am sure that a mixture of wines or the grapes before fermentation or even a mixed planting at a ratio of about 20 parts of the varieties with weak aroma to one part of the muscat like varieties would produce a very exportable wine.

2. The fact that most if not all the fruits and vegetables processed into pastes and concentrates in India are done so in open systems means that 90% of the odor active compounds are lost into the air. There is a very lucrative international market for natural fruit and vegetable essence. If only 10% of the total Indian processed fruit and vegetable production (200,000 tons) were concentrated using essence recovery systems the resulting 100 fold essence would be worth more than 100,000,000 rupees at $10 a gal. Volatile flavor analysis can play a role in developing such systems in India.

Appendix A

Schedule of Major Activities

Date	Activity
11-17	Arrive at New Delhi and stay at Taj Palace Hotel
11-19	Meet with Dr. McClung and Mr.Srinivasan at Winrock office . Go to USAID meet with Surgen Sighn. Go to ICAR and meet with Dr. G.L. Chadha, Dr. G.L. Kaul, & Dr. Amba Dan. Dinner with John Becker at Dr and Mrs McClung
11-20	Fly to Bangalore meet with Dr. Shanta Krishna Murty and Dr. Negi at IIHR. Discuss workshop.
11-21	Meet Dr. Murti from the Plant Hormone Lab and arrange to use his GC for Flavor Workshop and begin modifications necessary for flavor analysis. Meet with each member of PHT staff and hear their work in progress. Plan workshop schedule.
11-22	Spend morning giving General Lectures on Flavor Analysis Dr. Shanta Krishna Murty takes the group to here lab and they prepare polar and non-polar extracts of Mango Pulp. In between discussions with the students Dr. Murty and I begin to rebuild his GC to make it an HRGC.
11-23	Finish HRGC and give lectures on HRGC injector systems and start HRGC laboratory demonstration.
11-24	Run hydrocarbon standards and continue laboratory demonstrations. Modify GC for GCO analysis. Answer questions.
11-26	HRGC sniffing (GCO) lab and Discussion.
11-27	Run GCO of Mango extract. One-on-one w/ Dr. Maini from IARI.
11-28	One-on-one lectures and discussions with Dr Tanlon from CIHNP continue to give each participant an opportunity to sniff in a GCO Analysis.

11-29	Rebuild GC to an HRGC suitable for the analysis of plant Hormones
11-30	Meet with Dr. Selvaraj and discuss his research in the biochemistry of flavor at IIHR
12-1	Meet with Dr. Rao to discuss vegetable flavor chemistry. Give a Seminar on my Research in Flavor Chemistry
12-3	Visit CFTRI meet Sensory scientists, Juice Processing scientists, Natural Products Chemists etc.
12-4	Visit with other members of CFTRI and give seminar.
12-5	Visit Kissan Foods and two public fruit markets.
12-6	Plan Scientific Working Groups
12-7	Report to Director Negi
12-8	Fly to Delhi
12-10	Visit IARI
12-11	Visit IARI plan working groups
12-12	Write final report
12-13	Write final report
12-14	Meet with ICAR and USAID
12-17	Leave India

Appendix B

Flavor Workshop Handouts

Workshop
in

Flavor Research

November 22 - 28, 1990

Indian Institute of Horticultural Research

Hessaraghatta
Bangalore

Introduction

Through the sense of smell we discover changes in the chemistry of the air we breath. We interpret these chemical changes as signs of change in our immediate environment: a flower is blooming, bread is coming from an oven, leaves are burning, a bus stops, rain, coffee, leaking gas, fire, a barbecue, incense, musk, ginger snaps, and cola. We smell things like these and many more; we notice them; we remember them; we react to them. Smells invoke our memory, evoke our imagination and emote our feelings every day. We choose what we eat and drink to a large extent because we like the way it smells.

Gas chromatography-olfactometry

A smell is what it is because of its chemical composition. Although the perception of a single smell event, coffee or coke for example, often seems to be one distinct experience, it is, in fact, due to a combined response to several sensations produced by different chemical components. Gas chromatography-olfactometry, GCO, is an analytical technique that combines two different procedures: high resolution gas chromatography to separate the component odor-active chemicals extracted from a food, and olfactometry to combine the separate chemicals with purified and humidified air and deliver them to a human "sniffer" who breathes the air through his nose and record his perceptions. CharmAnalysis™ is a GCO method that measures smell-activity in terms of odor detection thresholds calculated from chromatograms. The chromatograms produced in CharmAnalysis™ are plots of odor-activity verses retention index and are generated by the software called Charmware™.

Sniffing

During GCO a single human subject, a sniffer, sniffs the air coming from the olfactometer and records perceptions. The gas chromatograph separates the components and the sniffer analyzes them for odor-activity. The same sniffer must be used during an experiment in order to minimize errors caused by differences in acuity between people. The only way to be sure that a sniffer is acute is to use standard odor-active chemicals. A set of standard chemicals are used to test subjects for their sensitivity.

Sniffing is conducted in runs or sessions of about 20 minutes duration. Longer sessions can fatigue the sniffers and is not recommended, but shorter runs are often devised. Sniffers require training in order to understand what to expect during the runs. A single run consists of the injection of one microliter of solvent, containing extracts of the substance to be analyzed, into the GCO. The gas chromatograph separates the components and delivers them as puffs into a rapidly moving stream of air. This causes the sniffer to experience a sudden rise in the concentration of the odor-active component: the concentration can double in just a few milliseconds. In its simplest form, GCO involves the sniffer detecting this sudden rise in concentration of the odorant when the compound exceeds its odor threshold. He records this event. In more complicated GCO procedures the sniffer reports his perception of the maximum odor intensity and/or associates a description with the kind of odor detected. Several of these runs must be conducted on serial dilutions and/or concentrations of the extracts in order to produce quantitative data based on

odor detection thresholds. Called "Aroma Extract Dilution Analysis" by Grosch *et al.* (1988) and "Charm Analysis" by Acree *et al.* (1984) this procedure yields chromatograms that are analogous to flame ionization, FTIR, and MS detection chromatography.

To understand what types of compounds are extracted by this scheme consider a mixture of ethyl 2-methylbutyrate, 2-phenylethanol, and 2,5-dimethyl-3-hydroxy-4(2H)-furanone (furaneol™) in 10% ethanol :water. Greater than 95% of the ethyl 2-methylbutyrate, about 50% of the 2-phenylethanol and less than 1% of the furaneol will be in the Freon™ or n-paraffin fraction. The ethylacetate fraction should have some of the small amount of remaining ethyl 2-methyl butyrate most of the remaining 2-phenylethanol, and more than 75% of the furaneol. However, if the odor-activity of interest is found exclusively in one fraction then the other step can sometimes be omitted.

The scheme shown in Figure 1 is just one of many methods used to prepare samples for GCO. The best method for any particular sample depends on the chemical and physical properties of, as well as, any safety issues raised by the sample. A review of sample preparation methods will be presented in Chapter 3 .

CharmAnalysis™

CharmAnalysis™ developed at Cornell University.is outlined in Figure 2 .

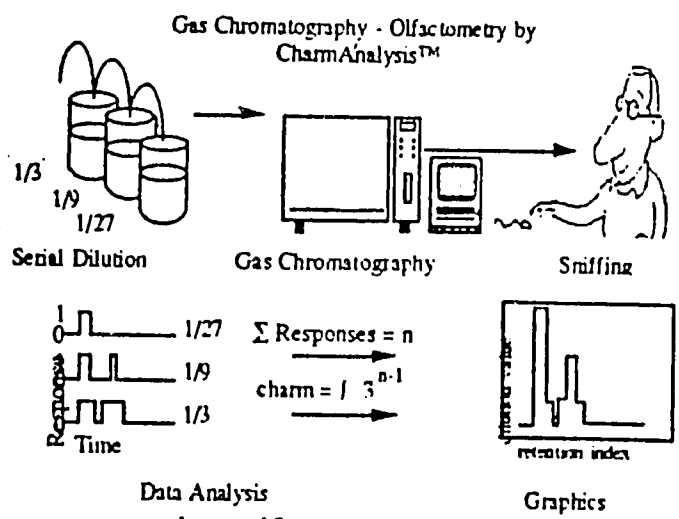


Figure 2. Gas chromatography - olfactometry by CharmAnalysis™ using Charmware™ Each dilution is then chromatographed separately and sniffed.

Serial Dilution

Samples consisting of gas chromatographable extracts must be diluted by a constant factor usually 2 or 3 resulting in a set of 7 to 10 dilutions. When a factor of 3 is used the samples are 1, 1/3, 1/9, 1/27, 1/81, 1/243, 1/729, 1/2187, 1/6561 concentration of the original. In order to determine the correct relationship between the component odors, the sample must be diluted until no odor is detected. Often the initial extract is too weak and must be concentrated in order to detect all odor-important components. In this case the concentrate

must be prepared such that a dilution series of it will match the dilutions prepared from the unconcentrated extract, e.g. 243x, 81x, 27x, 9x, 3x, 1. However, if it can be determined early in the analysis that the extract must be concentrated, then a serial dilution of the concentrate can be done directly. Obviously, data from one analysis is directly comparable with data from another only if the samples are handled in the same way. Future versions of Charmware™ will be able to accommodate different dilution schemes automatically yielding dilution values that are always comparable.

Gas Chromatography

The choice of gas chromatographic substrate and operating conditions is dependent upon the composition of the sample and the durability of the sniffers. We have found that non-polar substrates such as methyl silicons (OV101, SE30, etc.) elute most odor-active volatiles at the lowest possible temperature and can be temperature programmed at high rates (4-10 C/min). For example, a 12 m by .25 mm crosslinked OV101 on fused silica can be held at 35 C for 3 minutes then programmed at 6 C/min for 25 minutes. This allows the sniffer to experience volatiles that range between 700 and 1800 retention indices (between ethyl butyrate and ethyl tetradecanoate) in less than 30 minutes. It usually wise to survey the sample over as broad a range of volatilities as possible in order to locate the important odor-active compounds. If it can be determined that the odors of interest elute in a narrow range of volatilities as is sometimes the case with off-odors, then it best to examine the sample with longer columns using lower temperature program rates over narrow retention index ranges.

In some GCO configurations a splitter is used to divide the column effluent into two streams. One stream is sent to the olfactometer, the other is sent to chemical detector, generally a flame ionization detector (FID) allowing simultaneous sniffing and chemical detection. However, when a very dilute sample is chromatographed nothing will be detected by the chemical detector unless internal standards are added to the diluting solvents. These internal standards can be used to correct for changes in retention times that often occur when a chromatograph is operated over and over again for the week or more needed to produce a Charm chromatogram for one sample. Furthermore, internal standards with odor can be used to correct GCO data when no splitter is used. CharmAnalysis™ assumes that no splitter is used and that 100% of what is injected is transfered to the olfactometer.

Because charm data is often collected without the benefit of an internal standard it is absolutely essential that the chromatography be precisely reproducible and the same whether the column is attached to the olfactometer or the FID. Careful attention must be paid to the design and operation of the hardware to assure accurate results. For example, short open tubular columns are very sensitive to back pressures created by make-up gases in FIDs and olfactometers. This is because the pressure drop across short columns is very small. This is why the olfactometer in the CharmAnalysis™ System made by DATU, inc. looks exactly the same as the FID in terms of structure, pressure, temperature and gas flows at the end of the column.

Sniffing

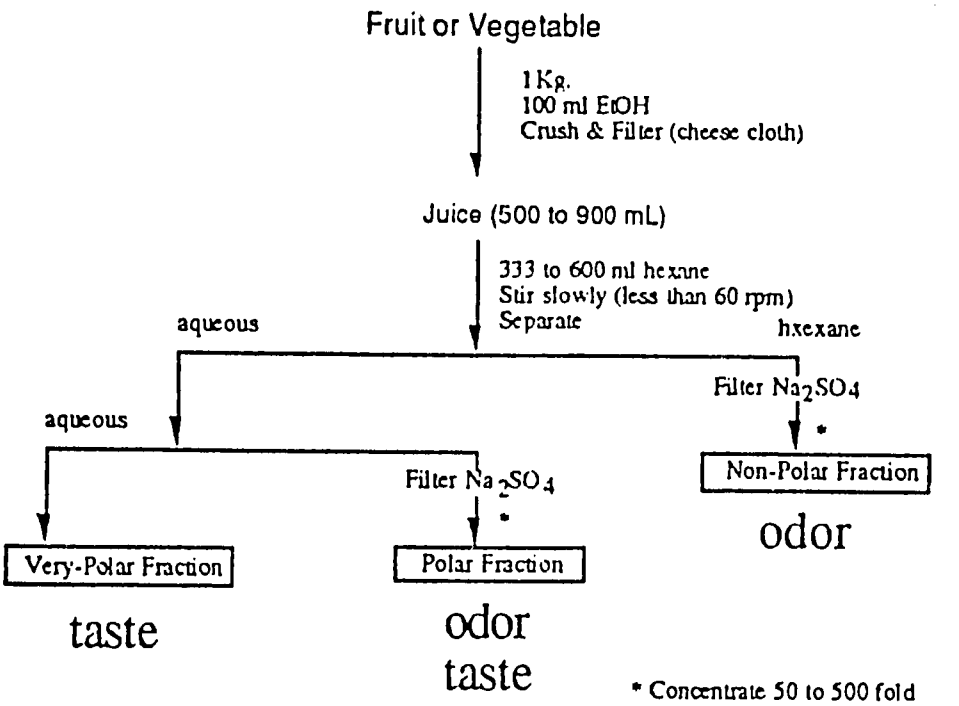
Although sniffers are sometimes expert sensory technicians, they are always human subjects in a psychophysical experiment and as such must be treated according to the recommended guidelines for the use of humans in experiments. Clearly, the most important consideration is the health and safety of the subjects including a complete disclosure of the materials the subject will come in contact with during the sniffing. Furthermore, sniffers, like human subjects in any psychophysical experiment, often bias their response if they know too much about the sample they are sniffing. Bias generated by the sniffers during CharmAnalysis™ can be minimized by the use of coded and randomized samples. The samples are best presented double blind, i.e. arranged and labeled by someone outside of the group doing the analysis.

Sniffing begins at the olfactometer after the solvent has eluted. The olfactometer delivers a stream of purified and humidified air (50 to 75% relative humidity) at a linear velocity equal to or greater than the carrier gas in the chromatographic column (20 to 30 cm/sec). For an olfactometer with a 1 cm cross section at the output this requires a volume flow of 20 L/min ! When the sniffer smells something in the olfactometer air stream he presses the mouse button (mouse down) and holds it down until the odor changes in character or vanishes. Then he releases the mouse button (mouse up). The computer records the time of these mouse events. Depending on the version of the software, the sniffer can choose a descriptive term by where the pointer is when the mouse is released or pointing and clicking at a word displayed on the screen just after the mouse is released. A more detailed discussion of descriptive analysis is presented in chapter 5.

EXTRACTION and CONCENTRATION of Fruits and Vegetables for Volatile Analysis.

Extraction is the process by which odor-active compounds are removed from a natural product and transferred to a volatile solvent. The most common solvents used for extraction are hexane, pentane or Freon 113 all non-polar solvents which extracts primarily non-polar components from the natural product, and Ethyl Acetate, which extracts the polar components. The non-polar extraction is always done first, followed by the Ethyl Acetate extraction of the remaining sample as shown in the scheme below.

Standard Flavor Extraction Scheme



Acid - Base Extraction of Concentrates

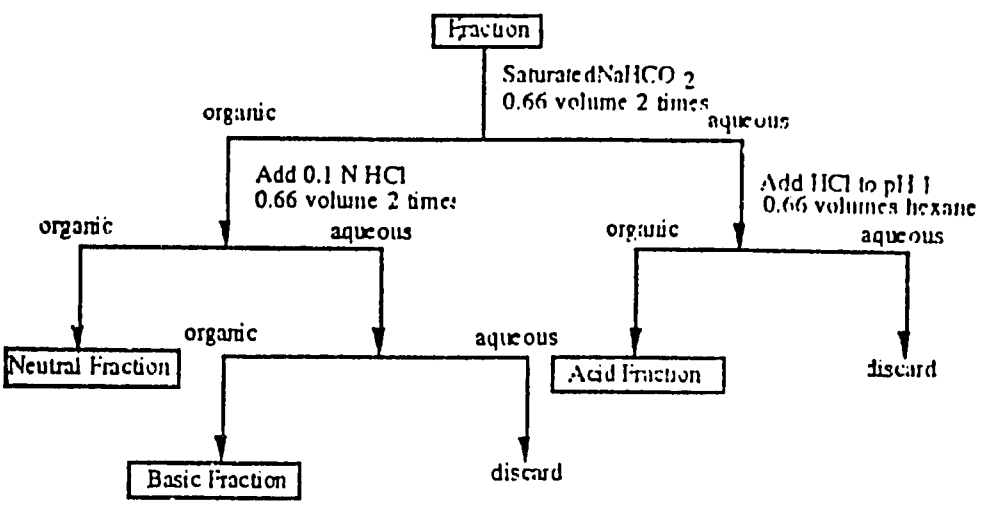


Figure 1 Typical extraction schemes for the isolation of odor-active volatiles from fruit and vegetables.

EQUIPMENT

Sample sizes vary, but you must maintain the ratio of 1 part sample to .666 (or 2/3) solvent to .1 (or 1/10) ethanol. For a 1200 ml. sample, 800 ml. solvent, and 120 ml. ethanol are added. A 500 ml. sample is convenient size. The size of your sample will dictate the various sizes of glassware that you'll be using. As a guideline, we'll start off with a list of the necessary equipment for extractions at 500 ml. and 1200 ml. and you can use your best judgement for different size extractions.

For a 1200 ml sample (assume liquid sample is provided)
Freon-Ethyl Acetate sequence

95% Ethanol
A 2 liter graduated cylinder*
A 2 liter media flask*
Two 1 liter graduated cylinder*
A 250 ml graduated cylinder*
3 in. stirring bar
Magnetic stirrer
Variable transformer such as a Powerstat
Ring stand & ring
A 2 liter separatory funnel with ground glass stopper*
Magnesium sulfate
Pleated filter paper (18.5 cm.)
Three 4 in. powder funnels
Two 4 in. powder funnels with curved stems.
Two 1-liter brown bottle for extracted sample*

For a 500 ml. sample, substitute the following for the glassware indicated above (*).

1 liter graduated cylinder
1 liter separatory funnel
Three 500 ml. graduated cylinder
Two 500 ml. brown sample bottles
50 ml. graduated cylinder

Set up ring stand or rack with a cast iron ring sized for a 2 liter separatory funnel, and place separatory funnel in ring. Plug magnetic stirrer into variable transformer. Set speed control on magnetic stirrer to mid-range. Stirring speed is adjusted with the variable transformer to 30 to 60 rpm.

Extractions of a 1200 ml. sample:

1. Measure 1200 ml. of sample juice using 2 liter graduated cylinder and pour into 2800 ml media flask.
2. Measure 800 ml. FREON 113 using 1 liter graduated cylinder and pour SLOWLY into media flask
3. Measure 120 ml. 95% Ethanol using 250 ml, graduated cylinder, and pour SLOWLY into media flask.
4. Tip media flask at 45° and carefully slide 3 in. magnetic stirring bar into the flask.
5. Place media flask on magnetic stirrer.
   a. Adjust stirring bar speed to 70-80 RPM. Continue to check the speed of the stirrer. The speed must be kept slow to prevent the formation of an emulsion.
   b. If the action of the stirring bar is erratic or hard to control, try placing an inch or so of paper towels under the flask.
   c. Continue stirring for 20 minutes.
6. Place a four inch powder funnel (with curved stem) on top of the separator funnel and SLOWLY pour the entire contents of the media flask into the separatory funnel. BE SURE THE STOPCOCK IS CLOSED (the handle is horizontal). Place the ground glass stopper in the top of the separatory funnel and leave until the layers (phases) in the sample are completely separated.
7. Rinse the Media flask with distilled water and hang it on the drying rack for further use.
8. Place a piece of pleated filter paper in a 4 in. powder funnel, add one heaping teaspoon of Magnesium Sulfate, and place the funnel on top of a CLEAN, DRY, one liter graduated cylinder. Position the graduated cylinder under the separatory funnel. REMOVE THE GROUND GLASS STOPPER FROM THE SEPARATORY FUNNEL. This is very important! If the stopper is in place when the stopcock is opened, a vacuum will form. Open the stopcock slightly -- the level of the solvent in the funnel should not rise above the level of the Magnesium Sulfate. -- You must monitor the progress of this step for you may need to adjust the flow of the solvent. When the level of the draining Freon nears the narrow portion of the separatory funnel keep your eye on it. You do not want the

next layer to drain into the graduated cylinder, and once it reaches the narrow portion it travels quickly. When you see the Freon reaching the end, turn off the stopcock. You can drain the remaining Freon with quick turns of the stopcock.

9. Record the amount of Freon that has been recovered and pour it into a brown sample bottle. Clearly mark the bottle with the amount of solvent, the date, the name of your supervisor, and the sample name.

ADAM'S APPLES

FREON EXTRACTION
725 ml. RECOVERED
5/20/89

ACREE

10. The sample layer remaining in the separatory funnel is now ready to be extracted with Ethyl Acetate. Drain the remaining sample from the separatory funnel into the rinsed Media flask. Measure 800 ml. of redistilled Ethyl Acetate using 1 liter graduated cylinder and pour SLOWLY into the media flask.
11. Tip media flask at 45° and carefully slide 3 in. magnetic stirring bar into the flask.
12. Place media flask on magnetic stirrer.

a. Adjust stirring bar speed to 70-80 RPM. Continue to check the speed of the stirrer. The speed must be kept slow to prevent the formation of an emulsion.
b. If the action of the stirring bar is erratic or hard to control, try placing an inch or so of paper towels under the flask.
c. Continue stirring for 20 minutes.
d. Ethyl Acetate extractions are susceptible to forming emulsions. The stirring speed must be carefully monitored at all times.

13. Place a four inch powder funnel (with curved stem) on top of the separator funnel and SLOWLY pour the entire contents of the media flask into the separatory funnel. BE SURE THE STOPCOCK IS CLOSED (the handle is horizontal). Place the ground glass stopper in the top of the

separatory funnel and leave until the layers (phases) in the sample are completely separated.

14. As Ethyl Acetate is lighter than water, the solvent is now the upper layer in the separatory funnel. REMOVE THE GROUND GLASS STOPPER. The aqueous (sample layer) is then drained down to the Ethyl Acetate interface. As with the Freon, be careful when the interface approaches the stopcock. If any emulsions have formed, they can also be drained off and disposed of with the remaining sample.-- You drain down to the clear Ethyl Acetate but not beyond.

15. Place a piece of pleated filter paper in a 4 in. powder funnel, add THREE heaping teaspoons of Magnesium Sulfate or Sodium Sulfate, and place the funnel on top of a CLEAN, DRY, one liter graduated cylinder. Position the graduated cylinder under the separatory funnel. REMOVE THE GROUND GLASS STOPPER FROM THE SEPARATORY FUNNEL. This is very important! If the stopper is in place when the stopcock is opened, a vacuum will form. Open the stopcock slightly -- the level of the solvent in the funnel should not rise above the level of the Magnesium Sulfate. -- You must monitor the progress of this step for you may need to adjust the flow of the solvent. You may completely drain the separatory funnel.

16. Record the amount of Ethyl Acetate that has been recovered and pour it into a brown sample bottle. Clearly mark the bottle with a label following the noddle given above substituting Ethyl Acetate extraction for Freon extraction.

*17. Clean all the glassware and tidy up the work area.

CONCENTRATION

Concentration of the sample is the next step in the flavor isolation process. Using the Rotory evaporator "the rotovap," solvents are removed from the sample concentrating it . When concentrating a sample, one should always remeasure the amount of solvent recovered once again. Although it should be labeled on your sample bottle double-checking is important. (Always be aware if you are doing an Ethyl Acetate or Freon concentration.) Next, using a pipet, take out your 1:1 sample. -- Make sure you always put your samples in brown bottles with teflon caps to avoid losing them. -- Label the sample and then you are ready to start concentrating.

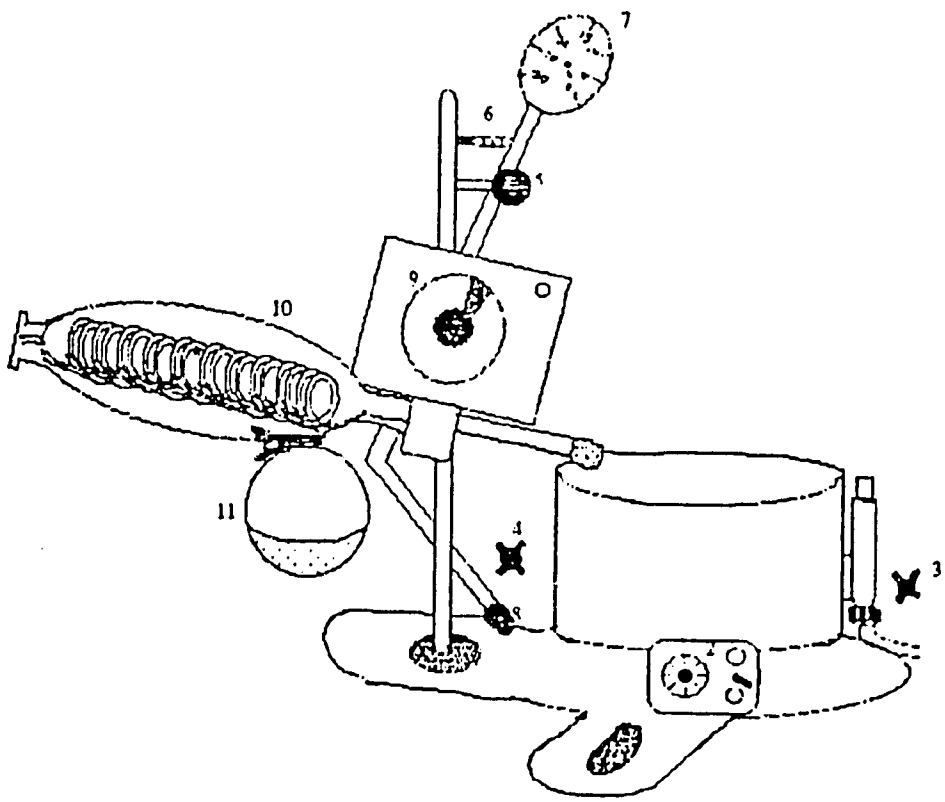


Figure 2 Typical rotary evaporator equipped with a pressure gauge. The numbers correspond to those with "#" in the following step by step procedure for solvent evaporation.

1. First turn on the heater to warm the water by switching #1 to the on position (up). A light should Come on.
2. Adjust the temperature of the water using dial #2 -- The normal temperature setting is around 30-40 °C denoted by a pen slash.

3. Next, choose the appropriate sized glassware and pour your remaining sample in it. Make sure your glassware is not too small-- this can cause the sample to be evaporated along with the solvent in one large "burp."

(It may be a good idea to choose two pieces of glassware that are the same size. Using distilled water, measure out the volume you are concentrating to and place it in the second flask. You can use the distilled water as a guide to know when your sample has reached the correct concentration.)

4. The water is turned on by turning valve #3 all the way on and turning valve #4 on about a quarter turn.
5. Place the glassware containing your sample on the Rotovap and be sure to hold it there while you turn on the vacuum by turning valve #5. Hold the glassware on until the gage #7 reads about 5-8 -- This should ensure that your sample will not be lost in the water. ***NOTE - Watch the gage carefully. If you are concentrating an Ethyl Acetate sample, the gage should reach 25. *If you are concentrating a Freon sample the gage should only reach 15.* The pressure can be adjusted by turning the small valve (#6). This valve is for fine tuning -- Turning it clockwise should increase the pressure.
6. Using handle #8, lower your sample into the water.
7. Turn dial #9 to spin your sample at the desired speed-- normally the speed is 3-5.
8. The solvent will evaporate up through #10 and then condense on the coils which have cold water running through them. The condensed solvent will drip into the waste container, #11.**One can perform other tasks while using the Rotovap but make sure you keep an eye on you sample and check it fairly often. The pressure sometimes changes without notice and the solvents can evaporate faster than you expect-- especially when your sample is small.

**While your sample is concentrating prepare a brown sample bottle with an appropriate label. Include the name of the sample, the solvent involved, the ml. amount contained, the concentration value (ex. 9x), and the date.

9. When your sample has been concentrated to the desired volume Stop the spinning motion, Lift the sample out of the water, and WHILE YOU CONTINUE TO HOLD ON TO GLASSWARE, turn the large knob (#5) toward you to stop the vacuum. When the gage reads zero the glassware should be easily removed.
10. Measure the sample volume -- if it is a bit below the desired level, pour a bit of the appropriate redistilled solvent into the glassware until the correct volume is obtained.

11. Record in your notebook the amount of sample you take out , and use this to calculate your next concentration value.
12. If you need to go home before you are done concentrating shut down the rotovap and place a stopper on your rotovap flask. You can place the entire flask in a large beaker as it will not spill and then label the beaker -- It is a good idea to label to what volume you are currently concentrating and what solvent is involved in case another lab worker picks up where you have left off. Make sure to also mark your container with ACREE. Place the entire beaker and its contents in the cooler.
13. When you are done:

    TURN OFF THE WATER!

    Turn off the heater

    Empty the solvent collected in the contain r marked #11 and place it in the appropriate waste bottles

GAS CHROMATOGRAPHY of Fruit and Vegetable Extracts.

After you have had the HP 5890 GC installed, attached the 19251A Split/Splitless injector and completed training in its operation you can begin to analyze for those components in the extract that interest you. From the literature you will find components such as linalool in citrus to use as indicators of flavor potency and process performance. Just how you go about this and how for you can go with the equipment available will depend on many things but one thing you should always do is describe every component analyzed in retention index values, e.g. Kovats. Retention indexes are the best way to communicate between colleagues. The following is a brief example of the calculation of retention indexes from the samples we ran during the work shop.

The gas chromatogram of the Hydrocarbon Standards (c7 to c18 or heptane to octadecane) yielded 12 peaks. These were used to calculate the retention index of the Flavor Standards. For example, in the Flavor Standards chromatogram the peak at 3.58 minutes the peaks produced by heptane and octane in the Hydrocarbon Standards chromatogram. Therefore, the retention of the peak at 3.58 minutes is 700 plus the percent 3.58 is between 2.15 heptane and 3.76 octane or (3.58-2.15)(100)/(3.76-2.15) + 700 =788. This is very close to 785 determined for ethyl butenoate in my lab using an entirely different gas chromatograph. Use this method to determine which peak corresponds which standard compound in the Flavor Standards chromatogram.

Compound	Amt.	RI
Ethyl butenoate	10ng/ul	785
Ethyl 2 methyl butenoate	5 ng/ul	832
Ethyl hexanoate	140 ng/ul	982
1,8 cineole	50 ng/ul	1019
menthol	4,800 ng/ul	1169
L-carvone	60 ng/ul	1215
o-aminoacetophenone	1 ng/ul	1260
m-anthranilate	10 ng/ul	1310
Benzaldehyde	400 ng/ul	927
b-damascenone	5 ng/ul	1360

— FLAVOR STANDARDS —

HP RUN.# 10 NOV/24/90 TIME 12:36:44

-15-

074

ESCAPE
TEMP1 400 50 80
TEMP1 2 2 5 W
TEMP1 400 225 74
TEMP1 5 0 W

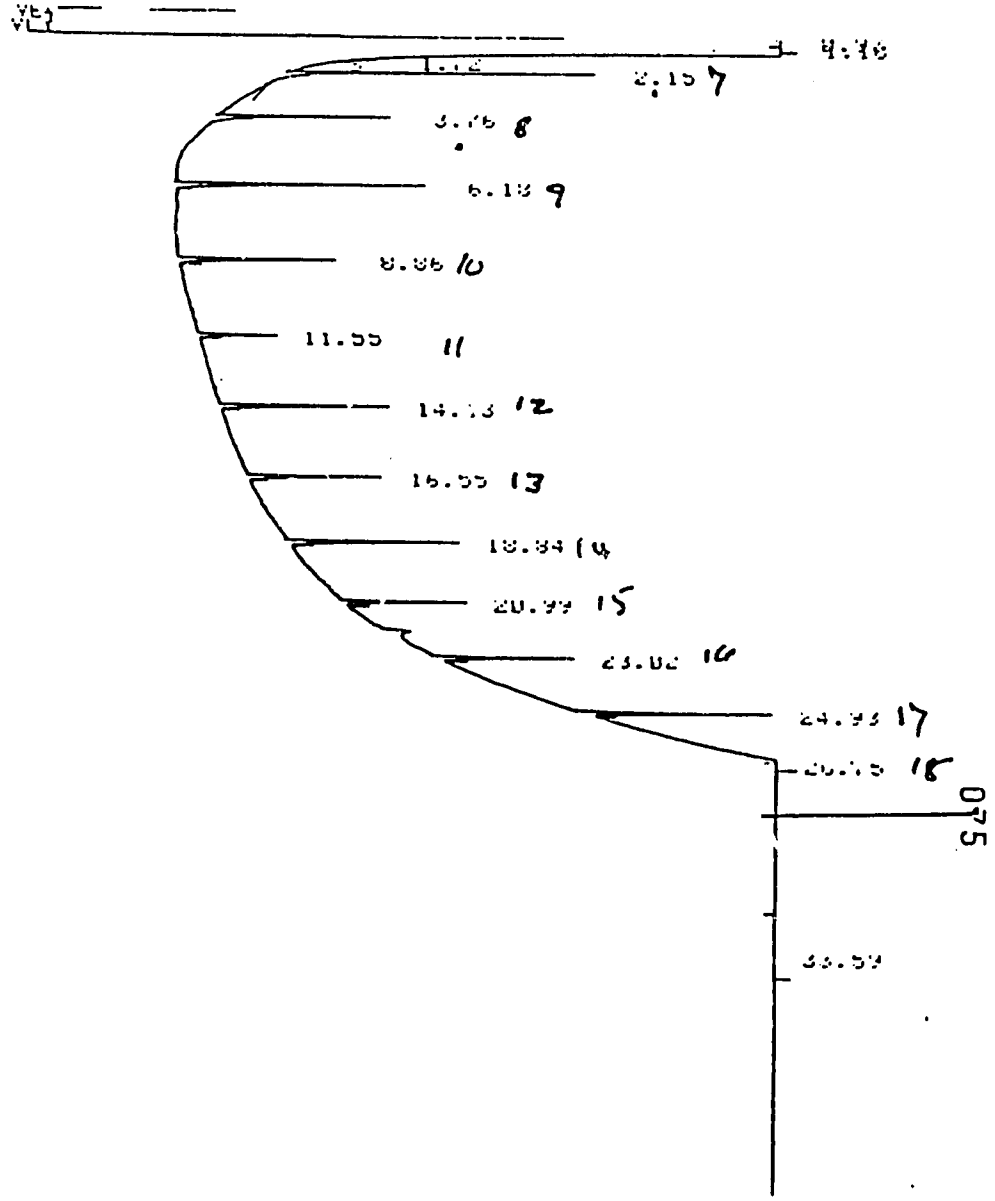
TEMP1 400 50 220
TIME1 3.00
RATE 6.00
TEMP2 400 225
TIME2 10.00
INJ TEMP 400 200 200
FID TEMP 400 250 250
AUX TEMP 400 200 200

CHT SPD 0.50
ZERO 20.0
MTH ZT 3
FID SGNL 48
SLP SENS 0.20
AREA REJ 0
FLOW A 0.0 57.6
FLOW B 0.0 28.4

0.00 VLV/EXT 1
0.50 VLV/EXT- 1

HYDROCARBON STANDARDS

POWER FAIL 13:18:55
TEMP1 400 50 114
FID TEMP 400 250 249
INJ TEMP 400 200 199
TEMP1 400 50 98
TEMP1 400 50 66
TEMP1 400 50 51
TEMP2V VE



075

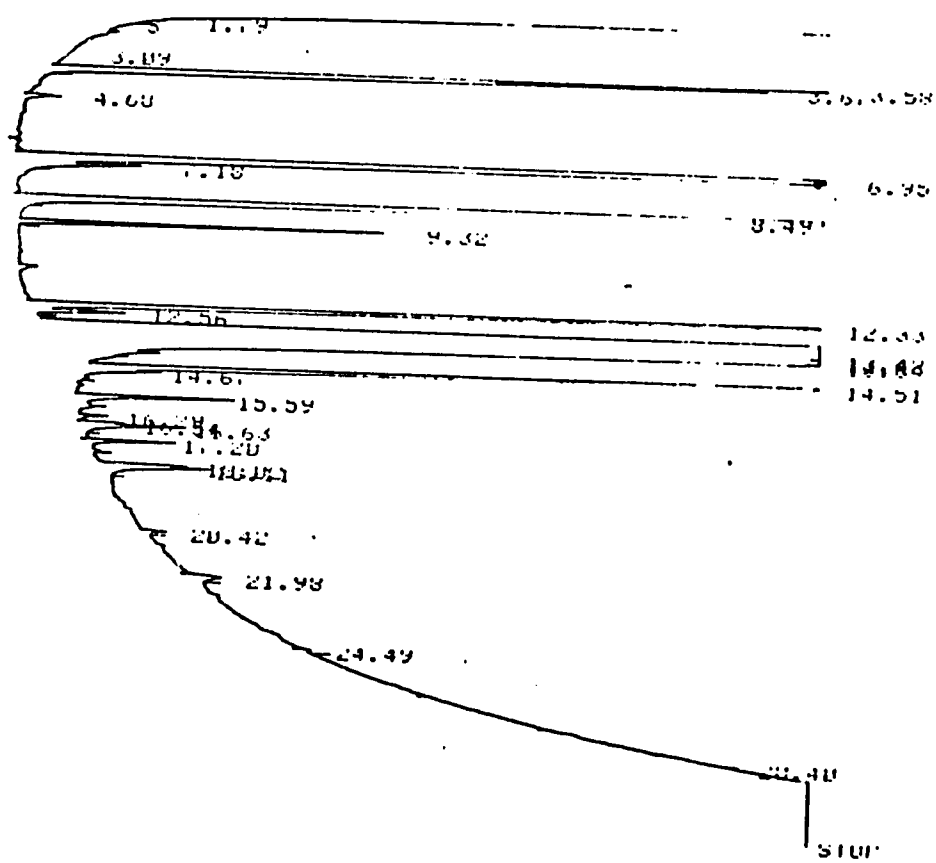
78

```
TEMP1      400    50    50
TIME1        3.00
RATE         6.00
TEMP2      400   225
TIME2       10.00
INJ TEMP   400   200   200
FID TEMP   400   250   250
HOX TEMP   400   200   200

CHT SPD      0.50
ZERO        20.0
HTIN 2T      3
FID SGNL     +8
SLP SENS     0.20
HKEH REJ
FLOW  H      0.0     0     57.2
FLOW  B      0.0     28.2

0.00 VLV/EXT
0.50 VLV/EXT-        1
                     1
ZERO 1 0 0
STRNET
```

Flavor Standards



HP RUN # 12

HP RUN # 11 NOV/24/90 TIME 13:25:12
RKEH %

MI	RKEH	RKEH %
0.93	131500000	96.025
1.18	5426000	3.962
1.72	49	0.000
2.15	831	0.001
3.76	583	0.000
6.18	1239	0.001
8.06	853	0.001
11.55	352	0.000
14.13	803	0.001
16.55	619	0.000
18.84	776	0.001
20.99	567	0.000
23.02	670	0.000
24.93	950	0.001
26.75	813	0.001
33.59	8230	0.006

DIL FACTOR: 1.0000 E+ 0

30

OCCURRENCE AND FORMATION OF ROAST-SMELLING ODORANTS IN WHEAT BREAD CRUST AND POPCORN

P. Schieberle

Deutsche Forschungsanstalt fur Lebensmittelchemie, Lichtenbergstraße 4, 8046 Garching, West Germany

1 Introduction

The characteristic roasty-sweet flavors of both wheat bread crust and popcorn are formed as a result of a heating process

By aroma extract dilution analysis (AEDA) and calculation of their odor units we could recently establish {1, 2} 2-acetyl-1-pyrroline (ACPY) and 2-acetyltetrahydropyridine (ACTPY) as the most important roast-smelling odorants in wheat bread crust Further studies revealed {2, 3} that during crust formation both flavor compounds are formed from proline and dihydroxyacetone phosphate supplied by the yeast.

In popcorn, fifty-seven volatile compounds were reported {4} Among them acetylpyrazine, which was identified in a steam distillate obtained from microwave popped corn {5}, was proposed as an important odorant on the basis of its popcorn-like flavor note. The contribution of the other volatiles to the popcorn flavor is yet unclear.

The purpose of this investigation was to evaluate the volatiles of popcorn by an AEDA and to compare the most important odorants to those earlier identified in wheat bread crust. Furthermore, the role of free amino acids in yeast and corn as precursors of roast-smelling flavor compounds was elucidated

2 Results and discussion

2.1 ODORANTS OF POPCORN

Corn (600 g; Purity Foods, Okemos, U.S.A.), popped in a commercial hot air corn popper, was frozen in liquid nitrogen, ground in a blender, extracted with dichloromethane and the volatiles isolated by sublimation in vacuo {1}. An AEDA of the flavor concentrate, which was freed from acidic compounds by treatment with sodium carbonate, revealed thirty-six odor-active fractions. The identification experiments in connection with the FD-factors revealed (Table 1) that 2-acetyl-1-pyrroline and (E,E)-2,4-decadienal, followed by 2-furfurylthiol and 2-methoxy-4-vinylphenol, were the most potent odorants of fresh popcorn. In addition, eleven further primary odorants were identified (Table 1, No. 5-13) among which the roast-smelling odorants 2-acetyltetrahydropyridine (No. 5) and 2-propionyl-1-pyrroline (No. 8) contributed with high FD-factors to the overall flavor. The latter compound, not yet reported among the food flavors, showed a similar odor quality and the same low odor threshold (0.02 ng/L air) as its homolog 2-acetyl-1-pyrroline. Details on the NMR and MS identification of this odorant will be published elsewhere {6}. The roast-smelling odorant acetylpyrazine, earlier identified in popcorn by Walradt et al. {5}, showed a low FD-factor.

TABLE 1. Odorants (FD ≥ 32) identified in popcorn {6}

No.	Compound[a)]	RI (SE-54)	Odor description	FD-factor
1	2-Acetyl-1-pyrroline	924	roasty	512
2	(E,E)-2,4-Decadienal	1318	fatty, waxy	512
3	2-Furfurylthiol	912	roasty, coffee-like	256
4	2-Methoxy-4-vinylphenol	1316	spicy	256
5	2-Acetyltetrahydropyridine	1049	roasty	128
6a	(E)-2-Nonenal	1161	green, tallowy, roasty, potato-like	128
6b	Diethylmethylpyrazine[b)]			
7	(Z)-2-Nonenal	1147	green	64
8	2-Propionyl-1-pyrroline	1026	roasty	64
9a	3-Ethyl-2,6-dimethylpyrazine	1086	roasty, potato-like	64
9b	6-Ethyl-2,3-dimethylpyrazine			
10	(E,E)-2,4-Nonadienal	1216	fatty, waxy	64
11	(E)-4,5-Epoxy-2-decenal	1381	metallic	64
12	ß-Damascenone	1386	cooked apple	32
13	2-Methoxyphenol	1091	burnt	32
14	Unknown	1281	hydrolyzed protein	32

a) The compound was identified by comparing it with the reference substance on the basis of the following criteria: RI on two stationary phases, mass spectra (MS/EI; MS/CI) and odor quality at the sniffing port.

b) The positions of the ethyl and methyl groups were not assigned.

2.2 COMPARISON OF PRIMARY ODORANTS IN POPCORN AND WHEAT BREAD CRUST

A comparison of the primary odorants in popcorn (Table 1) with those identified in wheat bread crust {1, 2} revealed that the roast-smelling flavor compounds ACPY and ACTPY are important odorants in the flavor profiles of both foodstuffs. 2-Furfurylthiol, 2-propionyl-1-pyrroline and 2-methoxy-4-vinylphenol were not identified as primary odorants of bread crust {1}. In contrast, the important crust odorants diacetyl (buttery) and 3-methylbutanal (malty) showed very low FD-factors in popcorn.

ACTPY is a relatively unstable compound. Furthermore, it was separated into two tautomers whose concentrations varied depending on the stationary phase of the GC capillary (unpublished results). To exactly determine its contribution to the flavor, the odor units of ACTPY were determined on the basis of quantitative data obtained by a stable isotope dilution analysis (SIDA) {6,7}. In Table 2 the odor units of ACTPY and ACPY in bread crust and popcorn are compared. The data revealed that, in contrast to bread crust, ACTPY is the most important roast-smelling odorant in popcorn.

2.3 PRECURSORS OF ACPY AND ACTPY

In recent studies {2} we could show that yeast supplies important precursors of the crust odorants ACPY and ACTPY. Model studies revealed {2, 3} that ACPY is formed from a reaction of proline and dihydroxyacetone phosphate via 1-pyrroline and 2-oxopropanal as intermediates.

To reveal whether further amino acids act as precursors of ACPY and ACTPY, the amounts of free amino acids present in bakers yeast were determined {8}. Eleven amino acids were identified, whose amounts ranged between 1290 mg/100 g dry yeast (glutamic acid) and 89 mg/100 g dry yeast (proline). The relatively

TABLE 2. Odor units of 2-acetyl-1-pyrroline and 2-acetyltetrahydropyridine in wheat bread crust and popcorn

Odorant	Odor unit [a]	
	Bread crust	Popcorn
2-Acetyl-1-pyrroline	4800	1600
2-Acetyltetrahydropyridine[b]	970	7280

[a] The odor units were determined on the basis of quantitative results {6} using the following odor thresholds: 0.02 ng/L air (ACPY); 0.06 ng/L air (ACTPY).

[b] The sum of both tautomers {cf. 2} is given.

rare amino acid ornithine amounted to 318 mg/100 g dry yeast.

In model experiments, each amino acid identified reacted then separately with 2-oxopropanal and the amounts of ACPY and ACTPY were determined by a SIDA {8}.

As detailed in Table 3, ACPY was generated from proline and ornithine in nearly equal amounts (No. 1 and 2), while ACTPY was exclusively liberated from proline. The resting amino acids were ineffective (data not shown).

The concentration of citrulline has been reported to increase in yeast under specific growing conditions {9}. Our data revealed (No. 3, Table 3) that this amino acid is a further important precursor of ACPY.

Replacement of 2-oxopropanal by fructose cancelled the formation of ACPY from proline, while the concentration of ACTPY increased by a factor of 2.5 (cf. No. 1 and 4; Table 3). This indicated that, in contrast to the formation of ACPY, the intermediate liberation of 2-oxopropanal was not necessary to generate ACTPY from proline and fructose.

Strecker degradation of ornithine does result in 4-aminobutyraldehyde as intermediate. The amino aldehyde rapidly cyclizes forming 1-pyrroline {10}. Further model experiments revealed (data not shown) that significant amounts of ACPY are liberated when 4-aminobutyraldehyde reacted with 2-oxopropanal. This results indicated that, as reported for the formation of ACPY from proline {2}, 1-pyrroline is also the key intermediate in the formation of ACPY from ornithine.

In the corn used for the experiments 155 mg/kg of free proline were determined, while ornithine was not detectable. This result provided an explanation for

TABLE 3. Amounts of 2-acetyl-1-pyrroline and 2-acetyltetrahydropyridine formed in amino acid/2-oxopropanal model solutions[a]

No.	Amino acid	2-Acetyl-1-pyrroline (μg)	2-Acetyltetrahydropyridine (μg)
1	Proline	41	160
2	Ornithine	43	<0.3
3	Citrulline	48	<0.3
4	Proline[b]	<0.3	430
5	Ornithine[b]	53	<0.3

[a] The amino acid (4 mmol), dissolved in 100 ml phosphate buffer (0.1 mol/L; pH 7.0), was reacted with 2-oxopropanal (0.1 mmol).

[b] 2-Oxopropanal was replaced by fructose (2 mmol).

the fact that ACTPY, which is the main roast-smelling odorant liberated from proline (cf. Table 3) predominated in popcorn. On the other hand, the amounts of free ornithine in yeast are four fold higher than the amounts of free proline. Therefore, in bread crust ACPY predominates (cf. Table 2).

2.4 FLAVOR ENHANCEMENT

To compare their effectiveness as enhancer of the roasty crust flavor note, the surface layer of a dough piece was spiked with either proline or ornithine, and the amounts of ACPY formed after baking were compared to a control bread crust without amino acid addition. As shown in Table 4 both amino acids led to an increase in the concentration of ACPY compared to the control (cf. No. 2 and 4 to No. 1). Ornithine was by a factor of two more effective than proline.

TABLE 4. Influence of additions of ornithine and proline to wheat doughs on the amounts of 2-acetyl-1-pyrroline formed during baking

Amino acid added[a] (1 mmol)	2-Acetyl-1-pyrroline[b] (µg/100 g of crust)
Without	1.9
Proline	5.9
Ornithine	9.4

[a] The amino acid, dissolved in 5 ml of phosphate buffer (0.1 mol/L; pH 7.0) was distributed in the surface layer of a dough piece.

[b] The compound was determined by a SIDA in an extract obtained by extraction with dichloromethane and sublimation in vacuo.

3 Conclusion

The results reveal that the roast-smelling crust odorants ACPY and ACTPY are also primary flavor compounds in popcorn. Higher FD-factors for 2-furfurylthiol and 2-methoxy-4-vinylphenol in popcorn but not in bread crust and higher FD-factors for 3-methylbutanal and diacetyl in bread crust but not in popcorn are undoubtedly responsible for the differences in both flavor notes.

Furthermore, it can be assumed that in popcorn ACPY is liberated from proline while in bread crust ornithine is its most important precursor.

REFERENCES

1. P. Schieberle and W. Grosch, Z.Lebensm.Unters.Forsch. 185, 111 (1987).
2. P. Schieberle in The Maillard Reaction in Food Processing, Human Nutrition and Physiology ed. by P.A. Finot, H.U. Aeschbacher, R.F. Hurrell and R. Liardon, Advances in Life Science, Birkhauser, Basel (1990), p. 187.
3. P. Schieberle in Thermal Generation of Aromas ed. by T.H. Parliment, R.J. McGorrin and C.-T. Ho, ACS Symposium Series 409, Washington (1989) p. 268.
4. S. van Straten and H. Maarse, Volatile Compounds in Food, qualitative data, TNO, Zeist, The Netherlands (1983)
5. J.P. Walradt, R.C. Lindsay and L.M. Libbey, J.Agric.Food Chem. 18,926 (1970).
6. P. Schieberle, J.Food Sci. (in progress).
7. P. Schieberle and W. Grosch, J.Agric.Food Chem. 35,252 (1987).
8. P. Schieberle, Z.Lebensm.Unters.Forsch. (in progress).
9. G.W. Malaney and D. Tanner, Biotech.Appl.Biochem. 10,42 (1988)
10. D.W. Lundgren, H. Tevesz and E.A. Krill, Anal.Biochem. 148,461 (1985).

J. Adda (Editor) *Progress in Flavour Research 1984*
Proceedings of the 4th Weurman Flavour Research Symposium, Dourdan, France, 9–11 May 1984
© 1985 Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

HEADSPACE ANALYSIS : TRAP DESORPTION BY MICROWAVE ENERGY APPLICATION TO THE VOLATILE COMPONENTS OF SOME TROPICAL FRUITS

B. Toulemonde and D. Beauverd
Développement Technologique Arômes
Nestlé Products Technical Assistance Co. Ltd.
CH-1814 La Tour-de-Peilz

ABSTRACT

In headspace analysis, the classical two-stage procedure with intermediate cold trapping can be avoided by using microwave energy. A device, acting as an injector and providing for near-instantaneous thermal desorption of the trapped components, has been tested and the method applied to the analysis of the volatiles from passion fruit, guava, litchi, starfruit and cherimoya. 60 and up to 110 compounds have been identified, a large number of substances not being previously described in these fruits.

INTRODUCTION

The information given by headspace analysis is well known and the advantages of this technique undisputed. In the study of flavours this method is irreplaceable since it is the only one that enables us to evaluate the exact composition of the vapour phase stimulating our olfactive receptors.

Numerous devices have been described, first of all direct introduction with a gas-tight syringe. Indeed this technique is very simple but it is not sensitive. For this reason one resorts to preconcentration on an adsorbent such as activated charcoal, Porapak or Tenax GC.

The desorption of trapped components can be carried out by a solvent or by heat, the latter solution being often preferred. In this case, the trap is generally heated by induction and the time to reach the correct temperature is too long to be consistent with direct injection. Therefore, most authors recommend intermediate cold trapping, requiring complex equipment and much handling, which can generate artefacts.

Recently, literature (ref. 1,2,3) described desorption using microwave energy, thus permitting a quick operation compatible with direct injection. We

equipped our laboratory with such a device and this paper gives the first results obtained with some tropical fruits (passionfruit, guava, litchi, starfruit and cherimoya)

EXPERIMENTAL SECTION

Trapping

A diagram of the apparatus is given in figure 1. The carrier gas (nitrogen, 20 ml/mn) is regulated by the needle valve (1), then purified by passing through a filter (2) filled with activated charcoal. The sample, 10-30 g of peeled fruit, is placed in the 200 ml vessel (3). The exhaust mixture passes through the ceramic cartridge (4) where the organic compounds are trapped on activated charcoal. Finally the flow is checked with a flowmeter (5).

In order to avoid any contamination, the whole system is purged and the trap desorbed before utilization. Depending on the amount of volatiles, the time of stripping varies from 3 to 90 minutes. The system operates at ambient temperature and a water trap was found unnecessary.

Desorption

The device (MW-1 from Rektorik analytical accessories) is set above the GC and acts as an injector. Apart from the pneumatic circuits, it mainly consists [illegible]rowave generator (2.45 GHz) and a waveguide (figure 2). The trap is [illegible]ted, then backflushed with helium for 25 seconds. After equilibration of the column pressure (0.8 bar), the generator is activated with 350 (arbitrary) units for 4 seconds and vaporization occurs. Whilst 2 ml of gas are admitted to the column, 20 ml are vented, thus giving a split ratio of 1:10. The temperature of the sampler-GC interface is kept at 200°C.

Gas Chromatography-Mass Spectrometry

The quadrupolar GC-EIMS HP 5995A is equipped with an open-split interface and a silica fused capillary column (30 m length, 0.32 mm i.diameter, OV-1, film thickness 0.32 um). The oven temperature is programmed from 20°C (3 mn) to 280°C with a rate of 4°C/mn. Identification occurs by comparing the mass spectra and the linear retention indices (ref.4) with those of authentic samples (either commercially available or synthetised in our laboratory).

RESULTS AND DISCUSSION

Figures 3-7 show the reconstructed chromatograms obtained by recording the total ion current. Except for acidic compounds (due to basic sites in the

column/interface), the peak shapes are correct. All experiments have duplicated and, after the end of the run, if the trap is desorbed again ignal is perceived. All these facts indicate that the injection is fast, lete and reliable.

:spite the splitless mode, sensitivity is good. The limit of adsorbtion is y the capacity of the activated charcoal, occasionally overloaded by some oric components.

e source of some aliphatic (C5-C16) and aromatic (benzene, toluene, e, styrene and naphtalene) hydrocarbons is not positive. It seems that are not due to contamination of the trap but really originate in the s. However, we found them in almost all tropical fruits and one can late on being in the presence of a pollution where fruits themselves act trap : further experiments should be done to confirm this statement: ile, although formally identified, these components are put in brackets les 1-5.

connection with this last remark, an unidentified substance (together wo isomers) occurs in passion fruit (peak 26), guava (26) and starfruit The main fragments of the mass spectrum are 43(100), 44(74), 101(47), 72(26), 73(25), 115(6).

Passion Fruit (Passiflora Edulis forma flavicarpa)

flavour of this fruit is very strong. It is maybe the reason why it has o extensively investigated. All species intermingled, the TNO compilation) gives 209 components to which one should add those newly described by (ref.6), Chen (ref.7), Engel (ref.8) and Tressl (ref.9). chromatogram is indicated in figure 3 and the identified components in table 1. Many new esters have been found, some being isomers sly described products.

Guava (Psidium Guajava L.)

The chromatogram is shown in figure 4 and the list of identified components in table 2.

Among the 83 products recorded by van Straten (ref.5), Wilson (ref.10) and McLeod (ref.11), only 24 have been found again. Surprisingly, we did not identify limonene nor myrcene and found far less ethyl acetate than did MacLeod. On the other hand we agree with the importance of hexanal, hexenal, ethyl hexanoate and hexenyl acetate. 42 new compounds are also described, mainly carbonyl containing chemicals.

Litchi (Litchi Chinensis Sonn.)

The chromatogram is given in figure 5 and the list of identified components in table 3.

In 1980 Johnston et al. (ref.12) found 42 components in a methylene chloride extract and attributed the floral odour to phenylethanol and to its derivatives. As for us, we only found traces of phenylethyl methyl ether. But we also identified methyl ethers form cyclohexyl, hexyl, benzyl, citronellyl and neryl alcohols. All these volatiles undoubtedly contribute to the typical floral odour of the litchi. The contribution of some other compounds such as limonene, rose oxyde, nonanal and decanal, citronellyl and geranyl alcohols and acetates, to fruity-floral and citrus notes is also unquestionable.

Starfruit (Averrhoa Carambola)

To our knowledge, no previous work has ever been published concerning the flavour composition of this fruit. At least 150 substances can be detected on the chromatogram (figure 7), 104 have been identified and are listed in table 5. The major components are esters, mainly methyl and ethyl esters of aliphatic acids. These products are known for their fruity notes and obviously, largely contribute to the overall impression.

Cherimoya (Annona Cherimolia)

We did not find any data regarding the volatiles' composition of cherimoya. Table 4 shows the 70 components identified in the chromatogram (figure 6). Here again, the headspace mainly consists of methyl and ethyl esters from aliphatic acids, especially from butanoic acid. Although it is important, this presence of butyrates is not sufficient to explain the unmistakable flavour of this peculiar fruit.

CONCLUSION

During these first trials, the desorption by microwave energy and the device used for this purpose showed good results, at least equivalent to those obtained by more conventional means. Its easy use and the time saved for the analysis are of particular interest. In our opinion, the main problem is presented by the adsorbant: the replacement of activated charcoal by a more selective polymer, such as Tenax-GC, should be studied.

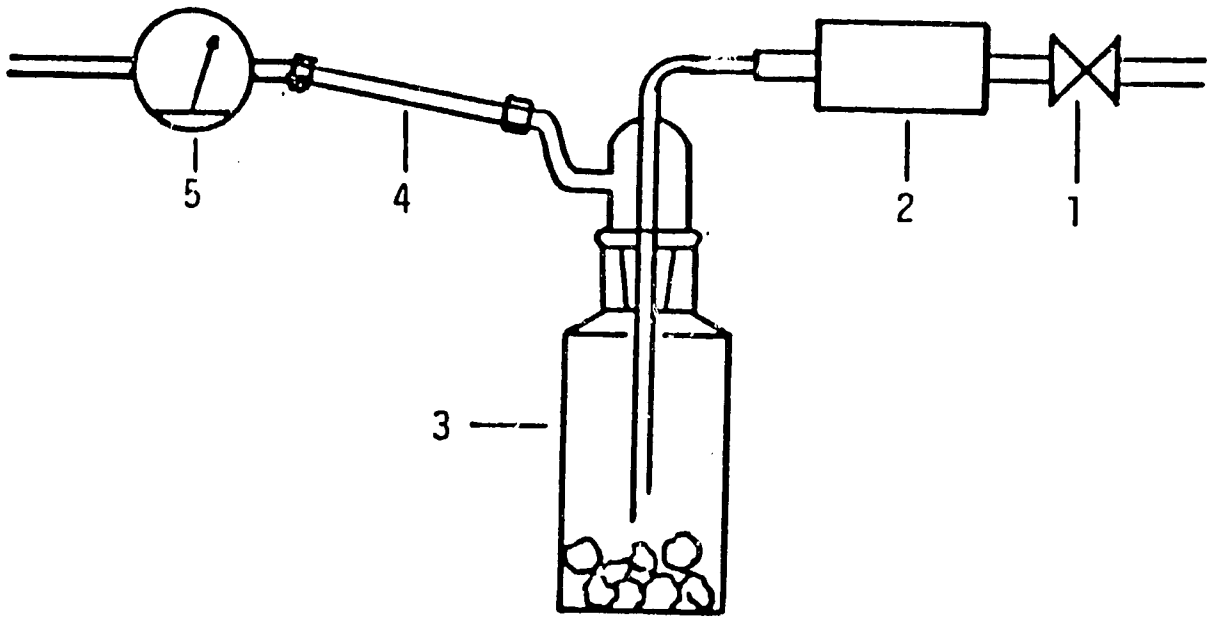


Fig. 1. Diagram of the trapping apparatus

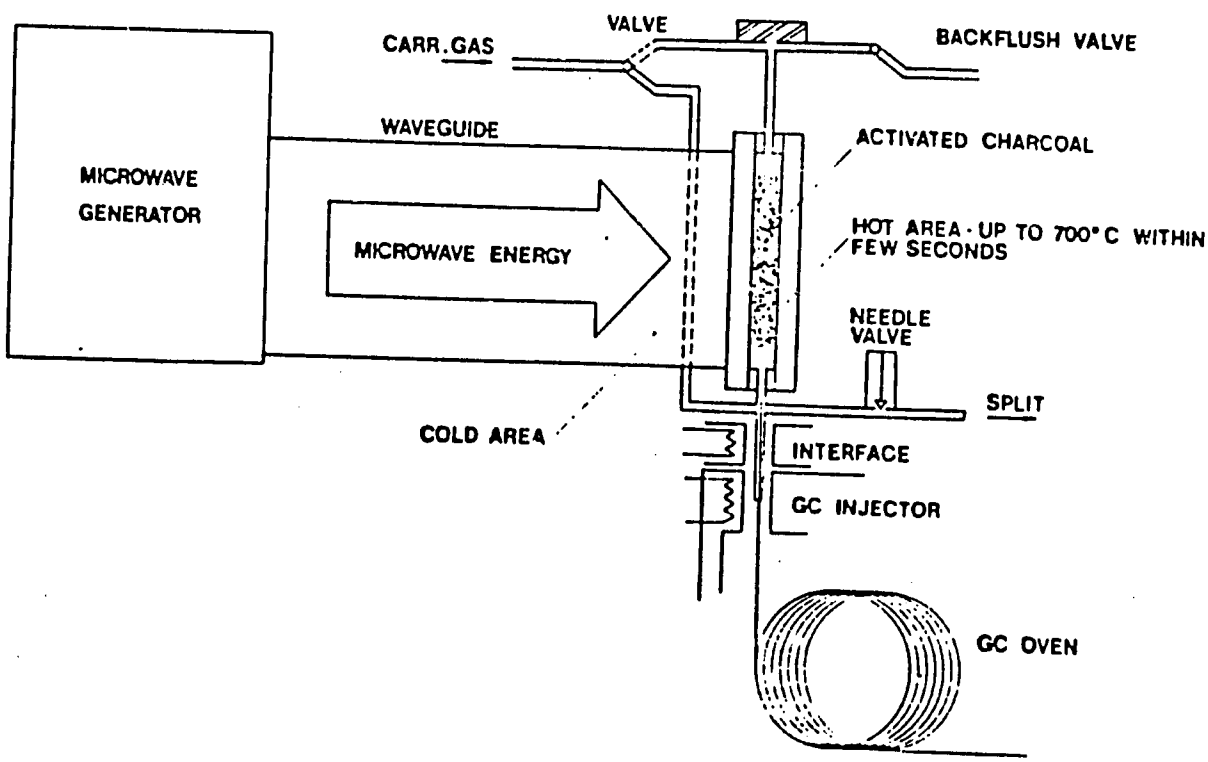


Fig. 2. Diagram of the desorption device
(by courtesy of J. Rektorik)

Fig. 3. Chromatogram from the headspace of yellow passion fruit

TABLE 1

Compounds identified from the headspace of yellow Passion Fruit

Peak	Compounds	Peak	Compounds
1	*Acetone	54	*α-Pinene
2	1-Pentene	57	(Trimethyl benzene)
3	*(Pentane)	58	Methylthiol hexanoate +
4	Carbon disulfide	59	(α-Methylstyrene)
6	2,3-Butanedione	61	(Trimethyl benzene)
7	2-Butanone	62	*Ethyl hexanoate
9	(Hexane)	63	*Hexyl acetate
10	*Ethyl acetate	64	(Decane)
11	*Acetic acid	65	*α-Terpinene
14	(Benzene)	66	para-Cymene
15	isoPropyl acetate	67	para-Menthene +
17	*2-Pentanone	68	*Limonene
19	*3-Pentanone	69	*Ethyl trans-2-hexenoate
20	*2-Pentanol/Ethyl acrylate	70	*cis-Ocimene
23	*(Heptane)	72	*trans-Ocimene
24	Propyl acetate	73	*γ-Terpinene
25	Methyl butanoate	76	3-Nonanone
26	ni (see text)	77	α, para-Dimethyl styrene
27	ni (isomer of 26)	78	*Terpinolene
28	ni (isomer of 26)	79	*Propyl hexanoate
29	*(Toluene)	80	*Ethyl heptanoate
30	*Cyclopentanone	81	*Linalool
31	*isoButyl acetate	82	*Rose oxyde
32	3-Hexanone	84	(Undecane)
33	*Diethyl carbonate	86	Methyl octanoate
35	*Hexanal	87	allo-Ocimene
36	*Ethyl butanoate	89	isoButyl hexanoate
37	*Butyl acetate	91	*(Naphtalene)
38	*Butanoic acid	92	*Terpinene-4-ol
39	*Ethyl trans-2-butenoate	93	*Ethyl 4,7-octadienoate +
40	isoPropyl butanoate	94	*Ethyl cis-4-octenoate
41	Ethyl 2-methylbutanoate	95	*Hexyl butanoate
42	Ethyl isopentanoate	96	*Ethyl octanoate
43	*(para-Xylene)	98	(Dodecane)
44	*(meta-Xylene)	99	Hexyl 2-Methylbutanoate
45	*isoAmyl acetate	100	isoAmyl hexanoate
46	sec.-isoAmyl acetate	105	(Methylnaphtalene)
47	*2-Heptanone	106	*Amyl hexanoate
48	*Propyl butanoate	107	Ethyl nonanoate
49	*Ethyl pentanoate	109	*Citronellyl acetate
50	Amyl acetate	110	*cis-3-Hexenyl hexanoate
52	Methyl hexanoate	111	*Hexyl hexanoate
53	Methyl cis-3-Hexenoate	114	(Tetradecane)
		119	(Hexadecane)

Peak number refers to the chromatogram (figure 3)
* Previously identified
\+ Tentative identification made on the sole mass spectrum
() Origin not elucidated (see text)

Fig. 4. Chromatogram from the headspace of guava

TABLE 2

Compounds identified from the headspace of guava

Peak	Compounds	Peak	Compounds
1	*2,3-Butanedione	52	2,4-Hexadienal
2	*2-Butanone	53	Butyl isobutanoate
4	*(Hexane)	54	Ethyl pentanoate
5	*Ethyl acetate	57	Amyl acetate
7	Acetic acid	59	*Methyl hexanoate +
9	*2-Butenal	62	*Benzaldehyde
13	*(Benzene)	63	Indane +
17	1-Penten-3-one	64	trans-2-Heptenal
18	2-Pentanone	67	(Trimethyl benzene
20	*Pentanal	73	Octanal
21	2-Pentanol	74	*Ethyl hexanoate
22	2-Ethyl furan	75	*cis-3-Hexenyl acetate
23	Ethyl propanoate	76	*Hexyl acetate
24	Propyl acetate	77	(Decane)
25	*Methyl butanoate	78	2,4-Hexadienyl acetate +
26	ni (see text)	79	Allyl benzene
27	cis-2-Pentenal	80	Indene +
29	trans-2-Pentenal	81	Ethyl trans-2-hexenoate
30	ni (isomer of 26)	82	trans-2-Octenal
32	*(Toluene)	83	*Methyl benzoate
33	ni (isomer of 26)	85	Nonanal
34	isoButyl acetate	89	ni 101(100) 55(39) 43(31) 73(26) 41(16) 45(13) 56(10)
36	*Hexanal		
37	*Ethyl butanoate	90	Methyl octanoate
38	*Butyl acetate	96	*Ethyl benzoate
39	Butanoic acid	97	(Naphtalene)
40	*cis-2-Hexenal	98	cis-3-Hexenyl butanoate
41	*trans-2-Hexenal	99	Hexyl butanoate
43	*cis-3-Hexenol	100	*Ethyl octanoate
45	(meta-Xylene)	102	(Dodecane)
47	*Hexanol	105	trans-2-Decenal
48	isoAmyl acetate	107	(Methylnaphtalene)
49	sec.-isoAmyl acetate	108	(Methylnaphtalene)
50	(Styrene)	111	Phenylpropyl acetate
51	(ortho-Xylene)	113	(Tetradecane)
		115	(Hexadecane)

Peak number refers to the chromatogram (figure 4)
* Previously identified
+ Tentative identification made on the sole mass spectrum
() Origin not elucidated (see text)

Fig. 5. Chromatogram from the headspace of litchi

TABLE 3

Compounds identified from the headspace of litchi

Peak	Compounds	Peak	Compounds
1	2,3-Butanedione	45	(Decane)
2	2-Butanone	46	*α-Terpinene
3	2-Butanol	47	para-Cymene
5	Ethyl acetate	48	*Limonene
6	2-Methyl-1-propanol	49	Ocimene
7	3-Methyl butanal	50	*γ-Terpinene
8	(Benzene)	51	*Methyl phenylethyl ether
9	Butanol	52	α, para-Dimethyl styrene
10	2-Pentanone	53	*Terpinolene
11	3-Pentanone	54	Nonanal
12	*3-Hydroxy 2-butanone +	56	cis-Rose oxyde
16	(Heptane)	57	(Undecane)
17	Propyl acetate	58	trans-Rose oxyde
18	3-Methyl 3-buten-1-ol	59	1,3,8-Menthatriene
19	2-Hexanone	60	Neroloxyde
20	2-Methylbutanol	62	(Naphtalene)
22	Dimethyl tetrahydrofuran +	63	*Terpinen-4-ol
23	(Toluene)	64	Citronellyl methyl ether
24	trans-2-Pentenal	65	Ethyl octanoate
25	isoButyl acetate	66	Decanal
26	Hexanal	67	Neryl methyl ether
27	Butyl acetate	68	(Dodecane)
28	Methyl cyclohexyl ether +	69	*Citronellol
29	Methyl hexyl ether	70	*Geraniol
30	(para-Xylene)	71	(Tridecane)
31	(meta-Xylene)	72	Citronellyl acetate
32	*Hexanol	73	Geranyl acetate
33	*isoAmyl acetate	74	Ethyl decanoate
34	Amyl acetate	75	Sesquiterpene
35	(Styrene)	76	(Tetradecane)
36	(ortho-Xylene)	77	β-Caryophyllene
37	Anisole	78	α-trans-Bergamotene
38	(Nonane)	80	Humulene
39	2-isoPentenyl acetate	82	ar-Curcumene
40	Cumene	83	Sesquiterpene mw=204
41	Benzyl methyl ether	84	Sesquiterpene mw=204
42	(Trimethyl benzene)	86	(Pentadecane)
43	*Myrcene	87	Sesquiterpene mw=204
44	α-Phellandrene	89	*(Hexadecane)

Peak number refers to the chromatogram (figure 5)
* Previously identified
\+ Tentative identification made on the sole mass spectrum
() Origin not elucidated (see text)

Fig. 6. Chromatogram from the headspace of cherimoya

TABLE 4

Compounds identified from the headspace of cherimoya

Peak	Compounds	Peak	Compounds
1	2,3-Butanedione	40	α-Pinene
2	2-Butanone	41	isoButyl butanoate
4	(Hexane)	43	isoAmyl propanoate
6	Acetic acid	44	β-Pinene
7	Methyl propanoate	46	(Trimethyl benzene)
8	isoPentanal	47	Butyl butanoate
9	(Benzene)	48	Ethyl hexanoate
10	Butanol	49	cis-3-Hexenyl acetate
11	2-Pentanone	50	Hexyl acetate
12	Methyl isobutanoate	52	para-Cymene
13	2-Pentanol	53	para-Menthene +
14	(Trichloroethylene)	54	Eucalyptole
15	Ethyl propanoate	55	Limonene
16	Methyl butanoate	56	Butyl 2-methylpropenoate +
17	Methyl methacrylate +	58	Butyl isopentanoate
18	isoPentanol	59	Ocimene
19	Methyl 2-butenoate	60	isoAmyl isobutanoate
20	(Toluene)	61	isoAmyl butanoate +
21	Methyl isopentanoate	68	Terpinolene
22	Hexanal	69	Amyl butanoate
24	Ethyl butanoate	70	ni 69(100) 41(72) 70(60)
25	Butyl acetate		55(48) 43(40) 87(27) mw=136
26	Butanoic acid	71	isoAmyl isopentanoate
27	Methyl pentanoate	72	Methyl octanoate
28	Ethyl 2-butenoate	73	allo-Ocimene
29	Ethyl 2-methylbutanoate	74	Methyl trans-2-octenoate
30	Ethyl isopentanoate	75	(Naphtalene)
31	(meta-Xylene)	77	Hexyl butanoate
32	Me 3-hydroxy-3-methylbutanoate	78	Ethyl octanoate
33	Hexanol	80	Ethyl trans-2-octenoate
34	isoAmyl acetate	82	Methyl decanoate
35	(Styrene)	83	Ethyl decanoate
36	(ortho-Xylene)	84	(Tetradecane)
37	Propyl butanoate	85	(Pentadecane)
38	Butyl propanoate	86	(Hexadecane)
39	Methyl hexanoate		

Peak number refers to the chromatogram (figure 6)
+ Tentative identification made on the sole mass spectrum
() Origin not elucidated (see text)

Fig. 7. Chromatogram from the headspace of starfruit

TABLE 5

Compounds identified from the headspace of starfruit

Peak	Compounds
1	2,3-Butanedione
2	2-Butanone
3	2-Butanol
4	(Hexane)
5	Ethyl acetate
6	Acetic acid
7	Methyl propanoate
10	(Benzene)
11	Propyl acetate
12	Butanol
14	2-Pentanone
15	Methyl isobutanoate
20	isoPropyl hexanoate
21	Methyl butanoate
22	ni (see text)
23	isoPentanol
24	2-Methylbutanol
25	ni (isomer of 22)
26	Methyl 2-butenoate
27	Ethyl isobutanoate
28	isoButyl acetate
29	Methyl 2-methylbutanoate
30	Diethyl carbonate +
31	Ethyl 2-methylprop-2-enoate
32	cis-3-Hexenal
33	Hexanal
34	Ethyl butanoate
35	(Tetrachlorethylene)
36	Butyl acetate
37	Butanoic acid
38	Methyl pentanoate
39	ni 41(100) 71(87) 99(56) 39(32) 43(30) 69(28) 114(19)
40	trans-2-Hexenal
41	Ethyl 2-butenoate
42	Ethyl 2-Methylbutanoate
44	(para-Xylene)
45	Methyl tiglate
46	(meta-Xylene)
47	2-Methylbutanoic acid
48	isoAmyl acetate
49	sec.isoAmyl acetate
50	(Styrene)
51	(ortho-Xylene)
52	trans-2,trans-4-Hexadienal
54	Propyl butanoate
55	Ethyl pentanoate
56	Butyl propanoate
57	Amyl acetate
58	Methyl hexanoate
68	(Trimethyl benzene)
70	(Trimethyl benzene)
71	β-Pinene
74	Hexanoic acid
75	Ethyl hexanoate
76	cis-3-Hexenyl acetate
77	Hexyl acetate
79	α-Terpinene
80	para-Cymene
81	ni 43(100) 93(87) 41(45) 91 (32) 108(30) 81(30) 154(25)
82	Limonene
83	Ethyl trans-2-hexenoate
84	Butyl 2-methylbutanoate
87	γ-Terpinene
89	Methyl benzoate
90	Ethyl 2,4-hexadienoate
91	Ethyl 2,4-hexadienoate
92	Terpinolene
93	Propyl hexanoate
94	Ethyl heptanoate
96	(Undecane)
97	Methyl octanoate
98	allo-Ocimene
99	Menthone
100	isoMenthone
101	Ethyl benzoate
102	(Naphtalene)
103	Terpinen-4-ol
104	Heptanoic acid
105	cis-3-hexenyl butanoate
106	ni mw=170
107	Hexyl butanoate
108	Ethyl octanoate
109	Octyl acetate
110	(Dodecane)
111	Methyl nonanoate
114	Piperitone
115	Ethyl trans-2-octenoate
116	isoAmyl caproate +
120	Ethyl nonanoate
121	(Tridecane)
122	Methyl decanoate
123	Methyl cinnamate
125	Methyl methylanthranilate
126	Ethyl decanoate
127	Sesquiterpene
130	(Tetradecane)
131	β-Caryophyllene
133	Ethyl cinnamate

49

TABLE 5 (continued)

Peak	Compounds	Peak	Compounds
59	Methyl cis-3-hexenoate	135	Humulene
60	Methyl trans-3-hexenoate	137	ar-Curcumene
61	Ethyl tiglate	138	Sesquiterpene mw=204
62	α-Pinene	139	Sesquiterpene mw=204
63	Propyl 2-methylbutanoate	141	(Pentadecane)
64	Phenylacetaldehyde	142	Sesquiterpene
65	Butyl isobutanoate	144	(Hexadecane)
66	(Trimethyl benzene)		

Peak number refers to the chromatogram (figure 7)
+ Tentative identification made on the sole mass spectrum
() Origin not elucidated (see text)

REFERENCES

1 J. Rektorik, Chimie Magazine, 5 (1982) 42-43

2 H.J. Neu, W. Merz and H. Panzel, HRC&CC, 7 (1982) 382-384

3 J. Rektorik, Proc. 5th Int. Symp. Capillary Chromatography, Elsevier ..dam, 1983, pp. 396-405

4 van den Dool and P. Dec. Kratz, J. Chromatog.,11 (1963) 463-471

5 S. van Straten and H. Maarse, volatile compounds in food, CIVO-TNO, Zeist-NL, 1983

6 D.J. Casimir, J.F. Kefford and B. WHitfield, Adv. Food Res., 27(1981) 243-296

7 C.-C. Chen, M.-C. Kuo, L.S. Hwang, J.S.-B. Wu and C.-M. Wu, J. Agric. Food Chem., 30(1982) 1211-1215

8 K.-H. Engel and R. Tressl, J. Agric. Food Chem. 31(1983) 998-1002

9 R. Tressl and K.-H. Engel, Instrumental Analysis of Food, Academic Press, New York, 1983, pp. 153-182

10 C.W. Wilson and P.E. Shaw, Phytochem., 17 (1978) 1435-1436

11 A.J. MacLeod and N. Gonzales de Tronconis, Phytochem., 21 (1982) 1339-1342

12 J.C. Johnston, R.C. Welch and G.L.K. Hunter, J. Agric.Food Chem. 28(1980) 859-861

50

J. Adda (Editor) *Progress in Flavour Research 1984*
Proceedings of the 4th Weurman Flavour Research Symposium, Dourdan, France, 9–11 May 1984
© 1985 Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

VOLATILE CONSTITUENTS OF ALGAE. ODORIFEROUS CONSTITUENTS OF SEAWEEDS AND STRUCTURE OF NOR-TERPENOIDS IDENTIFIED IN ASAKUSA-NORI FLAVOUR.

I. FLAMENT and G. OHLOFF

FIRMENICH SA, Research Laboratories, 1211 Geneva 8 (Switzerland)

ABSTRACT

The richness and the diversity of the chemistry of algae is illustrated by several publications on the identification of special sesquiterpenes, structure elucidation of hydrocarbons playing an important role in the sexual reproduction of brown algae, antimicrobial effect of some cyclic polysulphides, toxicity of halogen-containing constituents and analysis of water off-flavours produced by the degradation of algae. After a presentation of some aspects of harvesting, processing and consumption of edible algae, we discuss the structure of new nor-carotenoids which have been isolated from *Porphyra tenera*, known as Nori or Asakusa-nori.

INTRODUCTION

As pointed out by Chapman (ref. 1) seaweeds belong to a rather ill-defined assemblage of plants known as the algae. The term "seaweed" itself does not have any taxonomic value, but is rather a popular term used to describe the common large attached (benthic) marine algae found in the groups Chlorophyceae, Rhodophyceae, Phaeophyceae or green, red and brown algae respectively. The algae differ from the higher plants in that they do not possess true roots, stems or leaves, though some of the larger species possess organs presenting this appearance. With exception of Cyanophyceae (blue-green algae) which are true procaryotes and closer in evolution to the bacteria, macroscopic algae are formed of eucaryotic cells containing membrane-bounded organelles.

Seaweeds are widely distributed around the world: they grow in the cold waters of the Arctic as well as in the temperate climate of the central Pacific. Most of them have some commercial importance in food, cosmetic, textile, dye and paper industries for example. Their antibacterial, antibiotic, antifungal, antiviral, anti-inflammatory, and anticurare properties are of value in pharmacy and medicine. They are also a source of mannitol, polysaccharides (agars, carrageenans, gelans), emulsifying and thickening agents or stabilizers. Let us not forget, particularly in the context of the present symposium, that diatoms are fossil deposits of the silicified coats of minute brown algae.

Since ancient times, peoples living along the sea coasts have learned how to consume these "vegetables from the sea" either directly or after treatment. They are now being reconsidered in the hope of coping with future food shortages and even as a convenient way to maintain life in space ships.

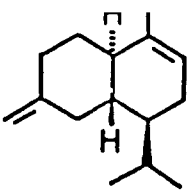
SOME ASPECTS OF THE CHEMISTRY OF ALGAE

Phycology (derived from the Greek word *phukos*, which means seaweed), has been enriched over the past twenty years, by the successful development of "phycochemistry", itself indebted to the tremendous progress of analytical techniques. The richness of the field can be illustrated by some original studies which have been conducted on different classes of chemical constituents.

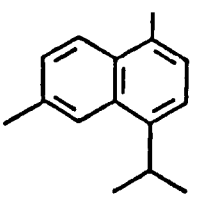
1. Identification of sesquiterpenes in the essential oil of *Dictyopteris divaricata*

In 1951, Takaoka and Ando (ref. 2) reported that an oil with a "beach-odour" can be obtained by steam-distillation of *Dictyopteris divaricata*, a brown alga that grows on the coasts of Japan. This extract consisted of sesquiterpene hydrocarbons and alcohols as shown by the azulene which was obtained on dehydrogenation. This result was reexamined in 1964 by Irie and co-workers (ref. 3) who confirmed the identity of (-)-α-copaene (1), (-)-γ_1-cadinene (2), cadalene (3), (-)-δ-cadinol (4), and (+)-ε-elemene (5).

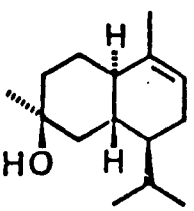
(1)



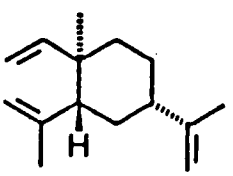
(2)



(3)



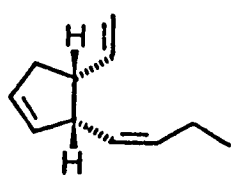
(4)



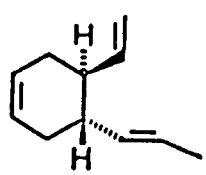
(5)

2. Structures of hydrocarbons playing an important role in the sexual reproduction of brown algae

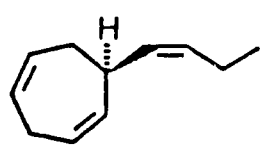
In contrast to the Japanese and Californian species of *Dictyopteris*, the Hawaiian *D. plagiogramma* (montagne) Vickes and *D. australis* Sonder elaborate non-isoprenoid C_{11} compounds in the essential oil instead of sesquiterpenes. These brown seaweeds, known as "limu lipoa" grow abundantly on the shores of the Hawaiian Islands; they are used as a condiment with raw fish and other foods (ref. 4). Some of the hydrocarbons present in these algae are chemical messengers for male gametes : they are mainly highly unsaturated C_{11} aliphatic or cyclic hydrocarbons with unsaturated side chains. Such hydrophobic compounds were isolated from cultures of mature gynogametes of Phaeophyceae to which they confer a fine and aromatic scent (ref. 5). For instance, multifidene (6), aucantene (7), and ectocarpene (8) show significant luring activity in the Mediterranean seaweed *Cutleria multifida* (ref. 6). 1,3,5-Undecatriene (9) was discovered in the volatile part of the Hawaiian seaweed *Dictyopteris* (ref. 7) and 1,3,5,8-undecatetraene (10) acts as a sex hormone in the brown alga *Ascophyllum nodosum* (ref. 8). Compound (9) also known as galbanolene was identified as the character impact compound in the essential of galbanum (ref. 9 and 10); it has also been found in yuzu peel oil (ref. 11) and, together with (10), in lavender oil (ref. 12).



(6)

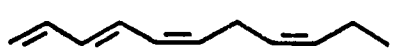


(7)



(8)

(9)

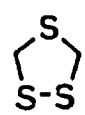


(10)

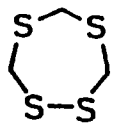
3. Cyclic polysulphides of *Chondria californica*

The Mexican red alga *Chondria californica* (ref. 13) possesses a strong sulphur odour and produces a crude extract exhibiting antimicrobial activity against *Vibrio anguillarium* (ref. 13). This effect is possibly due to a mixture of cyclic polysulphides and their oxidation products : 1,2,4-trithiolane (11)

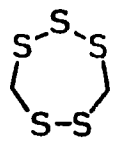
1,2,4,6-tetrathiepane (12), 1,2,3,5,6-pentathiepane (13), 1,2,4-trithiolane 1-oxide (14), 1,2,4-trithiolane 4-oxide (15), 1,2,4,6-tetrathiepane 1,1-dioxide (16), and 1,2,4,5,7,8,10,11-octathiacyclododecane (17).



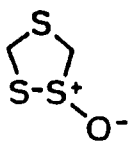
(11)



(12)

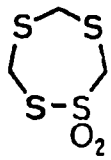


(13)

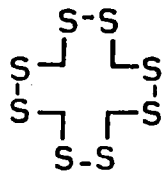


(14)

(15)



(16)

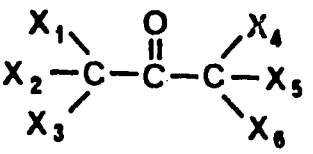


(17)

The major constituent, lenthionine (13), is an odoriferous antibiotic also isolated from the edible mushroom *Lentinus edodes* (ref. 14).

4. Identification and toxicity of halogen-containing constituents of *Asparagopsis*

The essential oil of *Asparagopsis taxiformis*, the favourite edible seaweed in Hawaii (limu kohu) contains haloforms and other halogenated compounds mainly containing bromine and iodine. Little is known about the physiological properties of most of these constituents although certain of them have been shown to be carcinogenic or toxic. Following Burreson and co-workers (ref. 15) whether limu kohu should be considered as a dangerous seaweed to eat (usually as a condiment) must await further study. The following list illustrates the wide variety of halogenated acetones (18) which have been identified in *Asparagopsis*.



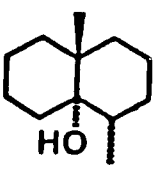
(18)

	X_1	X_2	X_3	X_4	X_5	X_6
a	Br	—	—	—	—	—
b	I	—	—	—	—	—
c	Br	Br	—	—	—	—
d	Br	—	—	Br	—	—
e	I	—	—	Br	—	—
f	Br	Br	Br	—	—	—
g	Br	Br	Cl	—	—	—
h	Br	Br	—	Br	—	—
i	I	—	—	Br	Br	—
j	Br	Br	—	Br	Br	—
k	Cl	Cl	Cl	Cl	Cl	Cl

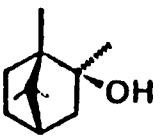
5. Analysis of water off-flavours produced by the degradation of algae

Sometimes undesirable flavours are produced by the formation of volatile excretion compounds resulting from the particular metabolism or the decomposition of certain varieties of algae.

It has been established that geosmin (19) and 2-methylisoborneol (20) are the major cause of the muddy flavour reported in various types of fish. The off-flavour has been associated with the presence of species of actinomycetes and blue-green algae in aqueous environments (ref. 16).



(19)



(20)

55

Some volatile compounds, such as hydrogen sulphide (21), methanethiol (22), dimethyl mono-, di-, and trisulphide (23-25) and the thioesters, *S*-methyl thioacetate (26) and *S*-methyl thiopropionate (27) have obvious undesirable effects. They are produced during the decomposition of algal mats (ref. 17) and have been noticed during bloom formation by Cyanophyceae or Chrysophyceae species which accidentally appear in drinkable waters confined in lakes and reservoirs (ref. 18 and 19).

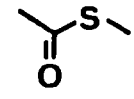
H_2S (21)

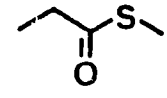
CH_3SH (22)

CH_3-S-CH_3 (23)

$CH_3-S-S-CH_3$ (24)

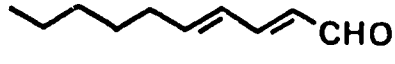
$CH_3-S-S-S-CH_3$ (25)


(26)


(27)

In addition to the noxious effect of the previous compounds (19) and (20), the origin of an offensive cod liver oil-like odour imparted to water contaminated by *Synura uvella* has been attributed to the formation of (*E*,*Z*)- and (*E*-*E*)-deca-2,4-dienal (28, 29) and of (*E*,*Z*)-hepta-2,4-dienal (30). These aldehydes which have very low odour thresholds are produced during cell lysis by the action of lipoxygenase on linoleic, γ-linolenic, and arachidonic acids (ref. 19).

(28)

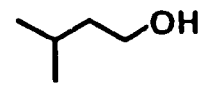

(29)

(30)

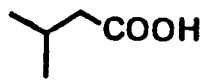
Yasuhara and Fuwa (ref. 20) attribute the aggressive odour of rotten blue-green algae to a mixture of acids, alcohols and esters (31) to (37).

(31)

(32)

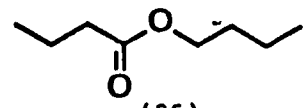


(33)

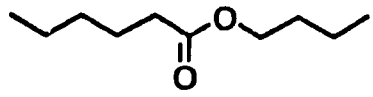


(34)

(35)



(36)

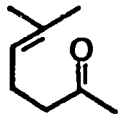


(37)

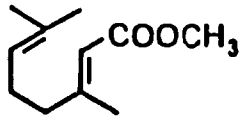
6. Structure of nor-carotenoids identified in non-edible algae

The odours of algal blooms have been described (ref. 21) as being fish musty and septic (explained by the presence of above mentioned products), also as being sweet and reminiscent of violet and geranium. This observation has been corroborated by the identification in such media, of degradation fragments of carotenoids; these compounds have long been well known to essential oil chemists.

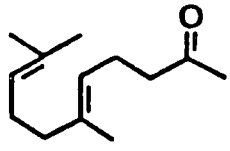
Acyclic nor-carotenoids such as 6-methyl-5-hepten-2-one (38), methyl geranate (39) and geranylacetone (40) have been identified by Juettner in the excretion products of *Cyanidium caldarium* (ref. 21 and 22), *Spirulina maxima* (ref. 23), and *Synechococcus 6911* (ref. 24). The relationship between polyene-carotene content and such volatile compounds, also identified in tomatoes, has been studied and discussed by Stevens (ref. 25).



(38)



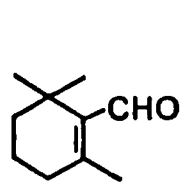
(39)



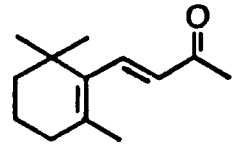
(40)

Cyclic nor-carotenoids have also been identified in microalgae : β-cyclocitral (41), β-ionone (42), (6E)-6-[(E)-2-butenylidene]-1,5,5-trimethylcyclohexene (43), 1,1,6-trimethyl-1,2-dihydronaphthalene (44), and dihydroactinidiolide (45). 5,6-Epoxy-β-ionone (46) and 2,6,6-trimethyl-2-cyclohexene-1,4-dione (47) are only hypothetical constituents of several *Synechococcus* species (ref.

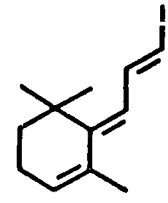
28). β-Cyclocitral (41) and β-ionone (42) have been identified in *Synura uvella* (ref. 23 and 18) and *Anabaena cylindrica* (ref. 26). Compound (41) was also detected in *Microcystis aeruginosa* and *M. wesenbergii* (ref. 27), and (42) in *Synechococcus* (ref. 28) as well as (45). The four compounds (41) to (44) have also been found in *Cyanidium caldarium* (ref. 21).



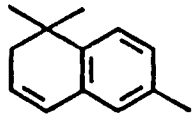
(41)



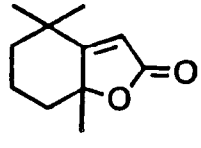
(42)



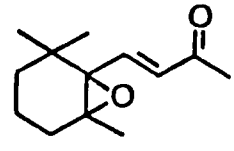
(43)



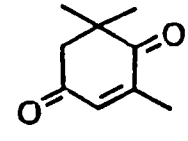
(44)



(45)



(46)



(47)

ANALYSIS OF "NORI", THE DRIED LAVER *Porphyra tenera*

1. Cultivation and preparation of nori

Following S. and T. Arasaki (ref. 29), people who, as a rule, consume seaweeds, live in Japan, China, Korea, Southeast Australia, New Zealand, Polynesia, and South America. So popular are these foods in Japan that in recent years natural crops of laver (nori), and of other popular algae, have been insufficient to the demand so that an artificial cultivation is now essential. Tables 1 and 2 indicate the production and consumption of seaweeds in Japan (ref. 29).

TABLE 1

Production of edible seaweeds in Japan (in tons)

Aonori	3,130
Hijiki	2,000
Kombu	32,042
Wakame	97,211
Mozuku	3,000
Tengusa	15,150
-Asakusa-nori	20,790

TABLE 2

Per capita annual consumption of seaweeds by the Japanese (1969-71, in grams)

Aonori (*Enteromorpha* sp.) and Hitoegusa (*Monostroma nitidum*)	31
Hijiki (*Hizikia fusiforma*)	20
Kombu (*Laminaria* sp.)	320
Wakame (*Undaria pinnatifida*)	972
Mozuku (*Nemacystus decipiens*)	30
Asakusa-nori (*Porphyra* sp.)	208
Total	1581

After wakame and kombu, asakusa-nori is the most popular seaweed used in japanese gastronomy. In 1973, 9.6 billion sheets of dried laver (25,920 t if each sheet weighs 2.7 g) was produced, the per capita consumption being about 96 sheets or nearly 259 g annually. The paper-thin sheets of asakusa-nori are produced from *Porphyra* species, especially *Porphyra tenera*. The method devized for their cultivation is to construct an artificial substratum by immerging fixed or floating nets in the littoral areas near river mouths at about the end of september or the beginning of october. A comprehensive study of the complex life cycle of the plant has led to the possibility of wide-scale artificial cultivation. The spores settled on the nets begin to grow rapidly and are nipped off for processing when they reach about fifteen centimeters in length. *Porphyra* disappears in the summer, but from ten to twelve harvests can be made each year. After removing from the sea, the fronds are thoroughly washed in fresh water, finely chopped, and while net, spread thinly on reed screens to dry. Today, the algae are mechanically shredded and dried in blowers at a temperature of 35-45°C for 2 to 3 hours. After drying, the water content is as low as 5%. In some cases, the seaweeds are submitted to a heat treatment at 60°C for two days to one week; the procedure combining the double advantage of reducing moisture content and

59

inactivating undesirable enzymes. This toasting procedure is identical to that used for the preparation of some kinds of tea. After this treatment Asakusa-nori acquires a fragrance, a crispness, and a typical light flavour which is particularly appreciated by the consumer. This treatment is certainly the cause of formation of the nor-carotenoids whose structures we shall later discuss, and which are identical or related to some typical constituents of tea. In general, the sheets of nori are cut or crumbled for addition to other dishes but one of the most important uses is as an outer covering for various kinds of rolled "sushi" (ref. 29).

2. Analysis of the volatile flavour constituents of nori

Relatively few studies have been devoted to the flavour constituents of edible algae. An interesting series of articles has been published by Katayama in the fifties and reviewed in 1962 (ref. 30). This work covers constituents of green algae as *Ulva*, *Enteromorpha*, and *Codium* species, brown algae as *Sargassum* and *Laminaria* species, and red algae as *Porphyra tenera*, the source of natural Asakusa-nori.

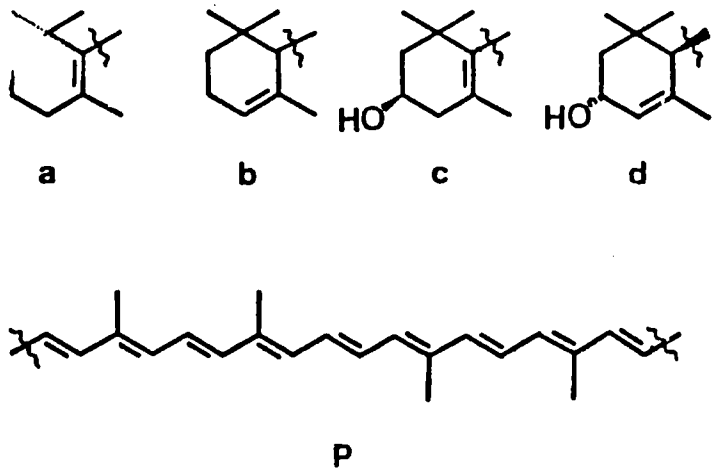
Literature on nori flavour substances is poor. In addition to the 22 compounds identified by the Katayama research group only three papers have appeared on the subject : Noda and co-workers successively measured the concentration of dimethyl sulphide in nori of various origins (ref. 31), analyzed their content in free amino acids and 5-nucleotides (ref. 32), and in sugars, organic acids and minerals (ref. 33). A recent article of Kasahara and Nishibori (ref. 34) mentions the identification of simple and trivial aldehydes and ketones.

The analytical work performed by our group has led to the identification of more than 100 new volatile constituents of nori flavour; the detailed procedure of extraction and the results of the analysis will be published elsewhere. This article is limited to the discussion of nor-carotenoid compounds.

3. Nor-carotenoids identified in nori flavour

Algal carotenoids have been particularly studied by Liaaen-Jensen (ref. 35). Carotenoids are usually yellow-red, isoprenoid, polyene pigments, widely distributed in nature, where they serve important functions such as protection against photodynamic damage and auxiliary light absorption for photosynthesis and phototaxis. β,β-Carotene is a common bicyclic C_{40}-carotene present in all algae. Its skeleton is formally made up of eight head-to-tail linked isoprenoid units, tail-to-tail linked in the centre of the molecule. Common carotenoids of red algae (Rhodophyceae), the family of *Porphyra* species, are β,β-carotene (48),

tene (49), zeaxanthin (50), and lutein (51)



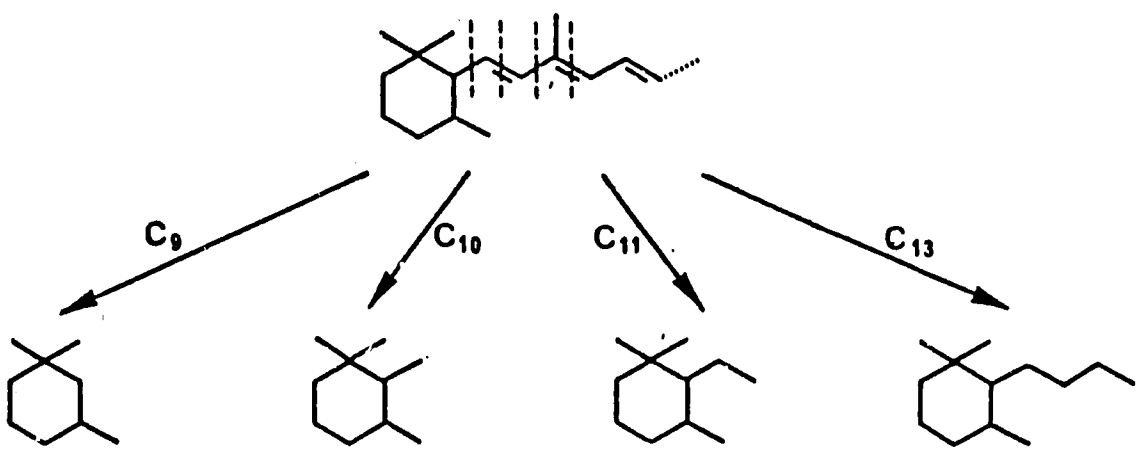
(48) a-P-a

(49) a-P-b

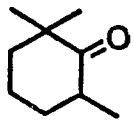
(50) c-P-c

(51) c-P-d

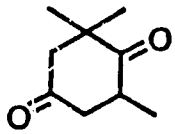
It has been observed that the thermal degradation of β,β-carotene produces polyenes (ref. 36) and aromatic hydrocarbons as toluene, *m*-xylene, 2,6-dimethylnaphthalene and β-ionene (ref. 37), products we have also identified in nori flavour. The mechanism of the thermal ionene formation from β-carotene has been explained by Ohloff (ref. 38). The structure of breakdown flavours derived from cyclic carotenoids of tobacco has been reviewed by Enzell and co-workers (ref. 39).



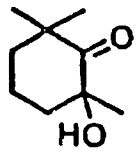
C_9 Nor-carotenoids



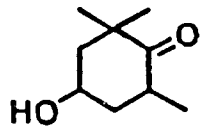
(52)



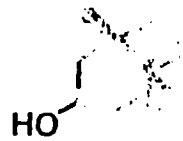
(53)



(54)



(55)

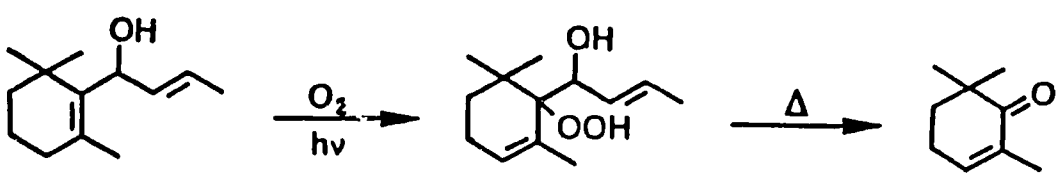


(56)

The five trimethylcyclohexanones (52) to (56) have been identified in nori flavour. 2,2,6-Trimethylcyclohexanone (52) has been previously identified in bilberry (ref. 40), tea (ref. 41), passion fruit (ref. 42), dill (ref. 43), and tobacco (ref. 44). 2,2,6-Trimethylcyclohexane-1,4-dione (53) has been identified in saffron (ref. 45) and in tobacco (ref. 46).

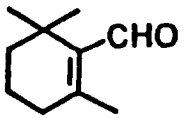
2-Hydroxy-2,6,6-trimethylcyclohexanone (54) has been identified in tomato (ref. 47), tea (ref. 48), and in tobacco (ref. 39). It has also been identified together with β-ionone (42) and dihydroactinidiolide (45) among the photo-oxygenation products of β-carotene (ref. 49). 4-Hydroxy-2,2,6-trimethylcyclohexanone (55) and 4-hydroxy-2,6,6-trimethyl-2-cyclohexen-1-one (56) had only been identified in tobacco (ref. 50).

The formation of these C_9 cyclic compounds can be explained by a mechanism similar to that proposed by Schulte-Elte and co-workers for the production of 2,6,6-trimethyl-2-cyclohexen-1-one (59) by photooxygenation of β-damascol (57) through the intermediate allylic hydroperoxide (58) (ref. 51). Subsequent oxidation, reduction, and hydration can explain the structural variation observed for compounds (52) to (56).

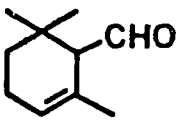


(57) (58) (59)

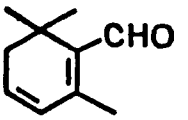
C_{10} Nor-carotenoids



(41)



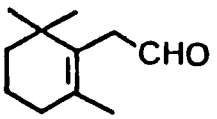
(60)



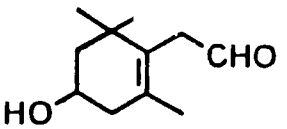
(61)

β-Cyclocitral (41) was discovered in peas (ref. 52), in rum (ref. 53), in tea (ref. 54), in Chinese cinnamon (cassia) (ref. 55), and in tobacco (ref. 56) where it is present at a concentration of 0.1 ppm. Juettner who identified it in various species of algae (ref. 27 and 23) mentions that β-cyclocitral which is released by the algal blooms is responsible for the tobacco-like odour observed in fresh reservoir waters. α-Cyclocitral (60) and safranal (61) are identified for the first time in a food system, but the latter (61) has also been isolated from tobacco (ref. 56).

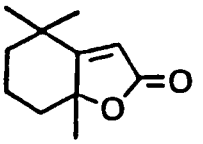
C_{11} Nor-carotenoids



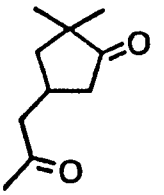
(62)



(63)



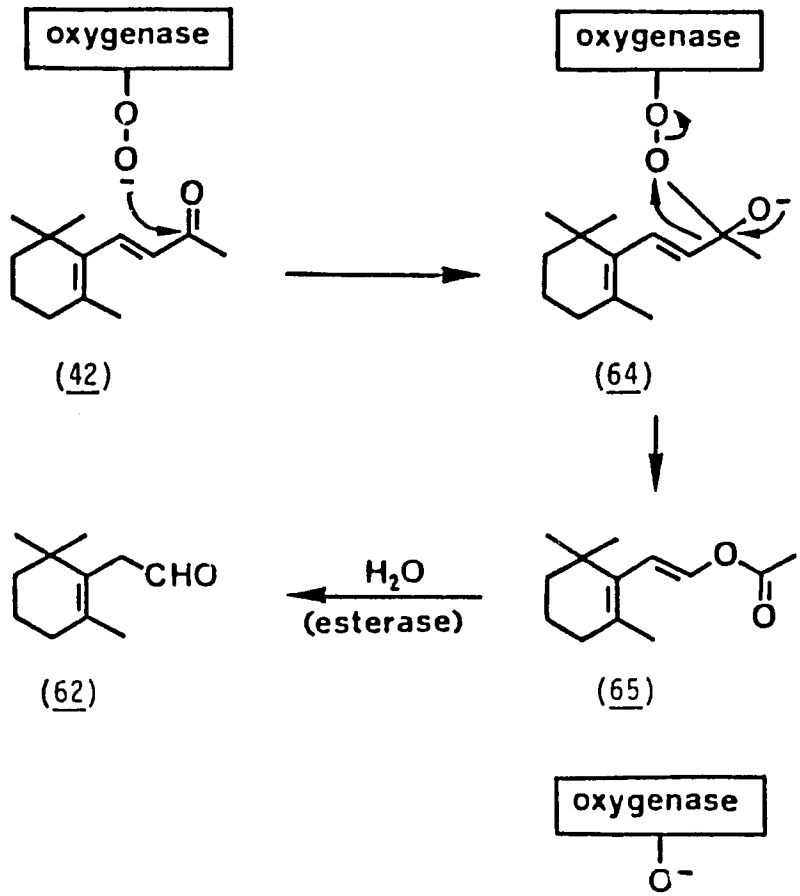
(45)



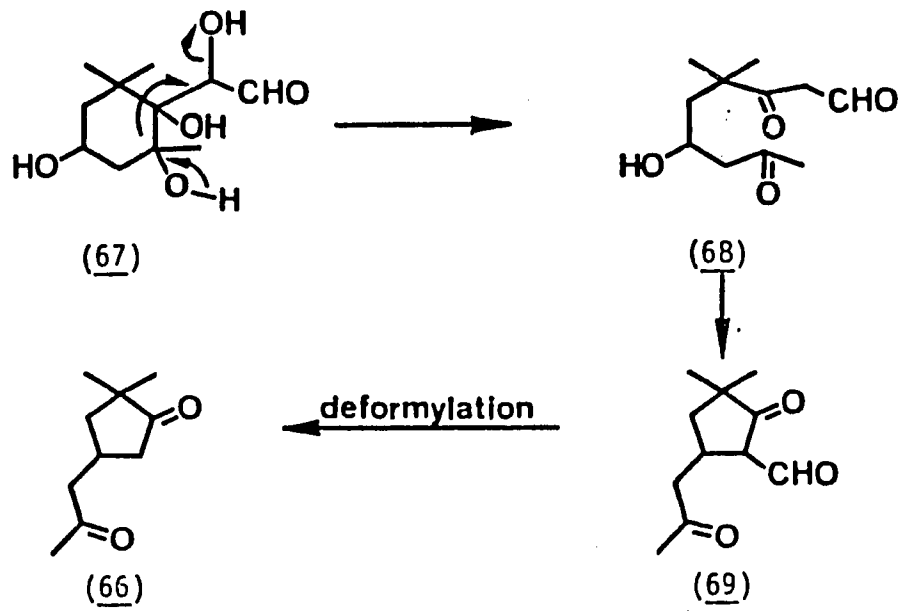
(66)

β-Homocyclocitral (62) and 3-hydroxy-β-homocyclocitral (63) are identified for the first time in a foodstuff. Compound (62) has been patented for its perfuming character; it imparts to cosmetics, detergents, and soaps an excellent woody, green, and earthy aroma (ref. 57). The corresponding acid (β-homocyclogeranic acid) has been identified in *Cistus labdaniferus* (ref. 11). The formation of (62) has been observed by Krasnobajew and Helmlinger (ref. 58) in the biotransformation products of β-ionone (42) by *Lasiodiplodia theobromae*. The intermediates are probably the peroxide (64) which rearranges by a Baeyer-Villiger reaction and the enol ester (65) which is hydrolyzed by an esterase to form β-homocyclocitral (62).

62

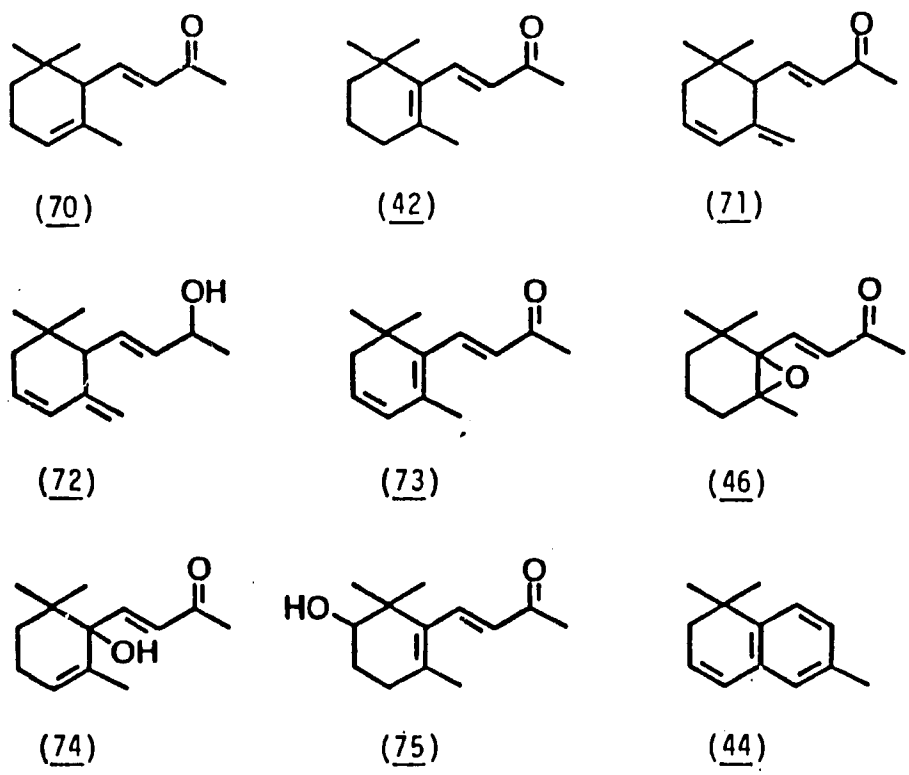


Two other C_{11} nor-carotenoids are also identified in nori : dihydroactinidiolide (45) which is a well known constituent of foods and essential oils, and 2,2-dimethyl-4-(2-oxopropyl)cyclopentanone (66), a product previously identified in tobacco (ref. 59). We might remember that the dihydroactinidiolide structure preexists in aurochrome, a carotenoid identified in Rhodophyceae (ref. 35). Various mechanisms of formation of (45) from β-ionone via its 5,8-epidioxide or its 5,6-epoxy derivative have been proposed (ref. 60, 61, and 62). The formation of (66) from a "xantophyll-type precursor" by exhaustive oxidation, ring cleavage, and intramolecular condensation in a C_5 ring via intermediates (67) to (69) has been explained by Demole (ref. 59). This view is supported by the work of Ohloff and Skorianetz in the ionone series, which provides evidence that polyols analogues to (67) can indeed undergo ring cleavage with acids (English-Zimmermann fragmentation (ref. 63).



C_{13} Nor-carotenoids

In the course of our investigation on nori flavour constituents, we also identified the following nine C_{13} compounds :



α-Ionone (70) has so far been identified in fourteen food systems and β-ionone (42) in twenty-eight food systems. Their flavouring effect is well known to the flavourists and perfumers and their presence partially explains the flowery and delicate note evolved from nori. 3,4-Dehydro-γ-ionone (71) has been identified in rum together with the corresponding alcohol (72) (ref. 53). 3,4-Dehydro-β-ionone (73), which is transformed by photoconversion to the dihydronaphthalene (44) (ref. 64), has also been identified in tobacco (ref. 50). 5,6-Epoxy-β-ionone (46), which yields (44) by acid-induced cyclization, has been identified in raspberry (ref. 65), rum (ref. 53), tea (ref. 48), mushrooms (ref. 66), dill (ref. 43), and tomatoes (ref. 47). So far as we know, compound (74) has not yet been described as a natural product but its presence can be explained by an acid-catalyzed rearrangement of the epoxide (46) as observed by Skorianetz and Ohloff (ref. 67). On the other hand, (75) has been identified in *Osmanthus* absolute by Kaiser and Lamparsky (ref. 68 and 69) and its optically active form has been synthetized by Escher and co-workers (ref. 70). Its racemate has been patented as a tobacco flavourant (ref. 71) and identified in the microbial transformation products of β-ionone by cultures of *Aspergillus*, *Rhizopus*, or *Penicillium* (ref. 58, 72, and 73).

The presence in nori of dehydroionene (1,1,6-trimethyl-1,2-dihydronaphthalene, 44) is not surprising. This product, which was previously identified in rum (ref. 74), wine (ref. 75), tobacco (ref. 76), and microalgae (ref. 21 and 22), has a relatively low threshold (20 ppb); it imparts to wine a "hydrocarbon, kerosene-like or bottle-aged" character (ref. 77 and 78).

We would like to thank Mrs V. Egger-Sigg and Mr R. Tonna who performed the experimental work with great skill and care.

REFERENCES

1 V.J. Chapman, Algal utilization. The algae (book), V.J. Chapman, St. Martin's Press, Mc Millan (1962).
2 M. Takaoka and Y. Ando, Composition of the oil of *Dictyopteris divaricata*, J. Chem. Soc. Jpn. 72, 999 (1951), Chem. Abstr. 46, 9261h (1952).
3 T. Irie, K. Yamamoto and T. Masamune, Sesquiterpenes from *Dictyopteris divaricata*. I, Bull. Chem. Soc. Jpn. 37, 1053 (1964).
4 R.E. Moore, Volatile compounds from marine algae, Acc. Chem. Res. 10, 40 (1977).
5 L. Jaenicke and W. Boland, Signal substances and their reception in the sexual cycle of marine brown algae, Angew. Chem., Int. Ed. Engl. 21, 643 (1982).

6 W. Boland, R. Terlinden, L. Jaenicke and D.G. Muller, Binding mechanism and sensitivity in gamete chemotaxis of the Phaeophyte *Cutleria multifida*, Eur. J. Biochem. 126, 173 (1982).
7 R.E. Moore, J.A. Pettus and J. Mistysyn, Odoriferous C_{11} hydrocarbons from Hawaiian *Dictyopteris*, J. Org. Chem. 39, 2201 (1974).
8 D.G. Muller, G. Gassmann, F.J. Marner, W. Boland and L. Jaenicke, The sperm attractant of the marine brown alga *Ascophyllum nodosum* (Phaeophyceae), Science 218, 1119 (1982).
9 Y.-R. Naves, Présence de *n*-undécatriènes-1,3,5 dans l'huile essentielle de la gomme-résine de Galbanum, Bull. Soc. Chim. Fr. 3152 (1967).
10 Y. Chrétien-Bessière, J. Garnero, L. Bénezet and L. Peyron, Sur un hydrocarbure à squelette non terpénique isolé à partir de l'essence de Galbanum, Bull. Soc. Chim. Fr. 97 (1967).
11 S. Escher (Firmenich SA), unpublished results.
12 R. Naef (Firmenich SA), unpublished results.
13 S.J. Wratten and D.J. Faulkner, Cyclic polysulfides from the red alga *Chondria californica*, J. Org. Chem. 41, 2465 (1976).
14 K. Morita and S. Kobayashi, Isolation and synthesis of lenthionine, an odorous substance of shiitake, an edible mushroom, Tetrahedron Lett. 573 (1966).
15 B.J. Burreson, R.E. Moore and P.P. Roller, Volatile halogen compounds in the alga *Asparagopsis taxiformis* (Rhodophyta), J. Agric. Food Chem. 24, 856 (1976).
16 M. Yurkowski and J.A.L. Tabachek, Geosmin and 2-methylisoborneol implicated as a cause of muddy odor and flavor in commercial fish from Cedar lake Manitoba, Can. J. Fish. Aquat. Sci. 37, 1449 (1980).
17 S.H. Zinder, W.N. Doemel and T.D. Brock, Production of volatile sulfur compounds during the decomposition of algal mats, Appl. Environ. Microbiol. 34, 859 (1977).
18 F. Juettner, Biologically active compounds released during algal blooms, Verh. Internat. Verein. Limnol. 21, 227 (1981).
19 F. Juettner and B. Hahne, Volatile excretion products of *Botryococcus braunii* *malhamensis*. Identification and formation, Z. Pflanzenphysiol. 103, 403 (1981).
20 A. Yasuhara and K. Fuwa, Characterization of odorous compounds in rotten blue-green algae, Agric. Biol. Chem. 46, 1761 (1982).
21 F. Juettner, Nor-carotenoids as the major volatile excretion products of *Cyanidium*, Z. Naturforsch. 34c, 186 (1979).
22 F. Juettner and K. Wurster, Simple device for adsorption of volatile constituents of algae on Tenax and their transfer to GC-systems, J. Chromatogr. 175, 178 (1979).
23 F. Juettner, Detection of lipid degradation products in the water of a reservoir during a bloom of *Synura uvella*, Appl. Environ. Microbiol. 41, 100 (1981).
24 F. Juettner, The algal excretion product, geranylacetone : a potent inhibitor of carotene biosynthesis in *Synechococcus*, Z. Naturforsch. 34c, 957 (1979).
25 M.A. Stevens, Relationship between polyene-carotene content and volatile compound composition of tomatoes, J. Amer. Soc. Hort. Sci. 95, 461 (1970).
26 F. Juettner, J. Leonhardt and S. Moehren, Environmental factors affecting the formation of mesityl oxide, dimethylallylic alcohol and other volatile compounds excreted by *Anabaena cylindrica*, J. Gen. Microbiol. 129, 407 (1983).
27 F. Juettner, β-Cyclocitral and alkanes in *Microcystis* (Cyanophyceae), Z. Naturforsch. 31c, 491 (1976).

67

28 J.J. Henatsch and F. Juettner, Volatile odorous excretion products of different strains of *Synechococcus* (Cyanobacteria), Nat. Sci. Tech. 15, 259 (1983).
29 S. Arasaki and T. Arasaki, Vegetables from the sea (book), Japan Publ. Inc. (1983).
30 T. Katayama, Volatile constituents of seaweed, Physiology and biochemistry of algae, R.A. Lewin Ed., Acad. Press (1962).
31 H. Noda and Y. Horiguchi, The flavor substances of nori, the dried laver *Porphyra tenera*. I. Dimethyl sulfide and dimethyl-β-propiothetin, Bull. Jap. Soc. Sci. Fish. 41, 481 (1975).
32 H. Noda, Y. Horiguchi and S. Araki, Studies on the flavor substances of nori, the dried laver *Porphyra* spp. II. Free amino acids and 5-nucleotides, Bull. Jap. Soc. Sci. Fish. 41, 1299 (1975).
33 H. Noda, H. Amano, K. Abo and Y. Horiguchi, Studies on the flavor substances of "nori", the dried laver *Porphyra* spp. III. Sugars, organic acids and minerals of "nori". Bull. Jap. Soc. Sci. Fish. 47, 57 (1981).
34 K. Kasahara and K. Nishibori, Flavoring of roasted laver. I, Bull. Jap. Soc. Sci. Fish. 41, 193 (1975).
35 S. Liaaen-Jensen, Algal carotenoids and chemosystematics, Marine Nat. Prod. Chem. (book), Faulkner & Fenical Ed., Plenum Press (1977).
36 J. Byers, Isolation and identification of the polyenes formed during the thermal degradation of 3-β-carotene, J. Org. Chem. 48, 1515 (1983).
37 W.C. Day and J.G. Erdman, Ionene : a thermal degradation product of β-β-carotene, Science, 141 (3583), 203 (1963)
38 G. Ohloff, Classification and genesis of food flavors, Third Int. Cong. Food Sci. Technol., Washington D.C. Aug. 9-14 (1970).
39 C.R. Enzell, I. Wahlberg and A.J. Aasen, Isoprenoids and alkaloids of tobacco, Prog. Chem. Org. Nat. Prod. (Zechmeister) 34, 1 (1977).
40 E. von Sydow and K. Anjou, The aroma of bilberries (*Vaccinium myrtillus* L.). I. Identification of volatile compounds, Lebensm.-Wiss. Technol. 2, 78 (1969).
41 H.A. Bondarovich, A.S. Giammarino, J.A. Renner, F.W. Shephard, A.J. Shingler, M.A. Gianturco, Some aspects of the chemistry of tea. A contribution to the knowledge of the volatile constituents, J. Agric. Food Chem. 15, 36 (1967).
42 M. Winter and R. Kloeti, The aroma of yellow passion-fruit (*Passiflora edulis, f. flavicarpa*), Helv. Chim. Acta 55, 1916 (1972).
43 P. Schreier, F. Drawert and I. Heindze, Volatile constituents of fresh dill herb. *Anethum graveolens*, Lebensm.-Wiss. Technol. 14, 150 (1981).
44 B. Kimland, A.J. Aasen and C.R. Enzell, Neutral volatile constituents of greek tobacco, Acta Chem. Scand. 26, 1281 (1972).
45 N.S.Zarghami and D.E.Heinz, The volatile components of saffron, Lebensm.-Wiss. Technol. 4, 43 (1971).
46 D.L. Roberts and W.A. Rohde, Isolation and identification of flavour components of *Burley* tobacco, Tob. Sci. 16, 107 (1972).
47 R. Viani, J. Bricout, J.P. Marion, F. Mueggler-Chavan, D. Reymond and R.H. Egli, The composition of tomato aroma, Helv. Chim. Acta 52, 887 (1969).
48 F. Mueggler-Chavan, R. Viani, J. Bricout, J.P. Marion, H. Mechtler, D. Reymond and R.H. Egli, On the composition of tea flavour. III. Identification of two ionone-related ketones, Helv. Chim. Acta 52, 549 (1969).
49 S. Isoe, S.B. Hyeon and T. Sakan, Photo-oxygenation of carotenoids. I. The formation of dihydroactinidiolide and 3-ionone from β-carotene, Tetrahedron Lett. 279 (1969).
50 E. Demole and D. Berthet, A chemical study of Burley tobacco flavor (*Nicotiana tabacum* L.). Volatile to medium-volatile constituents (b.p. 84°/0.001 Torr), Helv. Chim. Acta 55, 1866 (1972).

68.

51 K.H. Schulte-Elte, B.L. Müller and G. Ohloff, The dye-sensitized photo-oxygenation of β-damascol. A facile method of preparation of β-damascenone, Helv. Chim. Acta 54, 1899 (1971).
52 K.E. Murray, J. Shipton, F.B. Whitfield and J.H. Last, The volatiles of off-flavoured unblanched green peas (*Pisum sativum*), J. Sci. Food Agric. 27, 1093 (1976).
53 R. ter Heide, H. Schaap, H.J. Wobben, P.J. de Valois and R. Timmer, Flavor constituents in rum, The quality of foods and beverages, G. Charalambous Ed., Acad. Press (1981).
54 W. Renold, R. Näf-Müller, U. Keller, B. Willhalm and G. Ohloff, New volatile black tea constituents, Helv. Chim. Acta 57, 1301 (1974).
55 A.F. Thomas (Firmenich SA), unpublished results.
56 B. Kimland, R.A. Appleton, A.J. Aasen, J. Roeraade and C.R. Enzell, Neutral oxygen-containing volatile constituents of greek tobacco, Phytochemistry 11, 309 (1972).
57 Intern. Flavors & Fragrances, Food or flavor containing 2,6,6-trimethyl-1-cyclohexen-1-ylacetaldehyde, U.S. Pat. 3,940,499 (Feb. 24, 1976).
58 V. Krasnobajew and D.Helmlinger, Fermentation of fragrances : biotransformation of β-ionone by *Lasiodiplodia theobromae*, Helv. Chim. Acta 65, 1590 (1982).
59 E. Demole and C. Demole, Identification and synthesis of the novel terpenoid alkaloids 1,3,6,6-tetramethyl-5,6,7,8-tetrahydroisoquinolin-8-one and 3,6,6-trimethyl-5,6-dihydro-7*H*-2-pyrindin-7-one, Helv. Chim. Acta 58, 523 (1975).
60 C.S. Foote and M. Brenner, Chemistry of singlet oxygen. VIII. An unusual allenic oxygenation product, Tetrahedron Lett. 6041 (1968).
61 M. Mousseron-Canet, J.P. Dalle and J.C. Mani, Photooxydation sensibilisée de composés apparentés aux caroténoïdes. Analogie avec la structure des caroténoïdes oxygénés, alléniques et autres, Tetrahedron Lett. 6037 (1968).
62 U. Schwieter, W. Arnold, W.E. Oberhaensli, N. Rigassi and W. Vetter, Synthese in der Carotenoid Reihe. Ein Beitrag zur Chromsäure Oxydation von Polyenen, Helv. Chim. Acta 54, 2447 (1971).
63 W. Skorianetz and G. Ohloff, Über vinylogue English-Zimmerman-Spaltungen in der Jononreihe, Helv. Chim. Acta 58, 771 (1975).
64 V. Ramamurthy and R.S.H. Liu, Photochemistry of dehydro-β-ionone and related compounds, Tetrahedron Lett. 441 (1973).
65 M. Winter and P. Enggist, Aroma research. 17. The raspberry aroma. IV, Helv. Chim. Acta 54, 1891 (1971).
66 H. Pyysalo, Identification of volatile compounds in seven edible fresh mushrooms, Acta Chem. Scand. B30, 235 (1976).
67 W. Skorianetz and G. Ohloff, Über die Säurekatalysierte Umlagerung von β-Jonon-5,6-epoxid, Helv. Chim. Acta 57, 2439 (1974).
68 R. Kaiser and D. Lamparsky, Constituents of Geranium absolute. I. 2,5-Epoxy-megastigma-6,8-dienes, Helv. Chim. Acta 61, 373 (1978).
69 R. Kaiser and D. Lamparsky, Natural occurrence of new ionone derivatives and their structural assignments by synthetic approaches, Int. Congr. Essent. Oils, 7th, Japan. Flav. Fragr. Manuf. Assoc., Tokyo, Japan, 395 (1977).
70 S. Escher, W. Giersch and G. Ohloff, Proof of the absolute configuration of (-)-(*S*)-2-hydroxy-β-ionone by correlation with ursolic acid and with (-)-*trans*-verbenol, Helv. Chim. Acta 64, 943 (1981).
71 Japan Tobacco and Salt Public Corp., Hydroxyionone as tobacco flavorant, Jpn. Kokai Tokkyo Koho 78,107,497 (1978), Chem. Abstr. 90, 19280w (1979).
72 Japan Tobacco and Salt Public Corp., 2-Hydroxy-β-ionone by microbial transformation of β-ionone, Jpn. Kokai Tokkyo Koho 78,107,482 (1978), Chem. Abstr. 90, 70614k (1979).

69

73 Y. Mikami, Y. Fukunaga, T. Hieda, Y. Obi and T. Kisaki, Formation of (2*S*,6*R*,7*R*)-2,7-epoxydihydro-α-ionone via asymmetric hydroxylation of β-ionone by *Aspergillus niger*, Agric. Biol. Chem. 45, 331 (1981).
74 H.M. Liebich, W.A. Koenig and E. Bayer, Analysis of the flavor of rum by GLC and MS, J. Chromatogr. Sci. 8, 527 (1970).
75 M. Bertuccioli and R. Viani, Red wine aroma : Identification of headspace constituents, J. Sci. Food Agric. 27, 1035 (1976).
76 B. Kimland, A.J. Aasen and C.R. Enzell, Volatile neutral constituents of greek tobacco, Acta Chem. Scand. 26, 2177 (1972).
77 R.F. Simpson, 1,1,6-Trimethyl-1,2-dihydronaphthalene : an important contributor to the bottle-aged bouquet of wine, Chem. Ind. 37 (1978).
78 R.F. Simpson and G.C. Miller, Aroma composition of aged Riesling wine, Vitis 22, 51 (1983).

70

J. Adda (Editor) *Progress in Flavour Research 1984*
Proceedings of the 4th Weurman Flavour Research Symposium, Dourdan, France, 9–11 May 1984
© 1985 Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

NEW RESULTS IN TROPICAL FRUIT FLAVOUR ANALYSIS

H. IDSTEIN and P. SCHREIER
Lehrstuhl für Lebensmittelchemie, Universität Würzburg,
Am Hubland, D-8700 Würzburg, FRG

ABSTRACT

HRGC-MS and HRGC-FTIR studies were carried out on the following tropical fruits: cherimoya (Annona cherimolia, Mill.), guava (Psidium guajava, L.), mango (Mangifera indica, L., var. Alphonso), and papaya (Carica papaya, L., var. Solo). Sample preparation, analytical techniques, and selected examples of additional volatiles identified in the pulps of the above-mentioned fruits will be presented and discussed.

INTRODUCTION

Since the last decade many tropical fruits others than the well-known pineapples and bananas have reached an increasing economic value at the European market (1). Besides their important content of different vitamins (2) they are especially desirous due to their intense and specific aroma impression completely different from that of our local fruits. Therefore it is necessary to provide an actual objective analytical background for the aroma composition of this type of fruits. During the course of our flavour research work on plant volatiles and their biogenesis our interest was focused on the volatile composition of cherimoya (Annona cherimolia, Mill.), guava (Psidium guajava, L.), mango (Mangifera indica, L., var. Alphonso), and papaya (Carica papaya, L., var. Solo) fruits.

RESULTS AND DISCUSSION

For sample preparation from fresh fruits we used the standard combination of high-vacuum distillation and solvent extraction followed by a silicagel preseparation step with a pentane-ether gradient (Fig. 1). For identification additionally to the combined fused silica capillary gas chromatography-mass spectrometry (HRGC-MS) we introduced the HRGC-FTIR technique, i.e. the coupling of capillary gas chromatography with a fast scanning Fourier transform infrared instrument for real sample analysis of volatiles (3). Methods and application in tropical fruit flavour analysis have already been discussed elsewhere (3), so in the following only some examples will be demonstrated.

71

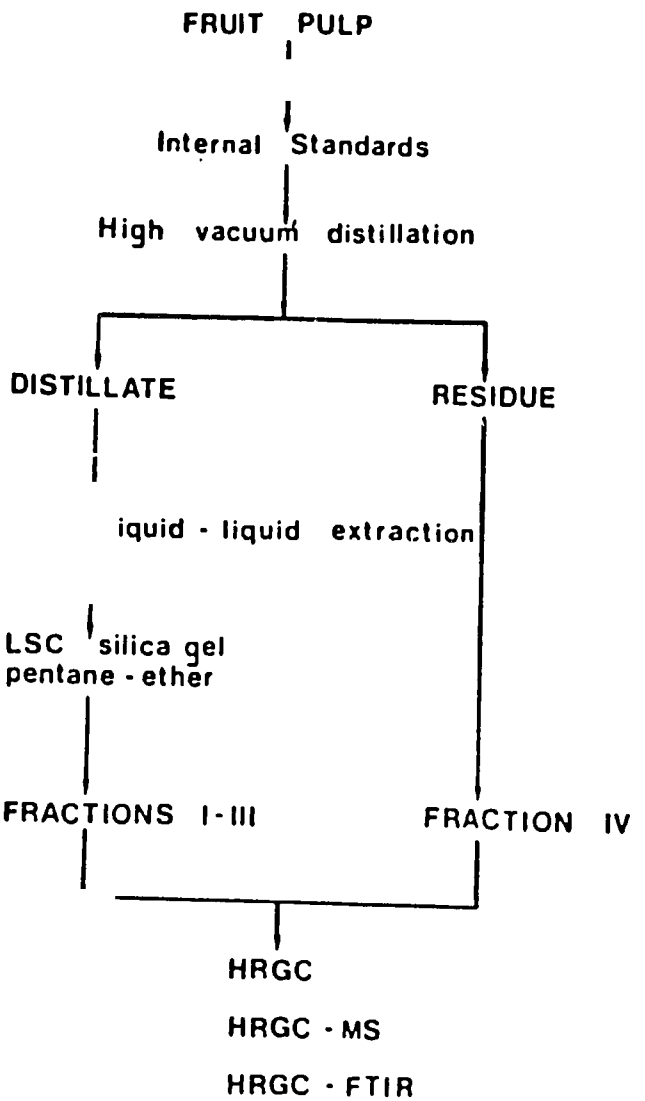


Fig. 1. Scheme of separation, concentration and analysis of tropical fruit volatiles.

In the course of our HRGC-MS studies on cherimoya volatiles, e.g. we obtained the mass spectrum shown in Fig. 2. At the first glance, it points to 2-pentenol, especially as this substance possesses a similar GC-retention. But the on-the-fly FTIR-spectrum of the same unknown (Fig. 3) showed absorption bands at 3082, 1755, 1650, 1177 and 895 cm^{-1}, clearly indicating an unsaturated Z-configurated ester. Comparison of the chromatographic and spectroscopic data with synthesized reference samples proved the identity of the unknown compound as (Z)-2-pentenyl butanoate.

In order to demonstrate the actual possibilities and limits of the HRGC-FTIR technique an overview of the main constituents of cherimoya aroma fraction II and III detectable with both methods is given in table 1. One can see that all the main components of cherimoya volatiles could be additionally detected by HRGC-FTIR (marked by an asterisk).

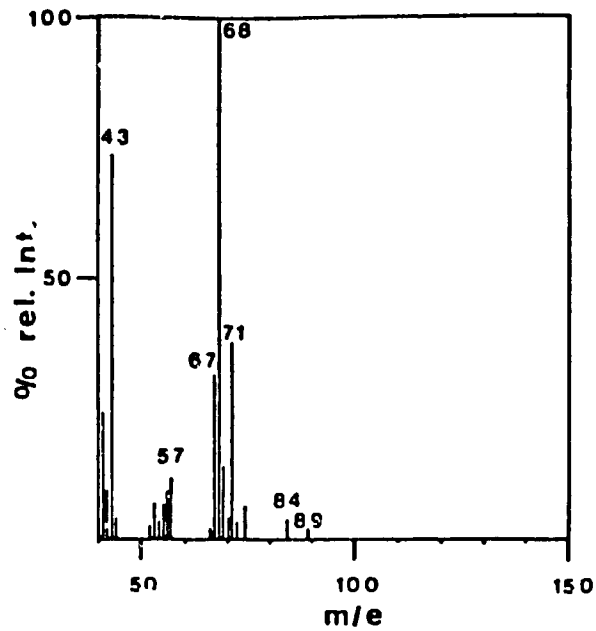


Fig. 2. Mass spectrum of unknown compound of cherimoya volatiles, explanation see text.

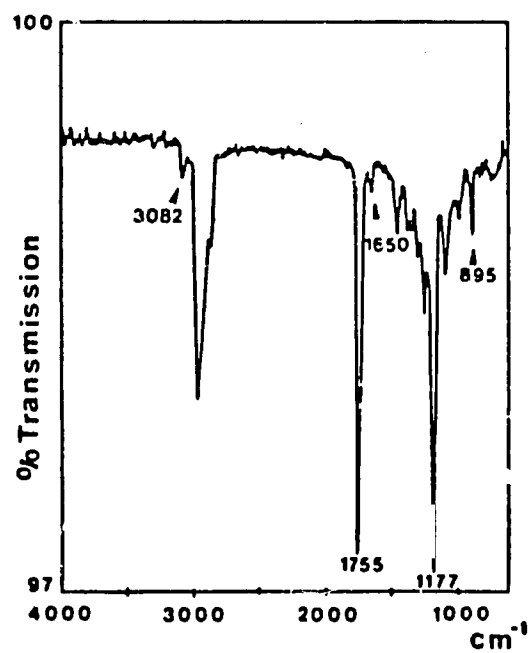


Fig. 3. Vapour phase FTIR spectrum of unknown compound from HRGC-FTIR analysis of cherimoya volatiles. Same compound as in Fig. 2, explanation see text.

In total 240, 199, and 134 volatiles were identified in the pulps of cherimoya, guava, mango, and papaya, respectively. On cherimoya (Annona cherimolia, Mill.) volatiles no previous reports had been provided (4). With guava, 156 volatiles could be newly identified. As to mango and papaya, we described 115 and 80 compounds, respectively, for the first time. Due to the high number of additional volatiles identified, in the following we have to make some choices.

TABLE 1

Quantitative composition of main constituents of cherimoya fruit volatiles.

10 - 50	50 - 100	100 - 500	500 - 1000	> 1000 µg.kg^{-1}
3-Methylbutyl 3-methylbutanoate*		Butyl butanoate*		
		3-Methylbutyl butanoate*		
		Hexyl butanoate*		
2-Methyl-1-propanol*	2-Methyl-2-butanol		1-Butanol*	3-Methyl-1-butanol*
2-Methyl-3-buten-2-ol	1-Pentanol*		Linalool*	1-Hexanol*
2-Pentanol	1-Penten-3-ol*			
(E)-2-Hexen-1-ol*	(E)-3-Penten-1-ol*			
2-Ethyl-1-hexanol	(Z)-2-Penten-1-ol*			
Benzylalcohol	(Z)-3-Hexen-1-ol*			
	α-Terpineol*			
Chrysanthenone				
(Z)-Linalooloxide, furanoid				

a) Standard-controlled capillary gas chromatographic determinations in fruit pulp without consideration of calibration factors, i.e. F = 1.00 for all compounds.

* Additionally identified by HRGC-FTIR analysis.

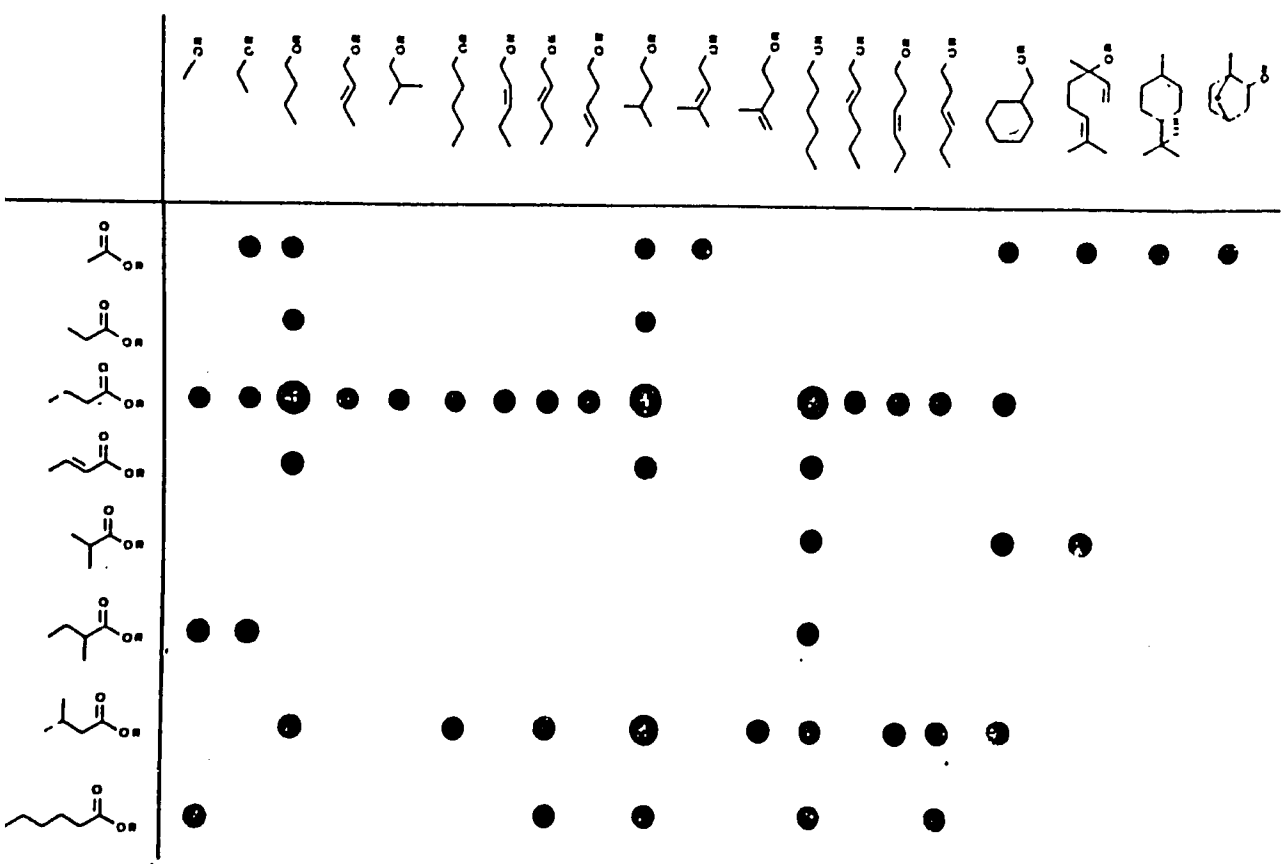


Fig. 4. Ester "spectrum" of cherimoya volatiles. Moieties arranged according to their chainlengths; alcohols from left to right, acids from top to bottom. (● : 100 - 500 ppb; ● : 10 - 50 ppb; ● : <10 ppb)

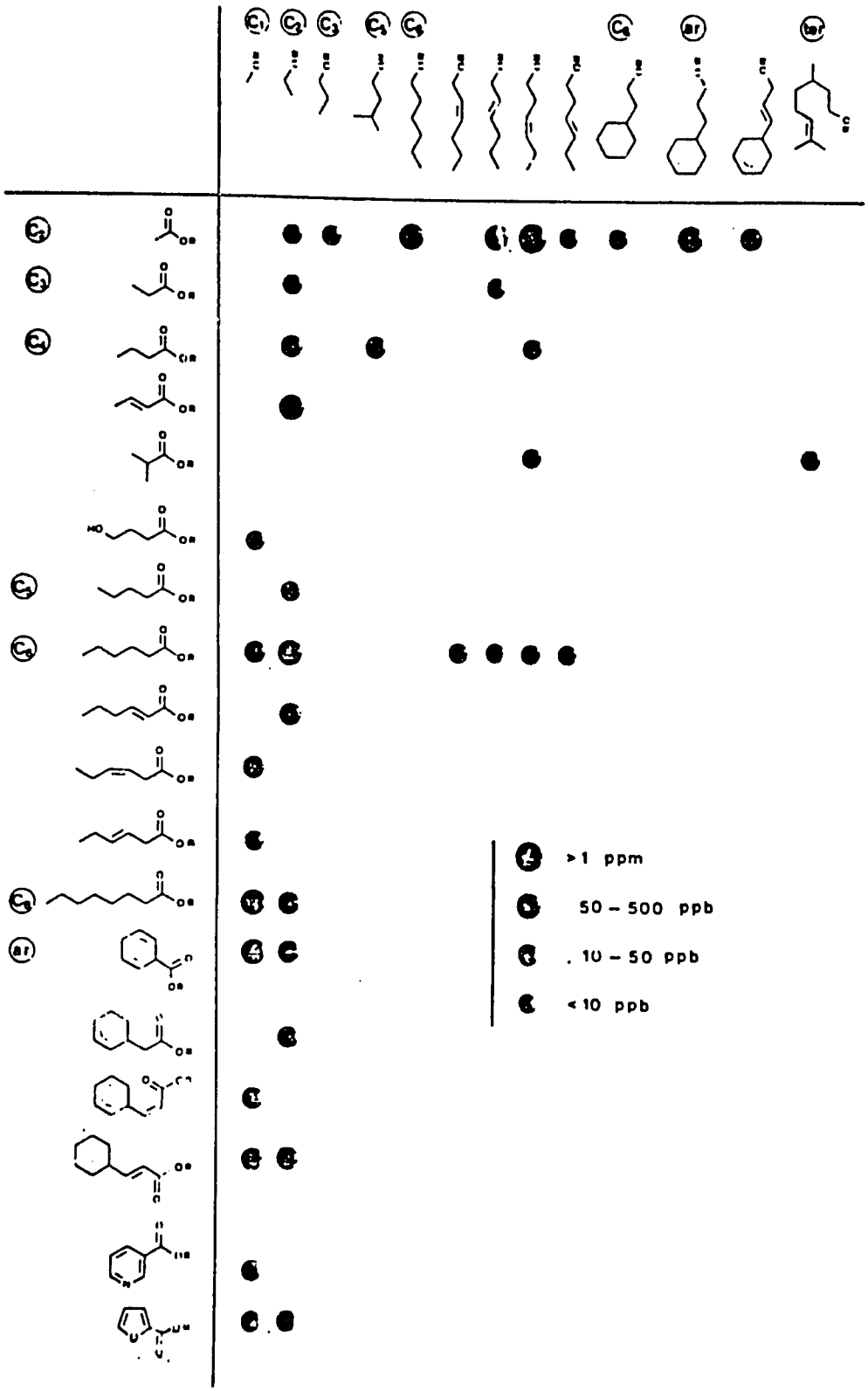


Fig. 5. Ester "spectrum" of guava, cf. legend to Fig. 4.

First of all, let us have a look at the distribution of esters in the different species of tropical fruits. The ester composition of cherimoya pulp (Fig. 4) is characterized by a lot of various butanoates, 3-methylbutanoates, and 3-methylbutyl esters. Especially with the butanoates the great variety of not

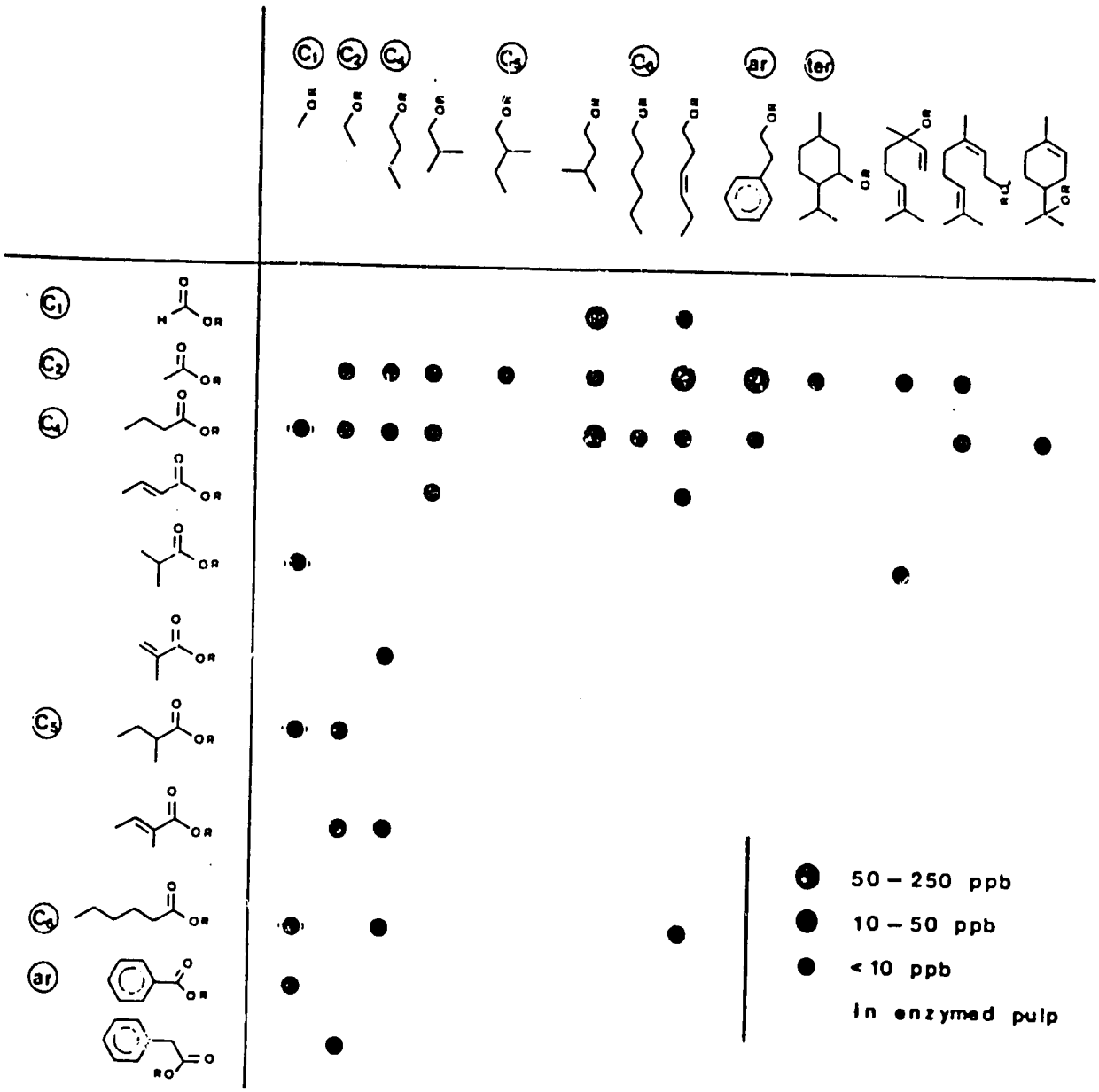


Fig. 6. Ester "spectrum" of Alphonso mango, cf. legend to Fig. 4.

very common C_5-alcohol functions has to be pointed out. The prevailing occurrence of 3-methylbutyl esters and 3-methylbutanoates indicates a forced leucine metabolism as it has already been described for banana (5).

Let us proceed to the ester "spectrum" of the guava (Fig. 5). Here C_2-chains dominate, i.e. ethyl esters and acetates, probably derived from ß-oxidation, besides a large variety of methyl esters. "Specialities" are the unsaturated C_6-hexanoates, the Z-isomer of methyl cinnamate, and some heterocyclic esters like methyl nicotinoate and the furoates.

The ester "spectrum" of mango (Fig. 6) mainly consists of acetates and butanoates including some terpene esters. Not very common is the occurrence of formates, we could describe for the first time in mango. The newly identified

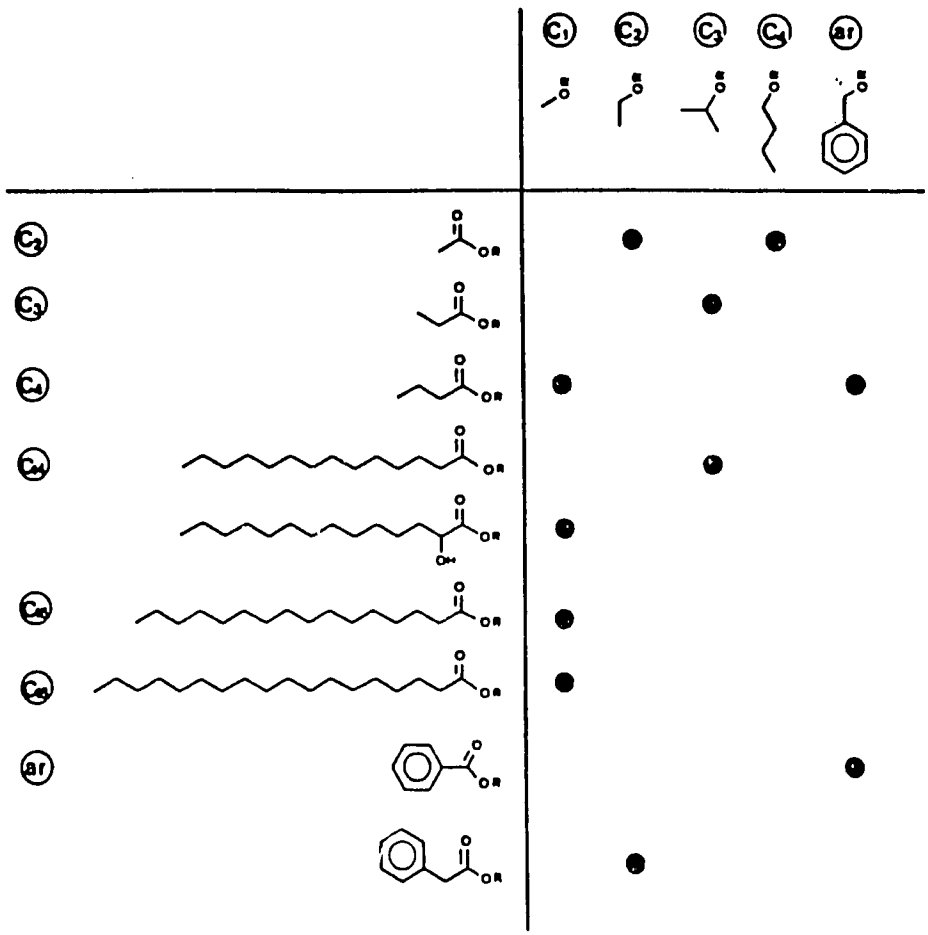


Fig. 7. Ester "spectrum" of papaya, cf. legend to Fig. 4

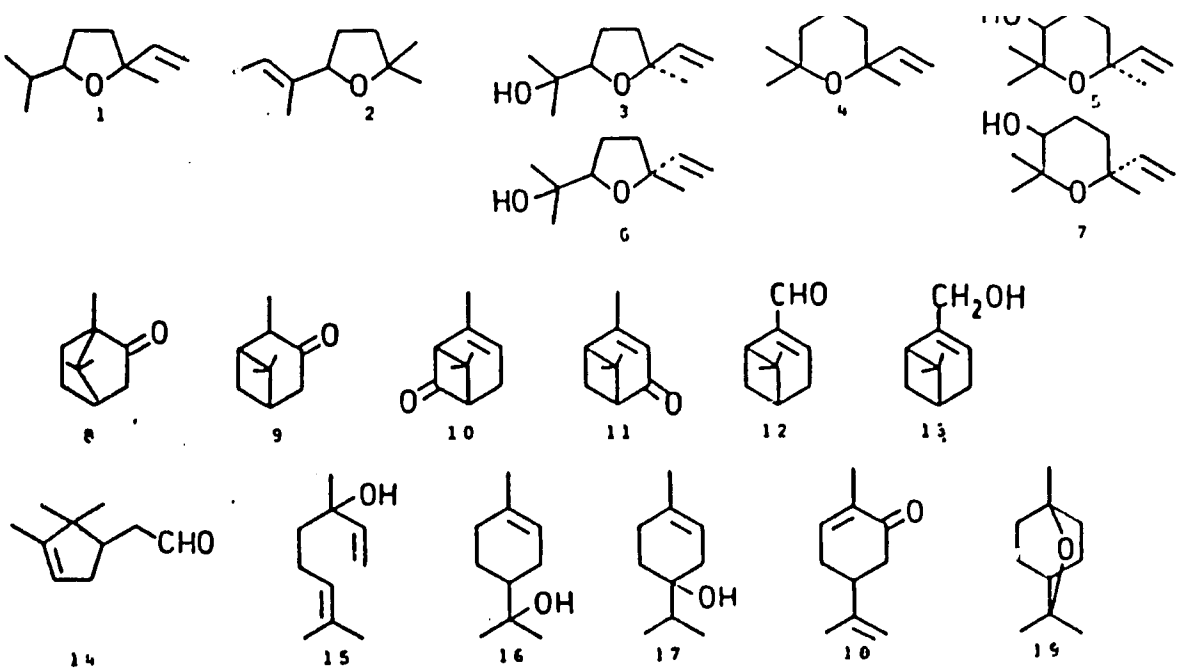


Fig. 8. Various terpenoids of cherimoya.

branched chain acid esters with isobutyl, methacryl, 2-methylbutyl, and tiglic skeleton may be derived from the metabolism of corresponding amino acids, i.e. valine and isoleucine (6).

In papaya fruit (Fig. 7) the ester distribution neither qualitatively nor quantitatively contributes to the overall aroma composition of papaya. All esters are trace compounds (<10 ppb). Mostly long chain acids are dominating together with the new detected aromatic esters, ethyl phenylacetate and benzyl benzoate.

Besides esters terpenoid compounds were found in a great variety in the tropical fruits investigated. Selected oxygenated monoterpenes of cherimoya fruit pulp are shown in Fig. 8. Within this group different volatiles often found together with linalool were detected; the well-known furanoid and pyranoid linalooloxides (Fig. 8; 3,5-7) as well as some other furanoid and pyranoid companions (Fig. 8; 1,2,4). Also various bicyclic terpene ketones and alcohols with pinane skeleton could be identified (Fig. 8, 8-13). The last row in the figure consists of known structures such as linalool, α-terpineol, 4-terpinol, carvone and 1,8-cineole (Fig. 8, 15-19). First in this row the structure of campholene aldehyde (Fig. 8, 14) is shown, recently described as constituent of Cistus ladanifer, L. (7).

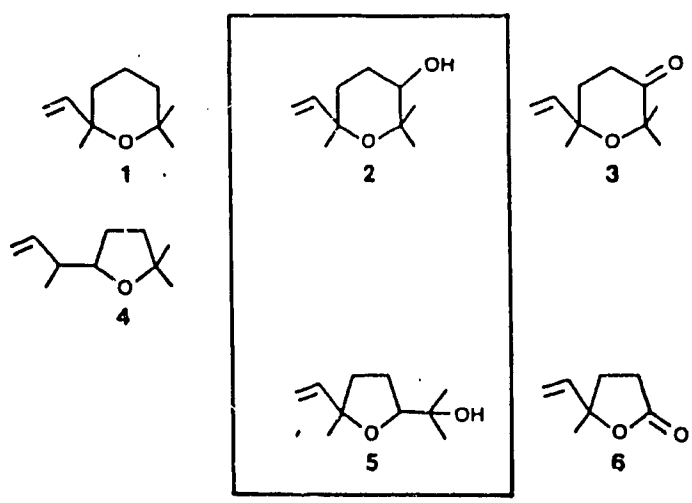


Fig. 9. Pyranoid and furanoid terpenoids of papaya

Terpene compounds are also characteristic for the papaya flavour composition where the isomer linalooloxides (Fig. 9; 2,5) are known to belong to the main aroma compounds (8). Additionally, we could identify oxygenated and reduced stages of these substances in papaya pulp (Fig. 9; 1,3,4,6). Further terpenes of the acyclic and the menthane type are shown in Fig. 10. In the first row the newly identified isomer neoalloocimenes (Fig. 10, 1 and 2) together with the acyclic monoterpene alcohol hotrienol (Fig. 10, 3) are outlined, the latter had

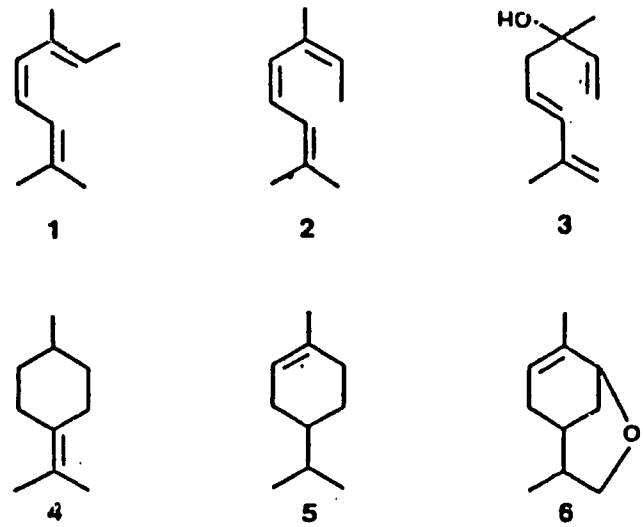


Fig. 10. Acyclic and menthane type terpenes of papaya.

been already described in the leaf oil of Japanese ho tree (9) and as grape and tea volatile (10,11). In the second row two hydrogenated monoterpenes are shown; Δ^4-p-menthene (Fig. 10, 4) and Δ^1-p-menthene (Fig. 10, 5). To the menthane class the terpene ether 2,9-epoxy-Δ^6-p-menthene also belongs.

To get some more knowledge about the metabolism of terpene compounds we have carried out some studies with enzyme-inhibited sample preparation. As this work is still in progress we would like to present here only some preliminary results. In order to look for glycosidically bound forms of terpenes, sample preparations with Hg^{2+}-inhibition were performed (12). The results are shown in Fig. 11 and 12. For a long time there has been evidence for the occurrence of thioglucosidically bounded benzylisothiocyanate, a main component of papaya flavour (13). So it is not striking that this compound disappeared after enzyme inhibition of the pulp. The same effect was observed as to the main terpene alcohols, i.e. especially linalool (Fig. 11, 5), and surprisingly -- shown in Fig. 12 -- as to all monoterpene hydrocarbons as well. As of the moment we don't have any explanation for the latter fact; as to the findings with the terpene alcohols we could tentatively identify a linalylglycoside in papaya pulp, from which the structure has still to be proven by synthesis (14). It seems possible that the terpene alcohols as well as the terpene hydrocarbons in papaya pulp may be regarded as technologically induced volatiles.

Let us now proceed to the results we found with guava volatiles. In Fig. 13, a selection of newly identified guava volatiles is shown. It has to be mentioned that with the exception of compound 1 all other components in this figure could only be detected in the distillation residues (Fig. 13, 2 - 6). In the first row some more or less hydrogenated 3-furanones, like 2-methyltetrahydrofuran-3-one (Fig. 13,1), 5-methyl-4-hydroxy-3(2H)-furanone (Fig. 13,2), and the well-

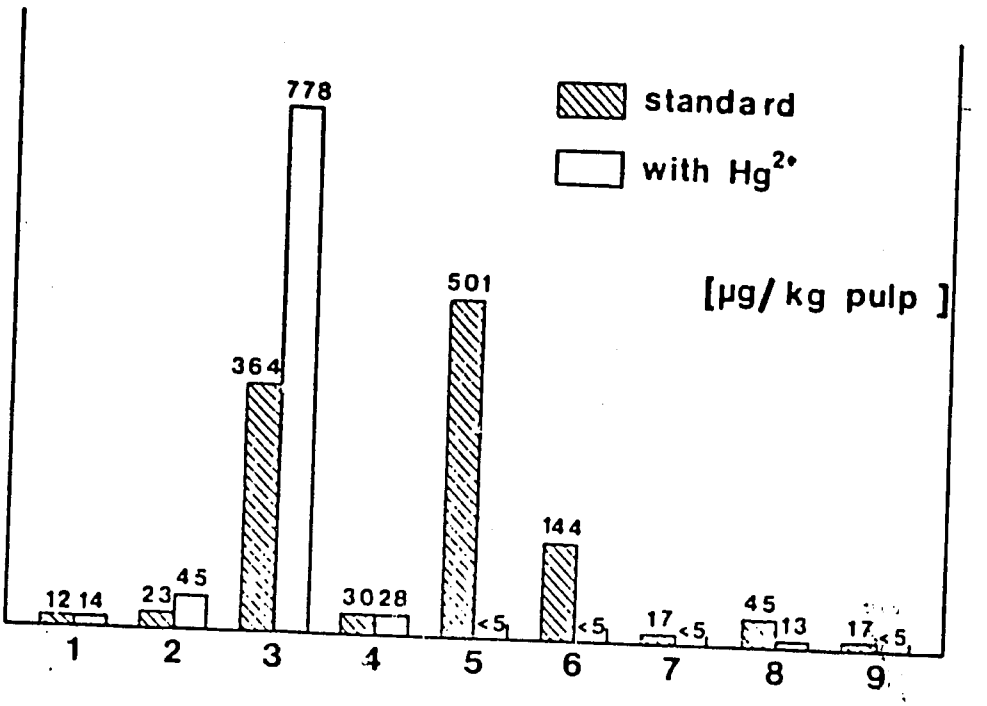


Fig. 11. Changes in the concentration of volatiles in papaya pulp with enzyme inhibition, aroma fraction III. 2-methylpropanol (1), 2-pentanol (2), 1-butanol (3), benzylalcohol (4), linalool (5), α-terpineol (6), geraniol (7), (Z)-linalooloxide, furanoid (8), (E)-linalooloxide, furanoid (9).

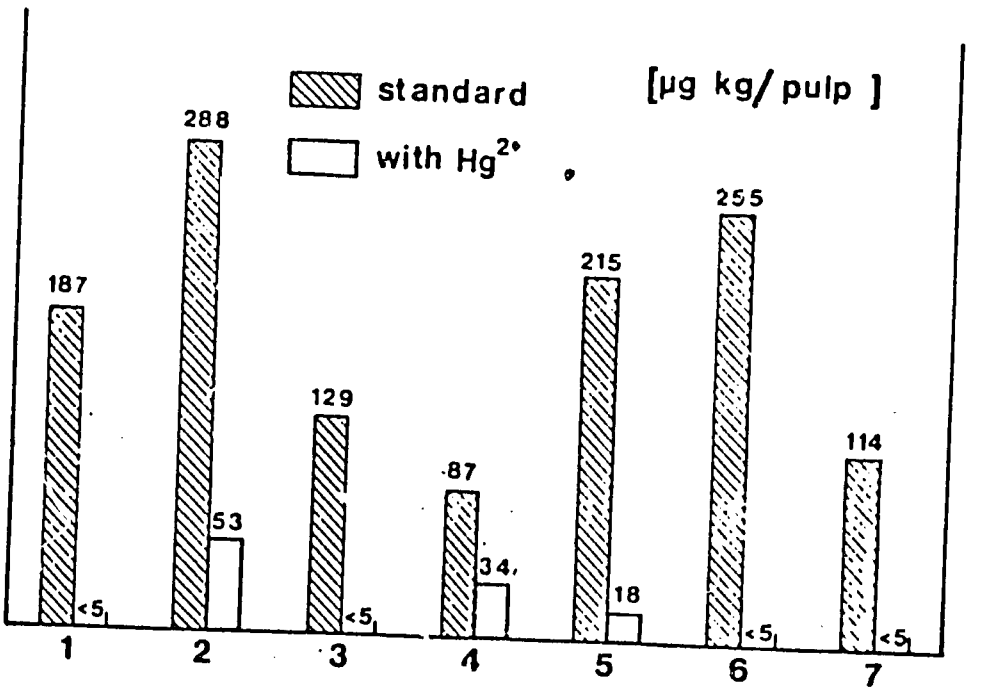


Fig. 12. Changes in the concentration of volatiles in papaya pulp with enzyme inhibition, aroma fraction I. β-myrcene (1), α-terpinene (2), limonene (3), β-phellandrene (4), (Z)-ocimene (5), (E)-ocimene (6), terpinolene (7).

-80

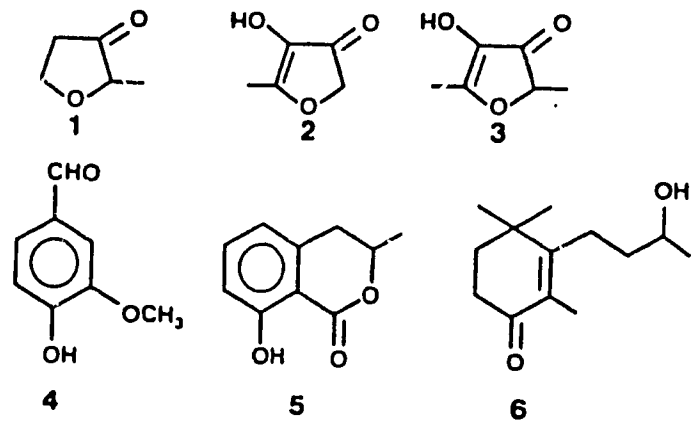


Fig. 13. Some newly identified guava volatiles.

known 2,5-dimethyl-4-hydroxy-3(2H)-furanone (Fig. 13,3) are outlined, whereas we failed to detect the corresponding methoxylated derivative of the latter compound. In the second row, besides vanillin (Fig. 13,4), 3-methyldihydrocoumarin (Fig. 13,5) and 4-oxodihydro-β-ionol (Fig. 13,6), a carotenoid degradation product, are presented.

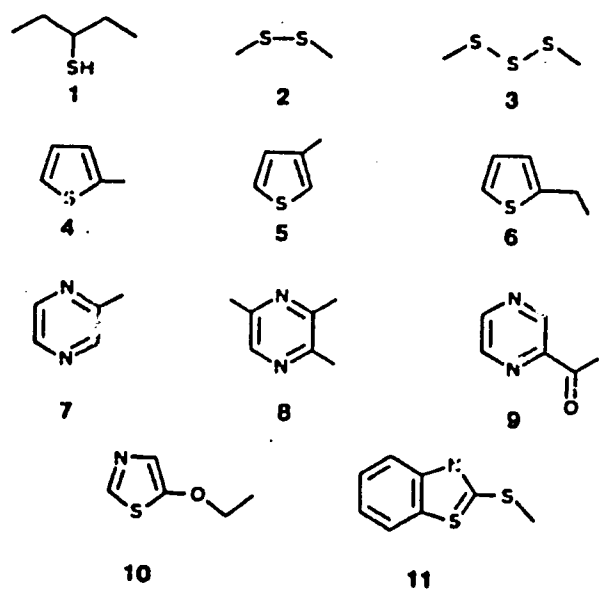


Fig. 14. New N- and S-heteroatomic substances in guava volatiles.

A certain content of different N- and S-heteroatomic substances seems to be characteristic for the composition of guava flavour; newly identified compounds are shown in Fig. 14. In low concentrations (10ppb) we could identify the low boiling 3-pentanethiol (Fig. 14,1), dimethyldisulfide as well as dimethyltrisulfide (Fig. 14, 2 and 3), together with some thiophenes (Fig. 14, 4-6) and pyrazines (Fig. 14, 7-9) including acetylpyrazine (Fig. 14, 9); additionally 2-

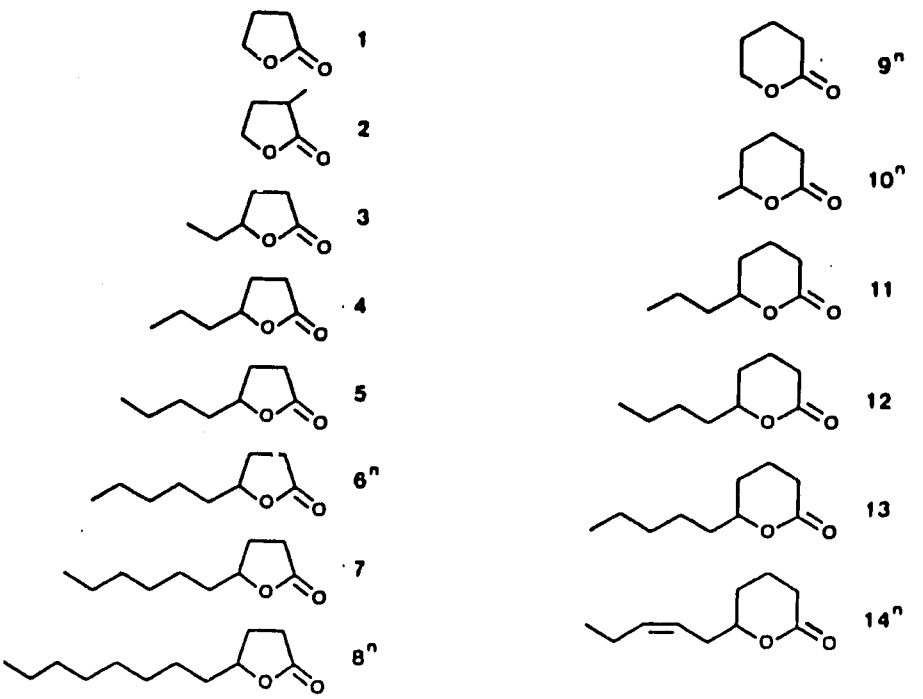


Fig. 15. Lactones in Alphonso mango volatiles.

ethoxythiazole (Fig. 14, 10) and 2-methylthiobenzothiazole (Fig. 14, 11) were found.

With the last fruit in the series we are presenting in this paper -- the mango -- distinct differences in the composition of the volatile constituents depending on the variety examined have been reported (15). We studied the genuine Alphonso type of Indian origin, regarded to be the most aroma impressive variety. Besides a lot of newly identified esters (cf. Fig. 7), carbonyl and alcohol compounds, we could complete the picture of the lactone "spectrum" of mango as shown in Fig. 15 (compounds marked with n were detected for the first time). It is well known that their contribution to the overall mango aroma cannot be neglected. Within this class of substances we could identify (Z)-jasmolactone (Fig. 15, 14) for the first time in mango, already known as important constituent of black tea flavour (16). The corresponding free acids in their Z- and E-configuration were also be detectable.

Together with the high amounts of 4-hydroxy-2,5-dimethyl-3(2H)-furanone (about 2ppm) the isomer ocimenes belong to the quantitatively dominating volatiles in this mango variety. Expanding our studies on metabolic pathways of terpene components to mango, similar sample preparation as with papaya (Hg^{2+}-inhibition) was carried out. In the experiment without enzyme inhibition together with the main terpene hydrocarbones (Z)- and (E)-ocimene (Fig. 16, 5 and 6) we identified a new trace compound 2,6-dimethylocta-1-(E)-3,7-diene (Fig. 16, 3), better known as α-hymentherene, an optically active terpene hydrocarbon, re-

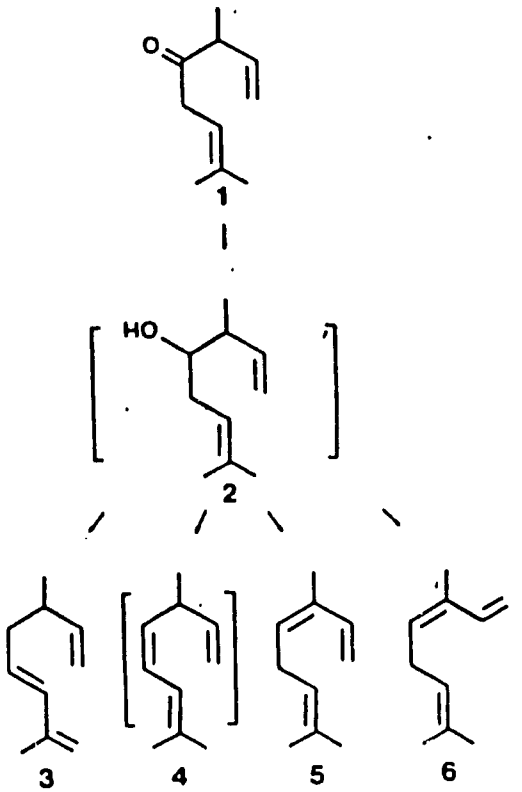


Fig. 16. Hypothetic pathway for the formation of ocimenes and α-hymentherene in mango pulp.

cently described as constituent of the seed oil of Seseli libanotis, L. (17). In the Hg^{2+}-inhibited fruit pulp we found a substantially increased concentration of 3,7-dimethyl-1,6-octadien-4-one (Fig. 16, 1), leading us to the proposal of a hypothetic genetic pathway for the main components (Z)- and (E)-ocimene as well as for the α-hymentherene via an postulated intermediate alcohol (Fig. 16, 2). The step from the ketone to the bounded (or not bounded) form of the alcohol and also the elimination to the ocimenes and -- not detected -- to β-hymentherene (Fig. 16, 4) could be well understood by enzymatic interaction. The reaction to α-hymentherene via the hypothetical β-isomer or by direct formation from the alcohol 2 must involve a rearrangement step, i.e. a shift of the 1,3-diene system. A chemical analogue of the reverse reaction has been described under photochemical conditions (18,19).

REFERENCES

1 K. Herrmann, Exotische Lebensmittel, Springer, Heidelberg, 1983.
2 G. Dünnenberger, D.M. Kopp and A. Blumenthal, Mitt. Geb. Lebensm. Hyg., 70 (1979) 294.
3 W. Herres, H. Idstein and P. Schreier, J. HRC & CC, 6 (1983) 590.
4 H. Idstein, W. Herres and P. Schreier, J. Agric. Food Chem., 32 (1984) in press.

5 R. Tressl and F. Drawert, J. Agric. Food Chem. 21 (1973) 560.
6 G. Ohloff, in W. Herz, H. Grisebach, and G.W. Kirby (Eds.), Fortschritte der Chemie organischer Naturstoffe , Springer, Wien, 35 (1978) 431.
7 P. Proksch, P.G. Gülz and H. Budzikiewicz, Z. Naturforsch., 35c (1980) 529.
8 R.A. Flath and R.R. Forrey, J. Agric. Food Chem., 25 (1977) 103.
9 T. Yoshida, S. Muraki, H. Kowamura and A. Komatsu, Agr. Biol. Chem., 33 (1969) 343.
10 P. Schreier, F. Drawert and A. Junker, Z. Lebensm. Unters. Forsch., 155 (1974) 98.
11 Y. Nakatani, S. Sato and T. Yamanishi, Agr. Biol. Chem., 33 (1969) 967.
12 T. Takeo, Phytochemistry, 20 (1981) 2145.
13 C.S. Tang and M.M. Syed, Phytochemistry, 11 (1972) 2531.
14 J. Heidlas, M. Lehr, H. Idstein and P. Schreier, J. Agric. Food Chem., 32 (1984) in press.
15 K.H. Engel and R. Tressl, J. Agric. Food Chem., 31 (1983) 796.
16 P. Cazenave and I. Hormann, Helv. Chim. Acta, 57 (1974) 209.
17 M. Ashraf, P.J. Sandra, T. Saeed and M.K. Bhatty, Pak. J. Sci. Ind. Res., 22 (1979) 322.
18 K.H. Schulte-Elte, M. Gadola and G. Ohloff, Helv. Chim. Acta, 54 (1971) 181[illegible].
19 K.H. Schulte-Elte and M. Gadola, Helv. Chim. Acta, 54 (1971) 1095.

HEWLETT PACKARD

- APPendix C -

HP 5890A Accessories/Options

Cross-Reference List

Gas Chromatography

June 1989

Option	Accessory	Description
011	05890-60670	Signal cable for use with HP 339XA Series Integrator—when used with HP 3392A or HP 3393A Integrator (digital output) this cable is not necessary—see chart below.
012	05890-60790	Signal cable for use with HP 3350 Laboratory Automation System A/D or HP 3380A Integrator.
013	05890-60800	Signal cable for general purpose use (spade lugs)—provides two leads; one for -10 to 1 mV and one for 0 to 1 V operation.
014	05890-60780	Signal cable for use with HP 3388A Integrator or HP 5880A Gas Chromatograph.
021	05890-61050	Remote start cable for use with HP 3390A Integrator—requires HP 3390A with input/output board.
022	05890-61060	Remote start cable for use with HP 3392A or HP 3393A Integrator—if HP 5890A Option 550 is ordered for digital transmission only, this cable is not necessary.
023	05890-61070	Remote start cable for use with HP 19653B Lab Automation System S/ECM.
024	05890-61080	Remote start cable for general purpose use (spade lugs).
025	05890-61160	Remote start cable for use with HP 18652A Lab Automation System A/D.
026	05890-61170	Remote start cable for use with HP 5890A/HP 3388A.
027	05890-61180	Remote start cable for starting HP 5890A from HP 18762A ALS controller.

Option	Accessory	Description
221	19232E*	Single Thermal Conductivity Detector with makeup gas and digital gain. Requires SERIES II.
N/A	19232B	TCD with electrometer for use with packed/capillary columns (makeup gas included). (NOT for use with SERIES II.)
N/A	19232C*	TCD to FID in series connection. Does not include either detector.
N/A	19232D	Adds makeup gas to an existing TCD.
236	19233B	Single Electron Capture Detector, general license, with makeup gas.
N/A	19233C	Adds makeup gas to an existing ECD.
238	19235B	Single Electron Capture Detector, specific license, with makeup gas.
240	19256A	Single Flame Photometric Detector with makeup gas.
252	19234B	Single Nitrogen Phosphorus Detector with makeup gas.
N/A	19234C	Adds makeup gas to an existing NPD.
260	19261A	Analog Input Board.
N/A	19265A	Additional Heated Zone.
550	19242A	Data communications interface board. Provides second channel of analog output and one channel of digital communications with single transmission. Includes one Instrument Network (INET) cable for digital transmission with HP 3392A Integrator. One maximum.
560	19254A	RS-232-C Serial Interface.
570	19242A & 19255-60500	HP ChemStation Interface.
600	19243A & 19231E	Universal injection port for packed columns and single flame ionization detector.
N/A	19246A	Additional flow module providing one 100 psig pressure regulator and gauge two 60 psig pressure regulators and gauges. Field installation only.

*For operation with HP 5890 SERIES II GC or equivalent upgrades only

Option	Accessory	Description
038	19237A	Two channels of flow sensing—calibration H_2, He, N_2, and $ArCH_4$ carrier gases.
055	19239A	CO_2 cryogenic oven control—incompatible with Option 056 (cooling to -50°C).
056	19239B	N_2 cryogenic oven control—incompatible with Option 055 (cooling to -80°C).
057	19239C*	Cryoblast cooling of SERIES II on-column injection port and oven.
100	19243A	Universal injection port for packed columns.
102	19243C	Packed injection port with septum purge.
N/A	19243D	Upgrade for standard packed port to a septum purged packed port.
107	19251A	Capillary inlet system for split/splitless operation.
108	19251B	Capillary inlet system for split operation only.
115	19245B*	Temperature programmable cool on-column injector with manual pressure control.
116	19245C*	Temperature programmable cool on-column injector with electronic pressure control.
N/A	19245D	Needle guide assembly for manual on-column injection includes syringe and fused silica needle. For use with options 115, 116, 19245B, and 19245C.
120	N/A	No injection port. Incompatible with detector Options 220-252 (TCD, ECD, and NPD) and any other injection port options. No flow controller provided.
200	N/A	No detector (incompatible with any other detector options).
201	N/A	No detector. For use with MSD.
202	N/A	No detector. For use with IRD.
203	N/A	No detector. For use with AED.
211	19231B	Single Flame Ionization Detector with makeup gas—includes adapter for 1/8" columns.
N/A	19231C	Add makeup gas to an existing FID.

Option	Accessory	Description
N/A	19247A	Additional flow module with 1 psig pressure gauge. Recommended for use when HP Series 530 μ columns are used with Options 107 or 108. Field installation only.
N/A	19247B	Carrier Gas Miser.
N/A	19247C	Flow control module for use with Series 530μ columns in the on-column inlet with TCD and ECD. (Incompatible with Option 116—Temperature Programmed Cool On-Column with Electronic Pressure Control).
N/A	19247G	Chemical trap for carrier gas.
801	05890-61400	German Manual Kit.
	05890-91310	Quick Reference Card.
802	05890-61410	French Manual Kit (substitute for English version).
	05890-92310	Quick Reference Card.
803	05890-61420	Italian Manual Kit (substitute for English version).
	05890-93310	Quick Reference Card.
804	05890-61430	Spanish Manual Kit (substitute for English version).
	05890-94310	Quick Reference Card
N/A	19237-60510	Plumbing kit for electronic flow sensing.
N/A	19244-80600	FID and NPD jet for glass capillary columns.
N/A	19246-60620	0–12 psig back-pressure regulator with gauge.
N/A	19246-60630	0–60 psig back-pressure regulator with gauge.
N/A	19247-60510	Oven exhaust deflector kit.
N/A	19862-60510	0–[illegible]0 ml/min restrictor for manual flow controller.
N/A	19862-60520	0–60 ml/min restrictor for manual flow controller.
N/A	19862-60530	0–110 ml/min restrictor for manual flow controller (standard in packed injection ports).
N/A	19862-60540	0–400 ml/min restrictor for manual flow controller (standard in capillary inlet systems).

Option	Accessory	Description
N/A	19862-60550	0–750 ml/min restrictor for manual flow controller.
N/A	5080-8872	NPD recoating kit.
N/A	19230T	HP 5890A upgrade to SERIES II (past S/N break 2908AXXXXX and prior to 2921AXXXXX).
N/A	19230U	HP 5890A upgrade to SERIES II (prior to S/N break 2908AXXXXX).

Accessory	Option
19230T	N/A
19230U	N/A
19231E	211
19231C	N/A
19232B	221
19232C	N/A
19232D	N/A
19233B	236
19233C	N/A
19234B	232
19234C	N/A
19235B	238
19237A	038
19239A	055
19239B	056
19239C	057
19242A	550
19243A	100
19243C	102
19243D	N/A
19245A	114
19246A	N/A
19247A	N/A
19247B	N/A
19247C	N/A
19247G	N/A
19251A	107
19251B	108
19252A	N/A
19254A	560
19256A	240

Accessory	Option
19261A	260
19237-60510	N/A
19244-80600	N/A
19246-61620	N/A
19246-60880	N/A
19247-80510	N/A
19362-80510	N/A
19362-60520	N/A
19362-60530	N/A
19362-80540	N/A
19362-60550	N/A
05890-60760	011
05890-60780	014
05890-60790	012
05890-60800	013
05890-61050	021
05890-61060	022
05890-61070	023
05890-61080	024
05890-61160	025
05890-61110	026
05890-61170	027
05890-61400	801
05890-61410	802
05890-61420	803
05890-61430	804
5080-8872	N/A

NOV-29-1994 09:46 FROM HP CLIENSERVICE NY TO 17872397 P.06

CITRUS I

[1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35]

1. Altenburger, R. and P. Matile. Further observations on rhythmic emission of fragrance in flowers. Planta. 180(2): 194-7, 1990.
2. Ameodo, C., A. Baszkin, J. P. Benoit and C. Thies. Interfacial tension behavior of citrus oils against phases formed by complex coacervation of gelatin. Acs Symp. Ser. : 1988.
3. Berger, R. G. and F. Drawert. Biotechnological preparation of glycosides using plant cell cultures. Ger. Offen. : 1988.
4. Boelens, M. H. and F. Valverde. Aroma chemicals and citrus oils. Perfum. Flavor. 13(5): 5-8, 1988.
5. Braddock, R. J. and G. D. Sadler. Chemical changes in citrus juices during concentration processes. Acs Symp. Ser. : 1989.
6. Buchel, J. A. Flavoring with citrus oils. Perfum. Flavor. 14(1): 22-6, 1989.
7. Dervieux, D., G. Nouri and J. Munger. Aromatization of elastomeric products. Eur. Pat. Appl. : 1989.
8. Dugo, G., G. Lamonica, A. Cotroneo, A. Trozzi, F. Crispo, G. Licandro and D. Gioffre. Authenticity of citrus essential oils. Note XVII. Composition of the volatile fraction of the essential oil of the Calabrian bergamot. Essenze Deriv. Agrum. 57(3): 456-534, 1987.
9. Fellers, P. J. A review of limonin in grapefruit (Citrus paradisi) juice, its relationship to flavor, and efforts to reduce it. J. Sci. Food Agric. 49(4): 389-404, 1989.
10. Fischboeck, G., R. Kellner and W. Pfannhauser. Capillary GC-FTIR- and GC-MS studies of citrus flavor decomposition processes. Dev. Food Sci. :
11. Fischboeck, G., W. Pfannhauser and R. Kellner. Characterization of citrus and kiwi flavors and their degradation processes by combination of GC/FTIR and GC/MS. Mikrochim. Acta. 1(1-6): 27-30, 1987.
12. Garrard, V. G. and A. B. Hudson. Citrus-flavored tobacco articles comprising citral acetal. U.s. : 1989.
13. Gibbons, C. E., J. L. J. Kinsey and A. A. Whillock. Laminated polyolefin-polycarbonate-paperboard containers for storage of fruit juices. Eur. Pat. Appl. : 1988.
14. Ichimura, N. and T. Uchiyama. Characteristic flavor components in citrus fruits. Koryo. : 1989.
15. Inoma, S., Y. Miyagi and T. Akieda. Characterization of citrus oil (mainly orange oil, mandarin oil, tangerine oil). Kanzei Chuo Bunsekishoho. : 1989.
16. Kobayashi, A., K. Nohara, E. Ohsumi and T. Yamanishi. Identification of di(1-oxyalkyl)peroxides in the essential oil of Citrus iyo. Agric. Biol. Chem. 54(2): 561-2, 1990.
17. Kolodesh, M. S., D. Toms, J. C. Baird, E. Weinshenker and C. H. Japikse. Fruit juices made with natural citrus juice and juice sacs. Eur. Pat. Appl. : 1988.
18. Lin, Z. and Y. Hua. Changes in the volatile flavor constituents of Citrus sinensis (L.) Osbeck during storage. Zhiwu Xuebao. 30(6): 623-8, 1988.
19. Ma, L., Y. Zheng, Y. Sun, M. Liu and Z. Wu. Aroma volatile constituents of Citrus aurantium var. amara Engl. Beijing Daxue Xuebao, Ziran Kexueban. 24(6): 687-94, 1988.
20. Nagy, S., R. L. Rouseff and H. S. Lee. Thermally degraded flavors in citrus juice products. Acs Symp. Ser. : 1988.

21. Nigam, M. C., M. S. Siddique and S. P. S. Duhan. Terpenoid compositions of the essential oil derived by acid hydrolysis of non volatile portion of aromatic plants. Indian Perfum. 31(3): 182-4, 1987.
22. Nikolaishvili, A. A. and L. G. Kharebava. Volatile compounds in leaves and fruits of Meyer lemon affected by red citrus mite. Subtrop. Kul't. : 1989.
23. Norman, S. I., C. C. Gopsi'' and R. T. Stringfield. Adsorbent resins for removing bitter flavor components from citrus juices. Eur. Pat. Appl. : 1989.
24. Ogawa, J. and H. Tezuka. Removal of a bitter taste in citrus fruit juice by treatment with ethanol. Jpn. Kokai Tokkyo Koho. : 1988.
25. Ogihara, K., K. Munesada, T. Yamamitsu and T. Suga. Fragrant and biologically active constituents of the citrus cultivar Jyabon. Phytochemistry. 28(4): 1061-7, 1989.
26. Romruen, K., W. Drochner, H. Lueders and K. D. Guenther. Effect of increasing amounts of pectin given to laying hens on feed intake, nutrient digestibility, and some parameters of gut chyme and serum as determined by the pair feeding method. J. Anim. Physiol. Anim. Nutr. 60(5): 257-68, 1988.
27. Sawamura, M., S. Kuwahara, K. Shichiri and T. Aoki. Studies on the essential oils of Pummelos. Part III. Volatile constituents of several varieties of Pummelos and a comparison of the nootkatone levels in Pummelos and other citrus fruits. Agric. Biol. Chem. 54(3): 803-5, 1990.
28. Sawamura, M., T. Tsuji and S. Kuwahara. Studies on the essential oils of pummelo. Part II. Changes in the volatile constituents of pummelo (Citrus grandis Osbeck forma Tosa-bun tan) during storage. Agric. Biol. Chem. 53(1): 243-6, 1989.
29. Sepulveda, E., C. Saenz, C. G. Yamada and J. I. Reyes. Yield and characteristics of the essential oil of orange [Citrus sinensis (L) Osbeck] at different ripening stages. II. Valencia variety. Essenze Deriv. Agrum. 59(2): 190-7, 1989.
30. Shiota, H. Volatile components in the peel oil from fingered citron (Citrus medica L. var. sarcodactylis Swingle). Flavour Fragrance J. 5(1): 33-7, 1990.
31. Skorepa, J., P. Hrabak, P. Mares, A. Blacky, R. Foglar, J. List, A. Prikryl and V. Svoboda. Dietetic oil for the prevention of atherosclerosis, containing polyenoic acids from fish and vegetable oils. Czech. : 1988.
32. Sugisawa, H., R. H. Yang, C. Kawabata and H. Tamura. Volatile constituents in the peel oil of sudachi (Citrus sudachi). Agric. Biol. Chem. 53(6): 1721-3, 1989.
33. Tajima, K., S. Tanaka, T. Yamaguchi and M. Fujita. Analysis of green and yellow yuzu peel oils (Citrus junos Tanaka). Novel aldehyde components with remarkably low odor thresholds. J. Agric. Food Chem. 38(7): 1544-8, 1990.
34. Wolf, P. A. and M. J. Havekotte. Stable microemulsions using edible oils as flavoring materials. U.s. : 1989.
35. Wuelknitz, P., R. Lehmann, W. Ploeger, K. Hill and F. Foerg. Antimicrobial compositions containing biguanide compounds, C8-16-alkyl glycosides, aroma oils, and surfactants. Ger. Offen. : 1989.

CITRUS II

[1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66]

1. Food packaging materials for prevention of flavor change. Jpn. Kokai Tokkyo Koho. : 1984.
2. Removal of bitter taste compounds from fruits juices. Jpn. Kokai Tokkyo Koho. : 1984.
3. Stabilization of flavor oil emulsion. Jpn. Kokai Tokkyo Koho. : 1984.
4. Ahmad, M., M. A. Chaudry and I. Khan. Technological studies on citrus and other fruit based drinks. Nucleus (Karachi). 23(3-4): 41-5, 1986.
5. Ahmad, M., M. A. Chaudry and I. Khan. Studies on the standardization and storage stability of citrus and mango squashes. Nucleus (Karachi). 23(1-2): 45-8, 1987.
6. Alencar, J. W., A. A. Craveiro and F. J. A. Matos. Kovats' indexes as a preselection routine in mass spectra library searches of volatiles. J. Nat. Prod. 47(5): 890-2, 1984.
7. AlSaadawi, I. S. and A. J. AlRubeaa. Allelopathic effects of Citrus aurantium L. I. Vegetational patterning. J. Chem. Ecol. 11(11): 1515-25, 1985.
8. AlSaadawi, I. S., M. B. Arif and A. J. AlRubeaa. Allelopathic effects of Citrus aurantium L. II. Isolation, characterization, and biological activities of phytotoxins. J. Chem. Ecol. 11(11): 1527-34, 1985.
9. Ben, Y. S., B. Shapiro and D. Timmer. Effects of shrink seal-packaging with cross-linked polyolefin films on keeping quality of various citrus fruits. J. Plast. Film Sheeting. 2(3): 255-7, 1986.
10. Bender, F. G., C. W. Ever[illegible] and W. E. Swartz. Increasing the organoleptic acceptability of shank mea[illegible]s. : 1985.
11. Berk, Z. and C. H. Mannheim. The effect of storage temperature on quality of citrus products aseptically packed into steel drums. J. Food Process. Preserv. 10(4): 281-93, 1986.
12. Bernhardt, C. A. Method for making better tasting low calorie fat materials. Eur. Pat. Appl. : 1987.
13. Braddock, R. J., S. Nikdel and S. Nagy. Composition of some organic and inorganic compounds in reverse osmosis-concentrated citrus juices. J. Food Sci. 53(2): 508-12, 1988.
14. Brown, R. H. Non-volatile nematocides control of citrus nematode in lemon trees. Plant Prot. Q. 1(1): 21-3, 1985.
15. Buccellato, F. Flavor characteristics of the components of orange blossom Citrus aurantium. Instrum. Anal. Foods: Recent Prog., Proc. Symp. Int. Flavor Conf., 3rd. 2: 1983.
16. Clark, B. C. J., T. S. Chamblee and G. A. Iacobucci. HPLC isolation of the sesquiterpene hydrocarbon germacrene B from lime peel oil and its characterization as an important flavor impact constituent. J. Agric. Food Chem. 35(4): 514-18, 1987.
17. Di, C. F. and J. J. Cipriano. An enhanced citrus flavor. Eur. Pat. Appl. : 1985.
18. Dmitriev, M. T., E. G. Rastyannikov and A. G. Malysheva. Chromatography-mass-spectrometry study of aerostimulants of plant origin. Rastit. Resur. 22(1): 79-83, 1986.
19. Drochner, W., A. Orth and H. Lueders. Effect of increasing amounts of pectic substances on the metabolism of laying hens. Dtw, Dtsch. Tieraerztl. Wochenschr. 93(1): 11-14, 1986.
20. Fischboeck, G., R. Kellner and W. Pfannhauser. Capillary GC-FTIR- and GC-MS studies of citrus flavor decomposition processes. Dev. Food Sci. : 1988.

21. Fustier, P. and R. R. Dale. Citrus flavored beverage in polyethylene containers. U.s. : 1985.
22. Glass, M., V. Corsello, D. A. Orlandi and A. Guzowski. Process for preparing a chewing gum composition with improved flavor. S. African. : 1986.
23. Hayashi, Y. Citrus note. Koryo. : 1986.
24. Huet, R. Citrus fruits with low acidity. Fruits. 39(11): 689-98, 1984.
25. Iwanami, Y., A. Cho, S. Togashi and M. Asashima. Effects of exposure to volatile substances from plants on the behavior of Drosophila. Yokohama shiritsu Daigaku Ronso, Shizen Kagaku Keiretsu. 36(2): 15-33, 1985.
26. Japikse, C. H., B. L. P. Van, J. A. Hembree, R. R. Kitts and D. R. Meece. Process for the production of citrus flavor and aroma compositions. Eur. Pat. Appl. : 1986.
27. Kashiwagi, S., M. Shiigi, A. Saeki, K. Aritomi, K. Arimura, F. Tomonaga, S. Kimura, T. Yano and Y. Moroki. Studies on methane fermentation from citrus onshu peel (Part I). Volatile fatty acid production from citrus onshu peel in acidogenic fermentation. Kogai. 20(5): 257-67, 1985.
28. Kharebava, L. G. Volatile compounds in leaves of the Meyer lemon and some of its hybrids. Subtrop. Kul't. : 1983.
29. Kharebava, L. G. and V. V. Tsertsvadze. Volatile compounds of flowers of Citrus unshiu Marc. Subtrop. Kul't. : 1986.
30. Loutaty, R. and C. Rolland. Process and device for extracting natural flavor compounds from plant products. Eur. Pat. Appl. : 1984.
31. Maeda, H. Studies on the production of high quality citrus juice by resin adsorption methods. Fluess. Obst. 54(2): 65-6, 1987.
32. Maeda, H., Y. Takahashi, M. Miyake, N. Inaba and Y. Ifuku. Studies on the quality improvement of citrus juice and utilization of peels with ion exchange resins. IV. Recovery of sugar solution from press liquor of Satsuma mandarin (Citrus unshiu Marc.) peel residue with ion exchanges and adsorbents. Nippon Shokuhin Kogyo Gakkaishi. 32(7): 500-8, 1985.
33. Matile, P. and R. Altenburger. Rhythms of fragrance emission in flowers. Planta. 174(2): 242-7, 1988.
34. Matsumoto, Y., Y. Bessyo, S. Monya and M. Kodama. Utilization and processing of middle or late ripening variety citrus fruit (Part 7). Utilization of essential oil from Citrus iyo as a flavor for frozencake and jelly. Kenkyu Hokoku Ehime ken Kogyo Gijutsu Senta. : 1984.
35. Mazza, G. Gas chromatographic and mass spectrometric study of the aroma composition of bergamot (Citrus aurantium subsp. bergamia Risso and Poiteau Engler) essential oil. J. Chromatogr. 362(1): 87-99, 1986.
36. Mazza, G. Gas chromatography and mass spectrometry study of the aroma composition of mandarine essential oil. Sci. Aliments. 7(3): 459-79, 1987.
37. Mehta, U. and S. Bajaj. Changes in the chemical composition and organoleptic quality of citrus peel candy during preparation and storage. J. Food Sci. Technol. 21(6): 422-4, 1984.
38. Meyers, M. A. Interactions between citrus flavor solutes and low density polyethylene by static and inverse-high pressure liquid chromatographic methods. 48(8): 1988.
39. Misaki, M. Utilization of cyclodextrin for citrus fruit products. Denpun Kagaku. 31(2): 98-106, 1984.
40. Moshonas, M. G. and P. E. Shaw. Direct gas chromatographic analysis of aqueous citrus and other fruit essences. J. Agric. Food Chem. 32(3): 526-30, 1984.

41. Muhammad, I., M. A. Chaudry, M. Ahmad, J. Muhammad and I. Khan. Effect of heating and addition of pectin on cloudiness and other physicochemical characteristics of citrus squash during storage. Nucleus (Karachi). 23(3-4): 15-18, 1986.
42. Nagy, S. and M. Kim. Gas chromatography-mass spectral techniques in citrus essence research. Food Technol. (Chicago). 40(11): 95-8, 1986.
43. Nichols, W., R. Newsome, R. Thesing and W. Houck. Matrix entrapment of flavorings for smoking articles. Eur. Pat. Appl. : 1987.
44. Nicolosi, A. C., L. C. M. Cataldi, S. Campisi and C. Russo. Lipids in citrus fruit juice. 1. Lipid content during orange ripening in eastern Sicily. J. Agric. Food Chem. 35(6): 1023-7, 1987.
45. Nunez, A. J., H. Maarse and J. M. H. Bemelmans. Volatile flavor components of grapefruit juice (Citrus paradisi Macfadyen). J. Sci. Food Agric. 36(8): 757-63, 1985.
46. Paulson, D. J., R. L. Wilson and D. D. Spatz. Reverse osmosis and ultrafiltration applied to the processing of fruit juices. Acs Symp. Ser. : 1985.
47. Pino, J. and M. Fernandez. Analysis of the volatile constituents of grapefruit juice and nectar produced in the citrus factory Isla de la Juventud. Tecnol. Quim. 5(4): 129-37, 1984.
48. Powers, J. R., R. S. Moore, B. J. Anderson, G. E. Downton, J. A. Kennedy, D. R. Kearney and D. A. Strang. Citrus juice concentrate. Eur. Pat. Appl. : 1984.
49. Roellig, H., L. Banasiak, H. Lyr, M. Jahn and H. J. Ullrich. New volatile antifungal compound for controlling post-harvest diseases. Tagungsber. Akad. Landwirtschaftswiss. D. D. R. : 1987.
50. Saleeb, F. Z. and R. P. McKay. Method for fixing food ingredients on a magnesium salt substrate. U.s. (621): 1987.
51. Santo, T. and M. Ota. Bitter taste removal from whole citrus fruit juice. Jpn. Kokai Tokkyo Koho. : 1985.
52. Sawamura, M. and T. Kuriyama. Quantitative determination of volatile constituents in the pummelo (Citrus grandis Osbeck forma Tosa-buntan). J. Agric. Food Chem. 36(3): 567-9, 1988.
53. Sebastiani, E., G. Dugo and A. Cotroneo. Purity of citrus essential oils. Note V. Evaluation of some stationary phases for analysis of volatile fractions of lemon essential oils by high-resolution gas chromatography. Essenze Deriv. Agrum. 53(4): 501-14, 1983.
54. Shankaranarayana, M. L., B. Raghavan, K. O. Abraham and N. B. Shankaracharya. Fruit flavors. Pafai J. 7(1): 13-22, 1985.
55. Shaw, P. E. The flavor of non-alcoholic fruit beverages. Dev. Food Sci., 3B(Food Flavours, Pt. B). : 1986.
56. Shaw, P. E. and M. G. Moshonas. Mass spectrometry for the identification of citrus flavor components. Mass Spectrom. Rev. 4(4): 397-420, 1985.
57. Sugisawa, H., R. H. Yang, T. Matsuo and H. Tamura. Volatile compounds in peel oil of Shiroyanagi navel. Nippon Nogei Kagaku Kaishi. 61(9): 1101-6, 1987.
58. Swartz, W. E., C. W. Everson and F. G. Bender. Use of soy products having a reduced beany flavor in meat and other food products. U.s. (541): 1985.
59. Takei, Y. Changes in the aroma of citrus peels by marmalade processing. Kaseigaku Zasshi. 36(10): 747-53, 1985.
60. Takei, Y. Comparison of the aroma of fresh peels of marmalades between several species of citrus fruits. Kaseigaku Zasshi. 36(10): 754-62, 1985.

61. Tateo, F., F. Bersc, A. Bianchi, C. Gregotti and P. Richelmi. The reactivity of aspartame with aldehydes in flavoring ingredients: technological and toxicological aspects. Note I. Riv. Soc. Ital. Sci. Aliment. 15(1-2): 23-34, 1986.
62. Temelli, F., C. S. Chen and R. J. Braddock. Supercritical fluid extraction in citrus oil processing. Food Technol. (Chicago). 42(6): 145-50, 1988.
63. Tsai, C. H., M. A. Walsh and R. L. J. Swaine. Citrus concentrate and beverage with fresh taste and greater storage stability. Eur. Pat. Appl. : 1986.
64. Van, d. O. and A. M. Godefridus. Modifying the taste and aroma of citrus fruit compositions. Eur. Pat. Appl. : 1986.
65. Williams, A. A. The flavor of non-citrus fruits and their products. Colloq. Int. Aromes Aliment., [Actes], Meeting Date. : 1982.
66. Yang, R. K. Tabletted chewing gum containing improved delivery system for flavor or other active ingredients. S. African. : 1987.

CITRUS III

[1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48]

1. Preparing citrus fruit sections with fresh fruit flavor and appearance. U. S. Pat. Appl. : 1980.
2. Alcoholic beverages leaving inoffensive breath. Jpn. Kokai Tokkyo Koho. : 1981.
3. Babu, K. J., P. Laxminarayana and S. M. Reddy. Evaluation of different volatile compounds in the control of fruit-rot of lemon. Pesticides. 17(12): 1983.
4. Berry, R. E., P. E. Shaw, J. H. Tatum and C. W. I. Wilson. Citrus oil flavor and composition studies. Food Technol. (Chicago). 37(12): 88-91, 1983.
5. Bruemmer, J. H. Citrus fruit sections with fresh fruit flavor and appearance. U. S. Pat. Appl. (PAT): 1981.
6. Chang, K. J. and T. T. Fang. Studies on the quality of citrus fruit juice in Taiwan. (1). Extraction and quality evaluation of peel oil. Kuo Li Tai wan Ta Hsueh Nung Hsueh Yuan Yen Chiu Pao Kao. 19(2): 66-85, 1979.
7. Chen, M. C., C. B. Ammerman, P. R. Henry, A. Z. Palmer and S. K. Long. Citrus condensed molasses solubles as an energy source for ruminants. J. Anim. Sci. 53(1): 253-9, 1981.
8. Demole, E. and P. Enggist. Further investigation of grapefruit juice flavor components (Citrus paradisi Macfayden). Valencane- and eudesmane-type sesquiterpene ketones. Helv. Chim. Acta. 66(5): 1381-91, 1983.
9. Demole, E., P. Enggist and G. Ohloff. 1-p-Menthene-8-thiol: a powerful flavor impact constituent of grapefruit juice (Citrus paradisi Macfayden). Helv. Chim. Acta. 65(6): 1785-94, 1982.
10. Drawert, F. and R. G. Berger. Biogenesis of aroma compounds in plants and fruits. XIX. Comparison of aroma biosynthesis in segment, callus, and suspension cultures of citrus species. Chem., Mikrobiol., Technol. Lebensm. 7(5): 143-7, 1982.
11. Fisher, J. F. Review of quantitative analyses for limonin, naringin, naringenin rutinoside, hesperidin, and neohesperidin dihydrochalcone in citrus juice by high performance liquid chromatography. Proc. Int. Soc. Citric. 1977(3): 813-16, 1977.
12. Gevorkyan, A. A., P. I. Kazaryan, S. V. Avakyan, G. G. Filatova, V. R. Zananyants, Y. M. Blazhin, S. K. Ogorodnikov, V. A. Lakotkina, M. A. Somova and B. G. Levitina. 2-(3-Buten-1-yl)pyran derivative as an odorant with citrus scent and a mushroom and fruit note. U.s.s.r. From: Otkrytiya, Izobret., Prom. Obraztsy, Tovarnye Znaki. 1980(41): 1980.
13. Iwanami, Y. and H. Hyodo. Effects of volatiles evolved from excised plant tissues and lemon oil on the growth of mung bean seedlings. Plant Cell Physiol. 24(3): 473-7, 1983.
14. Kanako, K. and O. Katayama. Studies on volatiles from Satsuma mandarin juice. Part I. Difference of volatile components among each part of Satsuma mandarin (Citrus unshu Marc.) fruit. Shokuhin Sogo Kenkyusho Kenkyu Hokoku. : 1980.
15. Kanako, K. and O. Katayama. Studies on volatiles from Satsuma mandarin juice. Part III. Changes in volatile components of Satusma mandarine (Citrus unshu Marc.) juice by heat treatment. Shokuhin Sogo Kenkyusho Kenkyu Hokoku. : 1980.
16. Kanako, K., O. Katayama, K. Iino and T. Owada. Studies on volatiles from Satsuma mandarin juice. Part II. Influence of harvest time and processing on volatile components of Satsuma mandarin (Citrus unshu Marc.) juice. Shokuhin Sogo Kenkyusho Kenkyu Hokoku. : 1980.
17. Kaneko, K., T. Imai and O. Katayama. Utilization of Tenax GC column trapping technique for gas chromatographic analysis of headspace volatiles from Satsuma

mandarin (Citrus unshiu) juice. Nippon Shokuhin Kogyo Gakkaishi. 27(8): 407-10, 1980.
18. Kekelidze, N. A., M. I. Dzhanikashvili and V. V. Kutateladze. Petitgrain essential oil of the mandarin variety Unshiu. Maslo Zhir. Prom st. : 1981.
19. Kharebava, L. G. Volatile compounds in leaves of the Meyer lemon and some of its hybrids. Subtrop. Kul't. : 1983.
20. Kim, C. M., K. S. Kim, M. H. Kim and I. O. Huh. Taxonomical and phytochemical studies of Citrus plants native to Je Ju Island. I. Flavour patterns of the Citrus peel oils and one of the Citrus flavonoids. Saengyak Hakhoe Chi (Hanguk Saengyak Hakhoe). 10(1): 13-16, 1979.
21. Kim, H., D. H. Jo, Y. H. Park, C. Y. Lee and Y. H. Lee. Quantitative determination of flavor constituents of Korean Milgam (citrus unshiu) juice. Hanguk Nonghwa Hakhoe Chi. 23(2): 106-14, 1980.
22. Kobayashi, A., M. Matsumoto, K. Uchida and T. Yamanishi. Aroma development in Citrus iyo by pretreatment and storage. Proc. Int. Soc. Citric. : 1981.
23. Kodama, M., Y. Matsumoto and Y. Bessho. Off-flavor of Citrus unshiu juice caused by heat pasteurization. 3. Flavor changes of juice caused by heat treatment and storage conditions. Ehime ken Kogyo Shikenjo Kenkyu Hokoku. : 1981.
24. Kulesza, J., J. Podlejski, J. Kula, W. Brud, B. M. Czapczynska, M. Pilecki and M. Jastrzebski. Fragrance with a green citrus smell. Pol. : 1979.
25. Kusunose, H. and M. Sawamura. Aroma constituents of some sour citrus oils. Nippon Shokuhin Kogyo Gakkaishi. 27(10): 517-21, 1980.
26. Labows, J. N. and B. Shushan. Direct analysis of food aromas. Am. Lab. (Fairfield, Conn. 15(3): 56-61, 1983.
27. Lee, P. I. Membrane-forming polymer systems. Eur. Pat. Appl. : 1983.
28. Lund, E. D., C. L. Kirkland and P. E. Shaw. Methanol, ethanol, and acetaldehyde contents of citrus products. J. Agric. Food Chem. 29(2): 361-6, 1981.
29. Manabe, T. Dimethyl sulfide as a factor of off-flavor in heat-induced Satsuma mandarin orange (Citrus unshiu) juice and its precursor. Hiroshima Nogyo Tanki Daigaku Kenkyu Hokoku. 6(2): 145-52, 1979.
30. Mannheim, C. H. and N. Passy. Recovery and concentration of citrus aroma. Proc. Int. Soc. Citric. 1977(3): 756-62, 1977.
31. Marmo, D. and F. L. Rocco. Augmenting or enhancing the aroma or taste of smoking tobacco or a smoking tobacco article by adding a suspended flavoring composition. U.s. (17): 1981.
32. Moshonas, M. G. and P. E. Shaw. Citrus essential oils: effects of abscission chemicals and evaluation of flavors and aromas. Recent Adv. Phytochem. : 1980.
33. Nagy, S. and R. Rouseff. Evaluation and control of undesirable flavors in processed citrus juices. Anal. Control Less Desirable Flavors Foods Beverages, [Proc. Symp.], Y. : 1980.
34. Ohta, H. and Y. Osajima. Glass capillary gas chromatographic analysis of oil components extracted from Yuzu (Citrus junos) juice. J. Chromatogr. 268(2): 336-40, 1983.
35. Pino, J. and R. Tapanes. The use of citrus essential oils in food and pharmaceutical industries. Rev. Cubana Farm. 14(1): 51-5, 1980.
36. Rosenberg, M., I. J. Kopelman and C. Mannheim. Purification of citrus peel pigment preparate by gel permeation chromatography. Lebensm. Wiss. Technol. 17(5): 282-4, 1983

37. Rouseff, R. L., S. Nagy and J. A. Attaway. Less-desirable flavor components in processed citrus products. Proc. Int. Soc. Citric. : 1981.
38. Sakurai, K., T. Toyoda, S. Muraki and T. Yoshida. Chemical composition of fragrant materials. Part III. Odorous constituents of the absolute from flower of Citrus unshiu Marcovitch. Agric. Biol. Chem. 43(1): 195-7, 1979.
39. Shaw, P. E., J. M. Ammons and R. S. Braman. Volatile sulfur compounds in fresh orange and grapefruit juices: identification, quantitation, and possible importance to juice flavor. J. Agric. Food Chem. 28(4): 778-81, 1980.
40. Shaw, P. E. and S. Nagy. Analysis and flavor effects of volatile sulfur compounds in citrus juices. Qual. Foods Beverages: Chem. Technol., [Proc. Symp. Int. Flavor Conf.], 2nd. 1(Y): 1981.
41. Shaw, P. E. and C. W. I. Wilson. Importance of selected volatile components to natural orange, grapefruit, tangerine, and mandarin flavors. Acs Symp. Ser. : 1980.
42. Shaw, P. E. and C. W. I. Wilson. Volatile sulfides in headspace gases of fresh and processed citrus juices. J. Agric. Food Chem. 30(4): 685-8, 1982.
43. Shaw, P. E., C. W. I. Wilson and R. E. Berry. Some important flavor compounds in mandarin, grapefruit and orange juices and peel essential oils. Proc. Int. Soc. Citric. : 1981.
44. Soukup, R. J. and T. H. Parliment. Improving the character of the flavor and taste in a food product by addition of bitter principles. Fr. Demande. : 1984.
45. Uchida, K., M. Matsumoto, A. Kobayashi and T. Yamanishi. Composition of oxygenated compounds in peel oil from Citrus iyo and its variation during storage. Agric. Biol. Chem. 47(8): 1841-5, 1983.
46. Wawrzenczyk, C., Z. Przepiorka, A. Zabza and J. Gora. (E)-1-Cyano-2,6-dimethyl-3-heptene. Pol. : 1982.
47. Wilson, C. W. I. and P. E. Shaw. Quantitation of individual and total aldehydes in citrus cold-pressed oils by fused silica capillary gas chromatography. J. Agric. Food Chem. 32(3): 399-401, 1984.
48. Yamanishi, T., S. Fukawa and Y. Takei. Aroma characteristics of Citrus iyo. Nippon Nogei Kagaku Kaishi. 54(1): 21-5, 1980.

[Guava]
1. Beverage for oral rehydration. Belg. : 1989.
2. Ahmad, M., M. A. Chaudry and I. Khan. Technological studies on citrus and other fruit based drinks. Nucleus (Karachi). 23(3-4): 41-5, 1986.
3. Askar, A. New processes for the production of mango and guava juices. Int. Fruchtsaft Union, Wiss. Tech. Komm., [Ber.]. : 1986.
4. Askar, A., N. S. E. El and S. S. Bassiouny. Aroma constituents in white and pink guava fruits. Alimenta. 25(6): 162-7, 1986.
5. Binder, R. G. and R. A. Flath. Volatile components of pineapple guava. J. Agric. Food Chem. 37(3): 734-6, 1989.
6. Brouard, F. E. M. E. and B. d. S. R. J. M. P. De. Liquid flavoring materials from tropical fruit by enzyme treatment. Fr. Demande. : 1989.
7. Chyau, C. C., W. Y. Lin and C. M. Wu. Using purge and trap system to evaluate the changes of volatile compounds during clarified guava juice processing. Shih P'in K'o Hsueh (Taipei). 16(1): 14-21, 1989.
8. Chyau, C. C. and C. M. Wu. Differences in volatile constituents between inner and outer flesh-peel of guava (Psidium guajava, Linn) fruit. Lebensm. Wiss. Technol. 22(3): 104-6, 1989.
9. Davis, P. L., K. A. Munroe and A. G. Selhime. Laboratory bioassay of volatile naturally occurring compounds against the Caribbean fruit fly. Citrus Ind. 58(7): 24-6, 1977.
10. Hashinaga, F., Y. Shima and S. Itoo. Production of volatile components of guava during maturation. Gakujutsu Hokoku Kagoshima Daigaku Nogakubu. : 1987.
11. Idstein, H., C. Bauer and P. Schreier. Volatile acids from tropical fruits: cherimoya (Annona cherimolia, Mill.), guava (Psidium guajava, L.), mango (Mangifera indica, L., var. Alphonso), papaya (Carica papaya, L.). Z. Lebensm. Unters. Forsch. 180(5): 394-7, 1985.
12. Idstein, H. and P. Schreier. New results in tropical fruit flavor analysis. Dev. Food Sci. : 1985.
13. Idstein, H. and P. Schreier. Volatile constituents from guava (Psidium guajava, L.) fruit. J. Agric. Food Chem. 33(1): 138-43, 1985.
14. Kalra, S. K., D. K. Tandon and H. C. Lohani. Prevention of discoloration in guava beverage during storage. Indian Food Packer. 41(1): 21-5, 1987.
15. Luccarelli, D. J., B. D. Mookherjee, R. A. Wilson, M. J. Zampino and D. R. Bowen. Flavoring some tropical flavored foods with a mixture of C4-10-n-alkanoic acid and ethyl ester of 2-hydroxy-4-methyl pentanoic acid. U.s. : 1985.
16. MacLeod, A. J. and d. T. N. Gonzalez. Volatile flavor components of guava. Phytochemistry. 21(6): 1339-42, 1982.
17. Mookherjee, B. D., R. W. Trenkle, R. K. Wolff, R. M. Boden and T. Yoshida. Use of methyl-substituted pinyl oxopentenes, for augmenting, enhancing or modifying the aroma or taste of smoking tobacco and smoking tobacco articles. U.s. : 1984.
18. Nishimura, O., K. Yamaguchi, S. Mihara and T. Shibamoto. Volatile constituents of guava fruits (Psidium guajava L.) and canned puree. J. Agric. Food Chem. 37(1): 139-42, 1989.
19. Oliveros, B. L., R. M. Smith, J. M. Robinson and V. Albano. A chemical study of the essential oil from the fruit peeling of Psidium guajava L. Philipp. J. Sci. 115(1): 1-21, 1986.
20. Pattabhiraman, T. R., P. Rao and L. V. L. Sastry. Preliminary studies on the preparation of odor concentrates and identification of odorous ingredients in mango and guava. Perfum. Essent. Oil Rec. 59(10): 733-6, 1968.

21. Pattabhiraman, T. R., L. V. L. Sastry and K. O. Abraham. Preparation of odor concentrates and identification of odorous ingredients in mango and guava. II. Perfum. Essent. Oil Rec. 60(6): 233-8, 1969.
22. Pittet, A. O., R. Muralidhara, M. H. Vock, D. J. Luccarelli, K. P. Miller and C. Wiener. Thioalkanoic acid esters of phenylalkanols as food aroma and flavor enhancers. U.s. : 1987.
23. Prabha, T. N., M. S. Narayanan and M. V. Patwardhan. Flavor formation in callus cultures of guava (Psidium guajava) fruit. J. Sci. Food Agric. 50(1): 105-10, 1990.
24. Ramteke, R. S., W. E. Eipeson and M. V. Patwardhan. Behavior of aroma volatiles during the evaporative concentration of some tropical fruit juices and pulps. J. Sci. Food Agric. 50(3): 399-405, 1990.
25. Rodriguez, R., P. C. Agarwal and N. K. Saha. Effect of maturity of Safeda guavas on flavor preference. Indian Food Packer. 25(1): 13-20, 1971.
26. Schreier, P. and H. Idstein. Studies on the aroma composition of enzymically liquefied guava (Psidium guajava, L.) and Mango (Mangifera indica, L., var. Alphonso). Dtsch. Lebensm. Rundsch. 80(11): 335-40, 1984.
27. Schreier, P., H. Idstein and W. Herres. The use of HRGC-FTIR in tropical fruit flavor analysis. Anal. Volatiles: Methods Appl., Proc. Int. Workshop, Meeting Date. : 1983.
28. Schreier, P., E. R. Kittsteiner and H. Idstein. Studies on the enzymic liquefaction of tropical fruit pulps: guava (Psidium guajava, L.), papaya (Carica papaya, L.), mango (Mangifera indica, L. var Alphonso). Flucss. Obst. 52(7): 365-70, 1985.
29. Shiota, H. Review and study on guava fruits flavor. Koryo. : 1978.
30. Shiota, H., T. Minami and T. Tsuneya. Aroma constituents of guava-like fruits. Koryo. : 1980.
31. Stevens, K. L., J. E. Brekke and D. J. Stern. Volatile constituents in guava. J. Agr. Food Chem. 18(4): 598-9, 1970.
32. Torline, P. and H. M. B. Ballschmieter. Volatile constituents from guava. I. Comparison of extraction methods. Lebensm. Wiss. Technol. 6(1): 32-3, 1973.
33. Toulemonde, B. and D. Beauverd. Headspace analysis: trap desorption by microwave energy application to the volatile components of some tropical fruits. Dev. Food Sci. : 1985.
34. Van, R. P. C., W. P. De, W. C. J. Van and A. Tromp. Chenin blanc wine volatiles and the intensity of a guava-like flavor. S. Afr. J. Enol. Vitic. 3(1): 1-7, 1982.
35. Wilson, C. W. I. and P. E. Shaw. Terpene hydrocarbons from Psidium guajava. Phytochemistry. 17(8): 1435-6, 1978.
36. Yusof, S., S. Mohamed and B. A. Abu. Effect of fruit maturity on the quality and acceptability of guava puree. Food Chem. 30(1): 45-58, 1988.
37. Zheng, Y., Y. Sun, Z. Wu and M. Liu. Natural flavor components of Psidium guajava L. fruit. Zhiwu Xuebao. 29(6): 643-8, 1987.

Mango

1. Fragrant compositions containing .omega.-alkenyl methyl ketones for food and cosmetics. Jpn. Kokai Tokkyo Koho. : 1984.
2. Abd, E. B. M. M., A. Askar, S. S. K. El and E. F. M. G. Abd. Studies on mango flavor. Dtsch. Lebensm. Rundsch. 77(4): 139-42, 1981.
3. Aboul, E. A. M., H. M. Salem and M. M. Zaharan. Effect of gamma irradiation on mango volatiles during ripening. Chem., Mikrobiol., Technol. Lebensm. 8(2): 60-3, 1983.
4. Ackerman, L. G. J. and P. A. Torline. Volatile components in the headspace of eight mango cultivars. Lebensm. Wiss. Technol. 17(6): 339-41, 1984.
5. Adam, S. T. A simple and efficient cryogenic trapping device for odor evaluation of aroma fractions. Hrc Cc, J. High Resolut. Chromatogr. Chromatogr. Commun. 10(6): 369-70, 1987.
6. Ahmad, M., M. A. Chaudry and I. Khan. Technological studies on citrus and other fruit based drinks. Nucleus (Karachi). 23(3-4): 41-5, 1986.
7. Ahmad, M., M. A. Chaudry and I. Khan. Studies on the standardization and storage stability of citrus and mango squashes. Nucleus (Karachi). 23(1-2): 45-8, 1987.
8. Askar, A. New processes for the production of mango and guava juices. Int. Fruchtsaft Union, Wiss. Tech. Komm., [Ber.]. : 1986.
9. Askar, A., S. S. K. El and E. B. M. M. Abd. Preparation of mango juice concentrates. Ind. Obst Gemueseverwert. 66(2): 27-31, 1981.
10. Aspiras, A. B. and E. M. Tocino. Survey of the aroma composition of Philippine fruits. Philipp. Agr. 55(1-2): 67-82, 1971.
11. Bandyopadhyay, C. Contribution of gas chromatography to food flavor research. Pafai J. 5(3): 26-30, 1983.
12. Bandyopadhyay, C. and A. S. Gholap. Relation of aroma and flavor characteristics of mango (Mangifera indica) to fatty acid composition. J. Sci. Food Agr. 24(12): 1497-503, 1973.
13. Bartley, J. P. Volatile flavors of Australian tropical fruits. Biomed. Environ. Mass Spectrom. 16(1-12): 201-5, 1987.
14. Bartley, J. P. and A. Schwede. Volatile flavor components in the headspace of the Australian or "Bowen" mango. J. Food Sci. 52(2): 353-5, 1987.
15. Bautista, J. G. I., A. H. Balagot and E. J. Meimban. Flavor profile of Philippine mango (Mangifera indica L.), Carabao variety. Philipp. J. Food Sci. Technol. 6(1-2): 102-7, 1982.
16. Berger, R. G., F. Drawert, H. Kollmannsberger and S. Nitz. Natural occurrence of undecaenes in some fruits and vegetables. J. Food Sci. 50(6): 1655-6, 1985.
17. Bernreuther, A., N. Christoph and P. Schreier. Determination of the enantiomeric composition of .gamma.-lactones in complex natural matrices using multidimensional capillary gas chromatography. J. Chromatogr. : 1989.
18. Blakesley, C. N., J. G. Loots, P. L. Du and B. G. De. .gamma. Irradiation of subtropical fruits. 2. Volatile components, lipids and amino acids of mango, papaya, and strawberry pulp. J. Agric. Food Chem. 27(1): 42-8, 1979.
19. Chowdhery, K., P. S. Rehill, S. N. Khan and D. S. Rawat. Chemotropic responses of soft-rot fungi to wood volatiles. Indian J. For. 9(2): 178-81, 1986.
20. Craveiro, A. A., C. H. S. Andrade, F. J. A. Matos, J. W. Alencar and M. I. L. Machado. Volatile constituents of Mangifera indica Linn. Rev. Latinoam. Quim. 11(3-4): 1980.

21. Diaz, N. Preliminary study on the flavor and aroma components of four mango varieties. Annu. Congr. Am. Soc. Hortic. Sci., Trop. Reg. : 1976.
22. Diaz, N. A preliminary study on the flavor and aroma components of four mango varieties. J. Agric. Univ. P. R. 64(3): 357-62, 1980.
23. El, N. S. E. and A. Askar. Distribution of volatile aroma compounds between pulp and serum of mango juice. Dtsch. Lebensm. Rundsch. 82(12): 383-6, 1986.
24. El, N. S. E., I. A. Ismail and A. Askar. Aroma changes in mango juice during processing and storage. Food Chem. 30(4): 269-75, 1988.
25. El, S. S. K., A. Askar, E. B. M. M. Abd and E. F. M. G. Abd. Concentration of mango juice. 2. Aroma deterioration during the concentration. Chem., Mikrobiol., Technol. Lebensm. 7(4): 102-6, 1982.
26. Engel, K. H. and R. Tressl. Studies on the volatile components of two mango varieties. J. Agric. Food Chem. 31(4): 796-801, 1983.
27. Gholap, A. S. and C. Bandyopadhyay. Contribution of lipids to the aroma of ripening mango (Mangifera indica). J. Am. Oil Chem. Soc. 52(12): 514-16, 1975.
28. Gholap, A. S. and C. Bandyopadhyay. Fatty acid composition as a quality index of ripe mango (Mangifera indica L) pulp. Indian Food Packer. 30(5): 63-4, 1976.
29. Gholap, A. S. and C. Bandyopadhyay. Characterization of green aroma of raw mango (Magnifera indica L.). J. Sci. Food Agric. 28(10): 885-8, 1977.
30. Gholap, A. S. and C. Bandyopadhyay. Characterization of mango-like aroma in Curcuma amada Roxb. J. Agric. Food Chem. 32(1): 57-9, 1984.
31. Gholap, A. S., C. Bandyopadhyay and G. B. Nadkarni. Aroma development in mango fruit. J. Food Biochem. 10(3): 217-29, 1986.
32. Gonzalez, L. and R. Gra. Concentration of volatiles in the study of the aroma of Carazon mango pulp. Rev. Cienc. Quim. 15(2): 275-80, 1984.
33. Hunter, G. L. K., W. A. Bucek and T. Radford. Volatile components of canned Alphonso mango. J. Food Sci. 39(5): 900-3, 1974.
34. Idstein, H., C. Bauer and P. Schreier. Volatile acids from tropical fruits: cherimoya (Annona cherimolia, Mill.), guava (Psidium guajava, L.), mango (Mangifera indica, L., var. Alphonso), papaya (Carica papaya, L.). Z. Lebensm. Unters. Forsch. 180(5): 394-7, 1985.
35. Idstein, H. and P. Schreier. New results in tropical fruit flavor analysis. Dev. Food Sci. : 1985.
36. Idstein, H. and P. Schreier. Volatile constituents of Alphonso mango (Mangifera indica). Phytochemistry. 24(10): 2313-16, 1985.
37. Joshi, G. D. and S. K. Roy. Effect of existing method of harvesting on chemical composition and palatability of mango (Mangifera indica L.) cv. Alphonso fruits. Indian Food Packer. 39(4): 26-31, 1985.
38. Kapur, K. L. Volatile components of the Dashehari mango. J. Food Sci. Technol. 20(5): 242-3, 1983.
39. Karg, J. E. The mango fruit and its uses. Riechst., Aromen, Kosmet. 28(3-4): 84-6, 1978.
40. Labows, J. N. and B. Shushan. Direct analysis of food aromas. Am. Lab. (Fairfield, Conn. 15(3): 56-61, 1983.
41. Luccarelli, D. J., B. D. Mookherjee, R. A. Wilson, M. J. Zampino and D. R. Bowen. Flavoring some tropical flavored foods with a mixture of C4-10-n-alkanoic acid and ethyl ester of 2-hydroxy-4-methyl pentanoic acid. U.s. : 1985.
42. MacLeod, A. J. and d. T. N. Gonzalez. Volatile flavor components of mango fruit. Phytochemistry. 21(10): 2523-6, 1982.

43. MacLeod, A. J., G. MacLeod and C. H. Snyder. Volatile aroma constituents of mango (cv Kensington). Phytochemistry. 27(7): 2189-93, 1988.
44. MacLeod, A. J. and N. M. Pieris. Comparison of the volatile components of some mango cultivars. Phytochemistry. 23(2): 361-9, 1984.
45. MacLeod, A. J. and C. H. Snyder. Volatile components of two cultivars of mango from Florida. J. Agric. Food Chem. 33(3): 380-4, 1985.
46. MacLeod, A. J. and C. H. Snyder. Volatile components of mango preserved by deep freezing. J. Agric. Food Chem. 36(1): 137-9, 1988.
47. Mehata, P., A. Patil and B. Herlekar. Assessment of suitability of various oil blends for culinary purposes. Indian J. Nutr. Diet. 23(5): 126-31, 1986.
48. Mookherjee, B. D., R. W. Trenkle, N. Calderone, L. Schreck and K. P. Sands. Schiff base reaction products of mixtures of aldehydes including helional and alkyl anthranilates, derivatives thereof, and organoleptic uses thereof. U.s. : 1989.
49. Osman, A. M., M. E. G. Younes and A. E. Sheta. Chemical examination of local plants. III. Constituents of the volatile oils of the leaves, fruit-skins, and seeds of Egyptian Mangifera indica. Egypt. J. Chem. 15(3): 247-53, 1972.
50. Pattabhiraman, T. R., P. Rao and L. V. L. Sastry. Preliminary studies on the preparation of odor concentrates and identification of odorous ingredients in mango and guava. Perfum. Essent. Oil Rec. 59(10): 733-6, 1968.
51. Pattabhiraman, T. R., L. V. L. Sastry and K. O. Abraham. Preparation of odor concentrates and identification of odorous ingredients in mango and guava. II. Perfum. Essent. Oil Rec. 60(6): 233-8, 1969.
52. Pino, J., A. Rosado and R. Sanchez. Volatile components of three cultivars of mango from Cuba. Nahrung. 33(8): 709-15, 1989.
53. Ramana, K. V. R., H. S. Ramaswamy, B. A. Prasad, M. V. Patwardhan and S. Ranganna. Freezing preservation of Totapuri mango pulp. J. Food Sci. Technol. 21(5): 282-5, 1984.
54. Ramteke, R. S., W. E. Eipeson and M. V. Patwardhan. Behavior of aroma volatiles during the evaporative concentration of some tropical fruit juices and pulps. J. Sci. Food Agric. 50(3): 399-405, 1990.
55. Rao, A. S., B. Rajanikanth and R. Seshadri. Volatile aroma components of Curcuma amada Roxb. J. Agric. Food Chem. 37(3): 740-3, 1989.
56. Saha, N. K., P. C. Agarwal and R. Rodriguez. Effect of ripeness level, storage period, processing conditions and ascorbic acid on flavor retention in canned Dusehri mango. Indian Food Packer. 30(5): 87-91, 1976.
57. Sakho, M., J. Crouzet and S. Seck. Evolution of volatile components from mango during heating. Lebensm. Wiss. Technol. 18(2): 89-93, 1985.
58. Sakho, M., J. Crouzet and S. Seck. Volatile components of African mango. J. Food Sci. 50(2): 548-50, 1985.
59. Salles, C., H. Essaied, P. Chalier, J. C. Jallageas and J. Crouzet. Evidence and characterization of glycosidically bound volatile components in fruits. Bioflavour '87, Proc. Int. Conf., Meeting Date. : 1987.
60. Schreier, P. and H. Idstein. Studies on the aroma composition of enzymically liquefied guava (Psidium guajava, L.) and Mango (Mangifera indica, L., var. Alphonso). Dtsch. Lebensm. Rundsch. 80(11): 335-40, 1984.
61. Schreier, P., E. R. Kittsteiner and H. Idstein. Studies on the enzymic liquefaction of tropical fruit pulps: guava (Psidium guajava, L.), papaya (Carica papaya, L.), mango (Mangifera indica, L. var Alphonso). Fluess. Obst. 52(7): 365-70, 1985.

62. Selvaraj, Y. Studies of enzymes involved in the biogenesis of lipid-derived volatiles in ripening mango (Mangifera indica L.) fruit. J. Food Biochem. 12(4): 289-99, 1989.
63. Selvaraj, Y., R. Kumar and D. K. Pal. Changes in sugars, organic acids, amino acids, lipid constituents and aroma characteristics of ripening mango (Mangifera indica L.) fruit. J. Food Sci. Technol. 26(6): 308-13, 1989.
64. Shankaranarayana, M. L., B. Raghavan, K. O. Abraham and N. B. Shankaracharya. Fruit flavors. Pafai J. 7(1): 13-22, 1985.
65. Tressl, R., K. H. Engel, W. Albrecht and A. H. Bille. Analysis of chiral aroma components in trace amounts. Acs Symp. Ser. : 1985.
66. Van, d. B. S., L. E. Van't and J. Stoffelsma. Methylthiomethyl esters as flavor additives. U.s. : 1983.
67. Verma, R. A., V. K. Tripathi, M. P. Tripathi and S. Singh. Studies on the effect of packaging materials on mango pickle during storage. Indian Food Packer. 40(6): 22-5, 1986.
68. Wilson, C. W. I., P. E. Shaw and R. J. J. Knight. Importance of selected volatile compounds to mango (Magnifera indica L.) flavor. Dev. Food Sci. : 1988.
69. Wilson, C. W. I., P. E. Shaw and R. J. J. Knight. Importance of some lactones and 2,5-dimethyl-4-hydroxy-3(2H)-furanone to mango (Mangifera indica L.) aroma. J. Agric. Food Chem. 38(7): 1556-9, 1990.
70. Yamaguchi, K., O. Nishimura, H. Toda, S. Mihara and T. Shibamoto. Chemical studies on tropical fruits. Instrum. Anal. Foods: Recent Prog., Proc. Symp. Int. Flavor Conf., 3rd. 2: 1983.

Passion

1. Casimir, D. J., J. F. Kefford and F. B. Whitfield. Technology and flavor chemistry of passion fruit juices and concentrates. Adv. Food Res. : 1981.
2. Chen, C. C., M. C. Kuo, L. S. Hwang, J. S. B. Wu and C. M. Wu. Headspace components of passion fruit juice. J. Agric. Food Chem. 30(6): 1211-15, 1982.
3. Demole, E., P. Enggist, M. Winter, A. Furrer, E. K. H. Schulte, B. Egger and G. Ohloff. Megastigma-5,8-dien-4-one, an aroma constituent of the yellow passion fruit and Virginia tobacco. Helv. Chim. Acta. 62(1): 67-75, 1979.
4. Engel, K. H. and R. Tressl. Formation of aroma components from nonvolatile precursors in passion fruit. J. Agric. Food Chem. 31(5): 998-1002, 1983.
5. Fang, T. T., L. M. J. Chiou and H. E. Chen. Effects of heat treatment and subsequent storage on the quality of passion fruit (Passiflora edulis) juice. Shih P'in K'o Hsueh (Taipei). 12(3-4): 87-99, 1985.
6. Heusinger, G. and A. Mosandl. Chiral, sulfur-containing aroma substances of the yellow passion fruit (Passiflora edulis f. flavicarpa). Preparation of enantiomers and absolute configuration. Tetrahedron Lett. 25(5): 507-10, 1984.
7. Heusinger, G. and A. Mosandl. New results about chiral flavor compounds. Top. Flavour Res., Proc. Int. Conf. : 1985.
8. Kuo, M. C., S. L. Chen, C. M. Wu and C. C. Chen. Changes in volatile components of passionfruit juice as affected by centrifugation and pasteurization. J. Food Sci. 50(4): 1208-10, 1985.
9. Mosandl, A. and G. Heusinger. Stereoisomers of fruit flavor substances - some aspects of synthesis and analysis. Anal. Volatiles: Methods Appl., Proc. Int. Workshop, Meeting Date. : 1983.
10. Nitz, S. Multidimensional gas-chromatography in aroma research. Top. Flavour Res., Proc. Int. Conf. : 1985.
11. Parliment, T. H. Volatile constituents of passion fruit. J. Agr. Food Chem. 20(5): 1043-5, 1972.
12. Shankaranarayana, M. L., B. Raghavan, K. O. Abraham and N. B. Shankaracharya. Fruit flavors. Pafai J. 7(1): 13-22, 1985.
13. Shoseyov, O., B. A. Bravdo, D. Siegel, A. Goldman, S. Cohen, L. Shoseyov and R. Ikan. Immobilized endo-.beta.-glucosidase enriches flavor of wine and passion fruit juice. J. Agric. Food Chem. 38(6): 1387-90, 1990.
14. Singer, G., G. Heusinger, O. Froehlich, P. Schreier and A. Mosandl. Chirality evaluation of 2-methyl-4-propyl-1,3-oxathiane from the yellow passion fruit. J. Agric. Food Chem. 34(6): 1029-33, 1986.
15. Tressl, R. and K. H. Engel. Formation and analysis of optically active aroma compounds. Anal. Volatiles: Methods Appl., Proc. Int. Workshop, Meeting Date. : 1983.
16. Tressl, R. and K. H. Engel. Formation of esters and terpenoids in passion fruits and their importance to quality evaluation. Instrum. Anal. Foods: Recent Prog., Proc. Symp. Int. Flavor Conf., 3rd. 1(Y): 1983.
17. Whitfield, F. B. and J. H. Last. The flavor of the passion fruit. Prog. Essent. Oil Res., Proc. Int. Symp. Essent. Oils, 16th, Meeting Date. : 1985.
18. Whitfield, F. B., K. E. Murray, G. Stanley and G. Sugowdz. Structures of edulan I and edulan II and their role in the aroma of the juice of the purple passion fruit. Int. Congr. Essent. Oils, [Pap.], 6th. : 1974.

19. Whitfield, F. B. and G. Sugowdz. The 6-(but-2'-enylidene)-1,5,5-trimethylcyclohex-1-enes: important volatile constituents of the juice of the purple passionfruit Passiflora edulis. Aust. J. Chem. 32(4): 891-903, 1979.
20. Whitfield, F. B., G. Sugowdz and D. J. Casimir. (6E,2E) and (6Z,2E)-6-(but-2-enylidene)-1,5,5-trimethylcyclohex-1-ene: important volatile constituents of the juice of the purple passion fruit (Passiflora edulis Sims). Chem. Ind. (London). : 1977.
21. Winter, M. and R. Kloeti. Aroma of yellow passion fruit (Passiflora edulis f. flavicarpa). Helv. Chim. Acta. 55(6): 1916-21, 1916.
22. Winter, M., F. Naf, A. Furrer, W. Pickenhagen, W. Giersch, A. Meister, B. Willhalm, W. Thommen and G. Ohloff. Ethyl (Z)-4,7-octadienoate and (Z)-3,5-hexadienyl butyrate, two new aroma components of the purple passion fruit. Helv. Chim. Acta. 62(1): 135-9, 1979.
23. Winter, M., E. K. H. Schulte, A. Velluz, J. Limacher, W. Pickenhagen and G. Ohloff. Aroma constituents of the purple passion fruit. Two new edulan derivatives. Helv. Chim. Acta. 62(1): 131-4, 1979.
24. Yamaguchi, K., O. Nishimura, H. Toda, S. Mihara and T. Shibamoto. Chemical studies on tropical fruits. Instrum. Anal. Foods: Recent Prog., Proc. Symp. Int. Flavor Conf., 3rd. 2: 1983.
25. Yu, Z. R. and B. H. Chiang. Passion fruit juice concentration by ultrafiltration and evaporation. J. Food Sci. 51(6): 1501-5, 1986.
26. Yu, Z. R., B. H. Chiang and L. S. Hwang. Retention of passion fruit juice compounds by ultrafiltration. J. Food Sci. 51(3): 841-2, 1986.

[Papaya

1. Ashby, P. and P. J. Sayer. Proteinaceous composition. Eur. Pat. Appl. : 1990.
2. Aspiras, A. B. and E. M. Tocino. Survey of the aroma composition of Philippine fruits. Philipp. Agr. 55(1-2): 67-82, 1971.
3. Blakesley, C. N., J. G. Loots, P. L. Du and B. G. De. .gamma. Irradiation of subtropical fruits. 2. Volatile components, lipids and amino acids of mango, papaya, and strawberry pulp. J. Agric. Food Chem. 27(1): 42-8, 1979.
4. Chan, H. T. J., R. A. Flath, R. R. Forrey, C. G. Cavaletto, T. O. M. Nakayama and J. E. Brekke. Development of off-odors and off-flavors in papaya puree. J. Agr. Food Chem. 21(4): 566-70, 1973.
5. Chan, H. T. J., M. T. H. Kuo, C. G. Cavaletto, T. O. M. Nakayama and J. E. Brekke. Papaya puree and concentrate. Changes in ascorbic acid, carotenoids, and sensory quality during processing. J. Food Sci. 40(4): 701-3, 1975.
6. Flath, R. A. and R. R. Forrey. Volatile components of papaya (Carica papaya L., Solo variety). J. Agric. Food Chem. 25(1): 103-9, 1977.
7. Flath, R. A., D. M. Light, E. B. Jang, T. R. Mon and J. O. John. Headspace examination of volatile emissions from ripening papaya (Carica papaya L., Solo variety). J. Agric. Food Chem. 38(4): 1060-3, 1990.
8. Hylin, J. W. and B. H. Chin. Volatile metabolites from dimethyldithiocarbamate fungicide residues. Bull. Environ. Contam. Toxicol. 3(6): 322-32, 1968.
9. Idstein, H., C. Bauer and P. Schreier. Volatile acids from tropical fruits: cherimoya (Annona cherimolia, Mill.), guava (Psidium guajava, L.), mango (Mangifera indica, L., var. Alphonso), papaya (Carica papaya, L.). Z. Lebensm. Unters. Forsch. 180(5): 394-7, 1985.
10. Idstein, H., T. Keller and P. Schreier. Volatile constituents of mountain papaya (Carica candamarcensis, syn. C. pubescens Lenne et Koch) fruit. J. Agric. Food Chem. 33(4): 663-6, 1985.
11. Idstein, H. and P. Schreier. New results in tropical fruit flavor analysis. Dev. Food Sci. : 1985.
12. Idstein, H. and P. Schreier. Volatile constituents from papaya fruit (Carica papaya, L., var. Solo). Lebensm. Wiss. Technol. 18(3): 164-9, 1985.
13. Luccarelli, D. J., B. D. Mookherjee, R. A. Wilson, M. J. Zampino and D. R. Bowen. Flavoring some tropical flavored foods with a mixture of C4-10-n-alkanoic acid and ethyl ester of 2-hydroxy-4-methyl pentanoic acid. U.s. : 1985.
14. MacLeod, A. J. and N. M. Pieris. Volatile components of papaya (Carica papaya L.) with particular reference to glucosinolate products. J. Agric. Food Chem. 31(5): 1005-8, 1983.
15. Mookherjee, B. D., R. W. Trenkle, R. K. Wolff, R. M. Boden and T. Yoshida. Use of methyl-substituted pinyl oxopentenes, for augmenting, enhancing or modifying the aroma or taste of smoking tobacco and smoking tobacco articles. U.s. : 1984.
16. Morales, A. L. and C. Duque. Aroma constituents of the fruit of the mountain papaya (Carica pubescens) from Colombia. J. Agric. Food Chem. 35(4): 538-40, 1987.
17. Schreier, P., E. R. Kittsteiner and H. Idstein. Studies on the enzymic liquefaction of tropical fruit pulps: guava (Psidium guajava, L.), papaya (Carica papaya, L.), mango (Mangifera indica, L. var Alphonso). Fluess. Obst. 52(7): 365-70, 1985.
18. Schreier, P., M. Lehr, J. Heidlas and H. Idstein. Volatiles from papaya (Carica papaya, L.) fruit: indication of precursors of terpene compounds. Z. Lebensm. Unters. Forsch. 180(4): 297-302, 1985.

19. Schreier, P. and P. Winterhalter. Precursors of papaya (Carica papaya, L.) fruit volatiles. Acs Symp. Ser. : 1986.
20. Schwab, W., C. Mahr and P. Schreier. Studies on the enzymic hydrolysis of bound aroma components from Carica papaya fruit. J. Agric. Food Chem. 37(4): 1009-12, 1989.
21. Venter, P. J. and J. D. Malan. Extracting aroma materials from plants. Ger. Offen. : 1973.
22. Winterhalter, P., D. Katzenberger and P. Schreier. Aroma precursors: oxygenated terpene alcohols and their derivatives. Lebensmittelchem. Gerichtl. Chem. 41(1): 1987.
23. Yamaguchi, K., O. Nishimura, H. Toda, S. Mihara and T. Shibamoto. Chemical studies on tropical fruits. Instrum. Anal. Foods: Recent Prog., Proc. Symp. Int. Flavor Conf., 3rd. 2: 1983.

Pineappl

1. Adam, S. T. A simple and efficient cryogenic trapping device for odor evaluation of aroma fractions. Hrc Cc, J. High Resolut. Chromatogr. Chromatogr. Commun. 10(6): 369-70, 1987.
2. Adhikary, S. K., W. P. Harkare, K. P. Govindan, K. C. Chikkappaji, S. Saroja and A. M. Nanjundaswamy. Deacidification of fruit juices by electrodialysis. Part II. Indian J. Technol. 25(1): 24-7, 1987.
3. Aishima, T. and Y. Ozawa. A simple method for concentrating volatile flavor compounds in aqueous systems using RP-HPLC. Chromatographia. 28(7-8): 405-11, 1989.
4. Aspiras, A. B. and E. M. Tocino. Survey of the aroma composition of Philippine fruits. Philipp. Agr. 55(1-2): 67-82, 1971.
5. Barcelon, S. A., A. Oppenheimer and M. M. Hussein. Taste- and odor-masked edible oil compositions. Eur. Pat. Appl. : 1988.
6. Beernaert, H. and J. Gossele. Aroma study of essences in non-alcoholic soft drinks. Isolation and concentration of aromas. Mitt. Geb. Lebensmittelunters. Hyg. 69(4): 516-35, 1978.
7. Berger, R. G., G. R. Dettweiler, H. Kollmannsberger and F. Drawert. The peroxisomal dienoyl-CoA reductase pathway in pineapple fruit. Phytochemistry. 29(7): 2069-73, 1990.
8. Berger, R. G., F. Drawert and H. Kollmannsberger. Biogenesis of aroma compounds in plants and fruits. XXIII. Precursor-dependent aroma accumulation in pineapple fruit tissue. Dtsch. Lebensm. Rundsch. 80(10): 299-304, 1984.
9. Berger, R. G., F. Drawert, H. Kollmannsberger and S. Nitz. Natural occurrence of undecaenes in some fruits and vegetables. J. Food Sci. 50(6): 1655-6, 1985.
10. Berger, R. G., F. Drawert, H. Kollmannsberger, S. Nitz and B. Schraufstetter. Novel volatiles in pineapple fruit and their sensory properties. J. Agric. Food Chem. 33(2): 232-5, 1985.
11. Berger, R. G., F. Drawert and S. Nitz. Sesquiterpene hydrocarbons in pineapple fruit. J. Agric. Food Chem. 31(6): 1237-9, 1983.
12. Berger, R. G. and H. Kollmannsberger. Pineapple flavors. Biosynthetic routes and sensorial properties. Top. Flavour Res., Proc. Int. Conf. : 1985.
13. Binder, R. G. and R. A. Flath. Volatile components of pineapple guava. J. Agric. Food Chem. 37(3): 734-6, 1989.
14. Broderick, J. J. Pineapple. Some distance to go. Int. Flavours Food Addit. 6(6): 1975.
15. Buechi, G., E. Demole and A. F. Thomas. Syntheses of 2,5-dimethyl-4-hydroxy-2,3-dihydrofuran-3-one (furaneol), a flavor principle of pineapple and strawberry. J. Org. Chem. 38(1): 123-5, 1973.
16. Byrne, B. and L. M. L. Lawter. Pineapple ketone carbonate derivatives. Eur. Pat. Appl. : 1986.
17. Byrne, B. and L. M. L. Lawton. Pineapple ketone 1'-alkoxyalkyl derivatives. Eur. Pat. Appl. : 1986.
18. Chaveron, H. Chromatographic study of aroma products. Choc., Confiserie Fr., No. : 1972.
19. Cherukuri, S. R., Y. C. Wei, F. Hriscisce, D. Piccolo and K. P. Bilka. Elastomer encapsulation of flavors and sweeteners for long lasting flavored chewing gums. S. African. : 1986.
20. Chou and S. T. John. To find imitation flavor components contained in two yogurt (pineapple and strawberry) in the market. Koryo. : 1977.

21. Creveling, R. K., R. M. Silverstein and W. G. Jennings. Volatile components of pineapple. J. Food Sci. 33(3): 284-7, 1968.
22. Crochon, M., R. Tisseau, C. Teisson and R. Huet. Effect of a preharvest ethrel application on the flavor quality of pineapple from the Ivory Coast. Fruits. 36(7-8): 409-15, 1981.
23. Diaz, N. and J. Rivera. Tentative identification of volatile components of pineapple juice from varieties Red Spanish and PR 1-67. J. Agric. Univ. P. R. 73(3): 189-94, 1989.
24. Dupaigne, P. Aroma of the pineapple. Fruits. 25(11): 793-805, 1970.
25. Dupaigne, P. Pineapple flavor. Riv. Ital. Essenze, Profumi, Piante Off., Aromi, Saponi, Cosmet., Aerosol. 54(8): 559-67, 1972.
26. Engel, K. H., J. Heidlas, W. Albrecht and R. Tressl. Biosynthesis of chiral flavor and aroma compounds in plants and microorganisms. Acs Symp. Ser. : 1989.
27. Fehl, A. J. and C. Marcott. Capillary gas chromatography/Fourier transform infrared spectroscopy using an injector/trap and liquid-liquid extraction. Anal. Chem. 61(14): 1596-8, 1989.
28. Flath, R. A. and R. R. Forrey. Volatile components of Smooth Cayenne pineapple. J. Agr. Food Chem. 18(2): 306-9, 1970.
29. Fountain, M. W. Method for making solvent dilution microcarriers and their uses. Pct Int. Appl. : 1989.
30. Fujiwara, Y., Y. Inoue, M. Fujisawa and M. Hosokawa. Enhancement of pineapple-like flavor in foods and beverages using amino acids. Jpn. Kokai Tokkyo Koho. : 1989.
31. Germino, F. J., F. E. Kite and E. H. Christensen. Texturizing ungelatinized starch. U.s. : 1971.
32. Glass, M., V. Corsello, D. A. Orlandi and A. Guzowski. Process for preparing a chewing gum composition with improved flavor. S. African. : 1986.
33. Hayakawa, K., S. Nakamura, K. Inaki and M. Miyazaki. Determination of carboxyl esters by indirect photometric ion chromatography with an on-line enzyme immobilized column. Anal. Sci. 5(6): 691-5, 1989.
34. Howard, G. E. and A. Hoffman. Volatile flavoring constituents of canned Malayan pineapple. J. Sci. Food Agric. 18(3): 106-10, 1967.
35. Huet, R. Aroma retention in tropical fruit powders obtained in a micro-wave oven under vacuum. Fruits. 29(5): 399-405, 1974.
36. Ishiyama, J. and S. Sugiyama. Fruity flavor substance. Japan. Kokai. : 1978.
37. Jin, Z. and H. Zuo. Synthesis of pineapple ester from 4-vinylcyclohexene. Faming Zhuanli Shenqing Gongkai Shuomingshu. : 1986.
38. Kama, W. and S. M. Siegel. Volatile mercury release from vascular plants. Org. Geochem. 2(2): 99-101, 1980.
39. Karg, J. E. Exotic pineapple flavor - history, formulation, and future. R.a.k., Riechst., Aromen, Kosmet. 33(7-8): 163-5, 1983.
40. Kataoka, E., C. Tokue, T. Yamashita and W. Tanimura. Amino acids, organic acids, fatty acids, trimethylamine, and methional in improved fish sauce. Eiyogaku Zasshi. 45(2): 67-76, 1987.
41. Kishino, K. and S. Kobayashi. From our memoirs on study of pineapple flavor. Koryo. : 1981.
42. Kung, J. F. and M. F. Epstein. Use of 2-methyl-6-ethoxypyrazine for pineapple flavor. U.s. : 1974.

43. Litman, I. Method and apparatus for obtaining the combined aroma of several substances. U.s. : 1985.
44. Loutaty, R. and C. Rolland. Extracting, concentrating and purifying natural aromas of vegetable origin, and products obtained from them. Eur. Pat. Appl. : 1983.
45. Moshonas, M. G. and P. E. Shaw. Direct gas chromatographic analysis of aqueous citrus and other fruit essences. J. Agric. Food Chem. 32(3): 526-30, 1984.
46. Naef, M. R. and B. Willhalm. Volatile part of pinapple. Helv. Chim. Acta. 54(7): 1880-90, 1971.
47. Nakajima, K., A. Sato, Y. Takahara, T. Hosaka and M. Taniguchi. Microbial formation of new compound 4,6-dimethyl-3,6-heptadienoic acid from 2,4-dimethyl-1,4-nonadiene. Nippon Nogei Kagaku Kaishi. 54(1): 27-9, 1980.
48. Nicholas, H. J. Miscellaneous volatile plant products. Phytochemistry. 2(Y): 1973.
49. Nisperos, C. M. O., P. E. Shaw and E. A. Baldwin. Changes in volatile flavor components of Pineapple orange juice as influenced by the application of lipid and composite films. J. Agric. Food Chem. 38(6): 1382-7, 1990.
50. Ochsner, P. A. Aroma-imparting medium. Swiss. : 1976.
51. Ohta, H., S. Kinjo and Y. Osajima. Glass capillary gas chromatographic analysis of volatile components of canned Philippine pineapple juice. J. Chromatogr. : 1987.
52. Ramteke, R. S., W. E. Eipeson and M. V. Patwardhan. Behavior of aroma volatiles during the evaporative concentration of some tropical fruit juices and pulps. J. Sci. Food Agric. 50(3): 399-405, 1990.
53. Re, L., B. Maurer and G. Ohloff. Simple access to 4-hydroxy-2,5-dimethyl-3(2H)-furanone (Furaneol), aroma component of the pineapple and strawberry. Helv. Chim. Acta. 56(6): 1882-94, 1973.
54. Ryo, S., A. Fujita and K. Kogami. 2-[.omega.,.omega.-Bis(alkylthio)- or .omega..omega.-(alkylenedithio)alkyl]-3-methyl-2-cyclopenten-1-ones. Japan. Kokai. : 1978.
55. Sanchez, N. F. and I. Hernandez. Studies on the freezing of Red Spanish and Smooth Cayenne pineapples. J. Agric. Univ. P. R. 61(3): 354-60, 1977.
56. Shankaranarayana, M. L., B. Raghavan, K. O. Abraham and N. B. Shankaracharya. Fruit flavors. Pafai J. 7(1): 13-22, 1985.
57. Sharma, S. C. and R. K. Yang. Chewing gum compositions containing sweetener delivery systems. S. African. : 1986.
58. Shiga, M. and T. Tsuenya. p-Mentha-1,8-diene-2-ol-6-one for enhancement of food flavor. Japan. Kokai. : 1974.
59. Shu, C. K., B. D. Mookherjee and C. T. Ho. Volatile components of the thermal degradation of 2,5-dimethyl-4-hydroxy-3(2H)-furanone. J. Agric. Food Chem. 33(3): 446-8, 1985.
60. Siegel, S. M. and B. Z. Siegel. Vegetation and the atmospheric cycling of mercury. Adv. Space Res. 3(9): 135-9, 1983.
61. Silverstein, R. M. Pineapple flavor. Symp. Foods: Chem. Physiol. Flavors, [Proc.], Meeting Date. : 1965.
62. Silverstein, R. M. Biochemistry and physiology of commercially important fruits. 9B. Pineapple. Flavor. Biochem. Fruits Their Prod. 2: 1971.
63. Sprecker, M. A., W. L. Schreiber and R. P. Belko. Allyl 4-cyclooctenyl glycolate, organoleptic uses thereof, process for preparing same and intermediates useful in said process. U.s. : 1988.

64. Takeoka, G., R. G. Buttery, R. A. Flath, R. Teranishi, E. L. Wheeler, R. L. Wieczorek and M. Guentert. Volatile constituents of pineapple (Ananas comosus [L.] Merr.). Acs Symp. Ser. : 1989.
65. Takeoka, G., R. A. Flath, T. R. Mon, R. G. Buttery, R. Teranishi, M. Guentert, R. Lautamo and J. Szejtli. Further applications of permethylated .beta.-cyclodextrin capillary gas chromatographic columns. J. High Resolut. Chromatogr. 13(3): 202-6, 1990.
66. Tressl, R., K. H. Engel, W. Albrecht and A. H. Bille. Analysis of chiral aroma components in trace amounts. Acs Symp. Ser. : 1985.
67. Van, d. B. S., L. E. Van't and J. Stoffelsma. Methylthiomethyl esters as flavor additives. U.s. : 1983.
68. Viana, C. M. T. and H. I. T. De. Chemical composition of microsilages of fish by-products and agricultural waste products. Tec. Pecu. Mex. : 1983.
69. Washino, K., S. Imai, Y. Horikawa and M. Nishimura. Manufacture of hydroxyfuranones for flavoring materials. Jpn. Kokai Tokkyo Koho. : 1988.
70. Wiegers, W. J. Norbornylbutadiene-acrolein adducts, and their perfumery uses. U.s. : 1985.