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Postharvest Institute for Perishables

SEMINAR AND WORKSHOP ON FOOD TECHNOLOGY IN NICARAGUA

Report to
Laboratorio de Tecnologia de Alimentos (LABAL)
Ministerio de Industria
and to USAID/Managua

by
Dr. Marion Fields
for the Postharvest Institute for Perishables

GTS Report No. 81-04A
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University of Idaho

in cooperation with
United States Agency for
International Development
Project Title: Storage and Processing of Fruits and Vegetables
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Washington, D.C., U.S.A. 20523

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EXECUTIVE SUMMARY

AID/Managua cabled a request from Dr. Gonzalo Pertz, of the Ministry of Industry, for a 6-day visit to Nicaragua by Dr. Marion Fields, Department of Food Science and Nutrition, University of Missouri (Columbia). The request was for 2 days of Dr. Fields' time to conduct a seminar on food plant sanitation for managers and employees of dairy and meat industries. Also 4 days were to be spent teaching techniques for microbiological analysis of foods, especially baby products, to laboratory staff.

Dr. Fields went to Managua on November 30, 1981, presented a seminar on December 3 and 4, and conducted a class from December 9 to 12, 1981.

The seminar was very satisfactorily presented to 46 participants from diverse interests in the processing industries. Emphasis was placed on sanitation in various aspects, such as insect and rodent control, water supply and chlorination, and waste treatment and disposal. Because of the diverse interests of the many participants, it is believed that there will be maximum dissemination of the information presented.

All material taken as visual aids to Nicaragua for use in the seminar was left with Dr. Pertz. He will make use of it in future seminars.

Upon completion of the seminar, each participant was given a certificate of attendance.

The laboratory workshop employed 4 microbiological cultures that were taken to Nicaragua with the permission of the Ministry of Health. The students analyzed sorghum for niacin and obtained highly satisfactory results.

It is believed that the cultures and methodologies that were employed give the laboratory personnel the tools by which any food, including baby food, can be assayed for several important vitamins and amino acids. Also, the techniques employed in the analyses are reasonable from a cost/benefit ratio.

It is believed that the time spent by Dr. Fields in Managua was very productive.

I. Introduction

The USAID Mission in Managua, Nicaragua received a request for assistance from Dr. Marion Fields, Department of Food Science and Nutrition, University of Missouri, Columbia, Missouri in some aspects of food technology in Nicaragua. It was initiated by Dr. Gonzalo Pertz, the Director of the Laboratorio de Tecnologia de Alimentos (LABAL), (Laboratory of Food Technology) in the Ministerio de Industria (Ministry of Industry.) In part, the request specified that Dr. Fields was to travel to Nicaragua to conduct a two-day seminar on food plant sanitation for managers and employees of dairy and meat industries. Also, four days were to be spent teaching laboratory techniques for microbiological analyses of foods, especially baby products.

In response to the request, Dr. Fields arrived in Managua on November 30, 1981. A seminar was held in the Camara de Industrias de Nicaragua (Chamber of Industries of Nicaragua) on December 3 and 4, 1981, and a class on laboratory analyses was taught on December 9 to 12, 1981.

II. Seminar

A meeting was held with Dr. Pertz and some of his staff on November 30 and December 1 to plan an agenda for the seminar. A copy is attached as Appendix A. The program was arranged to focus on the following:

1. A sanitation program
2. Rodent control
3. Insect control
4. Sanitary significance of microorganisms
5. Water supply
6. Cleaning food plants
7. Chlorination of water
8. Construction and maintenance of building and equipment
9. Waste treatment and disposal

When the seminar was presented, Dr. Pertz translated into Spanish when necessary. The Nicaraguan participants represented various institutes within the country, as shown in Tables 1 and 2. Because of the great diversity of interests at the seminar, it is believed that there will be maximum dissemination and use of the information.

Because of the varied nature of the audience, an attempt was made to use practical examples from different industries. Also, examples were given from previous public health experiences. The audience was surveyed by Dr. Pertz on both days and he found that the audience liked the practical material being presented. Apparently the participants benefitted from both days of the program.

TABLE 1. Participants in Seminar by Type of Organization.

Organization	Number of Participants
Ministry of Health	5
Ministry of Industries	1
Ministry of Internal Trade	1
Ministry of Agricultural Development	1
Nicaraguan Center for Technical Information	1
Nicaraguan Institute for Fisheries	3
National University of Nicaragua	1
Chamber of Industry of Nicaragua	1
Animal Science Department, Central American University	1
Total	15
Canning Industry	2
Dairy Industry	4
Pepsi Cola	1
Coca Cola	1
Oils and Fat Industries	1
Meat Packing Industry	1
Baking Industry	3
Cereal Industry	2
Frozen Vegetable Industry	1
Pickle and Sauce Industry	1
Ice Making Plant	2
Total	19
Laboratorio de Tecnologia de Alimentos	12
GRAND TOTAL	46

TABLE 2. Food Plant Sanitation Seminar Participants

Lecturer: Dr. Marion L. Fields
Department of Food Science and Nutrition
College of Agriculture
University of Missouri (Columbia)

Organizer: Laboratorio de Tecnologia de Alimentos (LABAL)
Ministerio de Industria de Nicaragua

Participants:

Silvio Alonzo G.
Department of Quality Control
Industria Frutera del Gran Lago
(Tomato Processing Industry)

Katya Benavides G.
Central Laboratory
Ministry of Health

Ligia Blandon P.
Department of Quality Control
ESKIMO S.A.
(Dairy Industry)

Sergio Bravo
Head, Microbiology Laboratory
Central Laboratory
Ministry of Health

Ramon Antonio Cornavaca E.
Department of Quality Control
Embotelladora Nacional S.A.
(Pepsi Cola Bottling Plant)

Carlos Cuarezma P.
La Selecta
Production Department
(Dairy Industry)

Gema Doris Chacon
Ministry of Industry

Julio Cesar Lopez
Applied Research Division
LABAL

Humberto Lopez
Panificadora Industrial del Pueblo
Department of Production
(Baking Industry)

Jamyleth L. de Martinez
Applied Research Division
LABAL

Mariano Maradiaga
Industries AMOLOMCA
Quality Control Department
(Frozen vegetables)

Mariel Medal de Fajardo
Food Analyst
Central Laboratory
Ministry of Health

Marino Antonion Membrono
Hielera Polar
Production Department
(Ice making plant)

Denis J. Navas Vega
Food Industry Section
Nicaraguan Centre for Technical Information

Juan Ignacio Ocana
Panaderia Leon Dorado
Supervisor
(Baking industry)

Maria Nieves Perez
Hielera Polar
Production Department
(Ice making plant)

Ernestina Prado de Zuniga
Food Analyst
Central Laboratory
Ministry of Health

Maria Eugenia Espinoza O.
Department of Quality Control
Nicaraguan Institute for Fisheries

Yelva Espinoza F.
Analytical Services Division
LABAL

Leonor Garcia D.
Lecturer
Department of Food Technology
National University of Nicaragua

Juan Jose Gollan
Applied Research Division
LABAL

Carlos Jose Gomez R.
Department of Quality Control
Nicaraguan Institute for Fisheries

Marcos Antonio Gomez Ch.
Industrias El Caracol
Production Department
(Pickle and Sauces Manufacturer)

Daly Gutierrez
Lecturer
Centralamerican University
Animal Sciences Department

Alvaro Hernandez
Information Center
LABAL

Roberto Herrera
Applied Research Division
LABAL

Oscar Lanzas D.
Chamber of Industry of Nicaragua

Reynaldo Lazo
Applied Research Division
LABAL

Oscar Leon V.
Head, Stored Products Section
Ministry of Agricultural Development

Jacinto Prado Reyes
Grasas y Aceites S.A.
Refining Department
(Oils and Fats Industry)

Fernando Quezada
Productos CARNIC
Production Department
(Slaughter house)

Juan Alberto Ramos A.
Panificadora Industrial del Pueblo
Production Department
(Baking industry)

Emilia Reyna
Nicaraguan Institute for Fisheries
Quality Control Department

Amalia Rivera R.
Head, Chemistry Laboratory
Central Laboratory
Ministry of Health

Efrain Rodriguez A.
ESKIMO S.A.
Production Department
(Dairy industry)

Danilo Rodriguez G.
Embotelladora MILCA
Production Department
(Coca Cola Bottling Plant)

Danilo Ruiz J.
Quality Control Department
Ministry of Internal Trade

Mario Ruiz S.
Analytical Services Division
LABAL

Anselmo Ruiz S.
Industria Frutera del Gran Lago
Quality Control Department
(Tomato processing industry)

Ivan Saavedra Dolmus
La Completa
Quality Control Department
(Dairy industry)

Sergi Salazar V.
Analytical Services Division
LABAL

Maria Eugenia Salgado
Applied Research Division
LABAL

Leonor Sevilla D.
Industrias El Caracol
Head, Quality Control Department
(Cereal industry)

Hernan Balbuena M.
Applied Research Division
LABAL

The success of the seminar would not have been possible without the excellent assistance of Director Pertz. He not only provides sound technical assistance to Nicaraguan industry but also is involved in the training of various personnel from ministries within the Nicaraguan government. He is a valuable national asset.

In order to maximize the use of time and money for the seminar and to make the material useful in the future, all the material used in the seminar along with the additional material on rodent and insect control were left with Director Pertz. It is anticipated that LABAL will make special use of this material for holding future seminars and/or as a basis for information to be translated into Spanish and published in the bulletin of LABAL.

Mr. Armando Gonzalez, USAID, American Embassy, Managua attended a portion of the seminar. Also in attendance was a Cuban advisor for the Nicaraguan Institute for Fisheries.

A photograph of one of the seminar sessions is attached as Figure 2.

When the seminar was completed, each Nicaraguan participant was presented a Certificate of Attendance. (See Appendix B.)

Following the seminar, there were requests for additional information which will be provided to the people requesting via LABAL. For example, Amalia Rivera, Head, Chemistry Laboratory, Central Laboratory, Ministry of Health, requested information on detecting rodent hair in foods; also if there are tolerances on chemical substances used to fumigate foods. Anselmo Ruiz requested information on detergent washing of tomatoes and finally Dr. Enrique Guerrero of the Ministry of Industry requested information on producing citric acid from molasses. Attempts will be made to fulfill these requests.

The seminar and the scientific work at the laboratory are examples of continued cooperation between the Laboratorio de Tecnologia de Alimentos and the Department of Food Science and Nutrition at the University of Missouri, Columbia. This cooperation started with Dr. Fields' visit in January 1981 to the Laboratorio de Tecnologia de Alimentos.

III. Laboratory Workshop

In order to conduct the workshop, it was necessary to have the following bacterial and protozoan cultures:

1. Pedicoccus cerevisiae ATCC 8042 (lysine and methionine analyses)
2. Lactobacillus plantarum ATCC 8014 (niacin and tryptophan analyses)
3. Lactobacillus casei ATCC 7469 (riboflavin analsis)
4. Lactobacillus fermenti ATCC 9338 (thiamin analysis)

To get them to Nicaragua safely and on time for the workshop, AID/Washington made arrangments with the Nicaraguan Embassy in that city for Dr. Fields to carry the cultures to Managua. See Appendices C, D, and E for correspondence on the subject.

Upon arrival in Managua the cultures were placed in the care of Reynaldo Arroliga, bacteriologist for LABAL.

The University of Missouri supplied the cultures. Each would have cost about $30-35 if purchased from the American Type Culture Collection.

After the cultures were transferred and checked for growth, all bacterial cultures were placed under refrigerator storage until needed in analyses. Lactobacillus plantarum, a bacterial culture used to assay for niacin, was grown in preparation for an analysis of sorghum grain for niacin. Dr. Fields acted as an advisor to the participants during the analyses. They were started on Wednesday and completed on Saturday. The shape of the standard curve was good and the amount of the assay was 39 mg of niacin per 100 g of grain. From a table of the composition of foods, it was observed that a value of 40 mg was listed. This was considered an excellent beginning using a new technique.

It now appears that the cultures and methodologies give LABAL tools by which any food such as baby food can be assayed for thiamin, riboflavin, niacin, lysine, tryptophan, and methionine. Adequate vitamins and amino acids are necessary for good health in all ages of life but especially for babies in their formative years.

The application of more techniques on food quality is another example of the foresighted nature of LABAL's planning. The techniques employed in these analyses are reasonable from a cost/benefit ratio.

All details of analytical procedures and assay media are shown in Appendix F. Two photos of the workshop in action are shown in Figures 3 and 4.

APPENDIX A

GOBIERNO
DE
RECONSTRUCCION NACIONAL

Al Contestar Refiérase A

MINISTERIO DE INDUSTRIA

SEMINARIO SOBRE HIGIENE Y SANITACION EN LA INDUSTRIA DE ALIMENTOS

Oranizado por: LABORATORIO DE TECNOLOGIA DE ALIMENTOS (LABAL).
Expositor: Dr. Marion Fields.

P R O G R A M A

Jueves 3 de Diciembre de 1981:

8.00 - 9.00	Registro
9.00 -10.15	Organización de un Programa de Sanitación.
10.15-10.30	Café.
10.30-12.00	Control de Roedores.
2.00 - 3.00	Control de Insectos.
3.00 - 3.15	Café
3.15 - 5.00	Microorganismos Significantes.

Viernes 4 de Diciembre de 1981:

9.00 - 10.15	Suministro de Agua
10.15-10.30	Café
10.30-12.00	Limpieza y Clorinación
2.00 - 3.00	Diseño de equipo y edificios
3.00 - 3.15	Café
3.15 - 5.00	Manejo de desperdicios y desechos.

Financiado por: AGENCIA INTERNACIONAL PARA EL DESARROLLO (AID)
CAMARA DE INDUSTRIAS DE NICARAGUA (CADIN)
LABORATORIO DE TECNOLOGIA DE ALIMENTOS(LABAL)
MINISTERIO DE INDUSTRIA.

Costo por Participante: Totalmente gratuito.

LABORATORIO DE TECNOLOGIA DE ALIMENTOS
LABAL
Ministerio de Industria
MANAGUA, NICARAGUA

EL LABORATORIO DE TECNOLOGIA DE ALIMENTOS

DIRECCION TECNOLOGICA

MINISTERIO DE INDUSTRIA

Hace Constar que:

_____

ha participado en el

SEMINARIO SOBRE HIGIENE Y SANITACION EN LA INDUSTRIA DE ALIMENTOS

Impartido en Managua, del 3 al 4 de Diciembre de 1981

Managua, 4 de Diciembre de 1981.-

DIRECTOR LABAL

LABORATORIO DE TECNOLOGIA DE ALIMENTOS
LABAL
Ministerio de Industria
MANAGUA, NICARAGUA

Marcos Fields
COORDINADOR DEL EVENTO

APPENDIX C

UNITED STATES INTERNATIONAL DEVELOPMENT COOPERATION AGENCY
AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D.C. 20523

October 13, 1981

Gloria Tunnerman
Counsel
Embassy of Nicaragua
1627 New Hampshire Avenue, N.W.
Washington, D.C. 20009

Dear Ms. Tunnerman:

At the request of Dr. Gonzalo Pertz, the Director of the Food Laboratory of the Ministry of Industry, Dr. Marion Fields of the University of Missouri will be visiting Nicaragua on a short-term assignment to assist the Ministry of Industry in techniques for microbiological analysis of foods, especially baby products.

In order to assist this work, Dr. Fields will be hand-carrying five lactobacillus and one protozoan live cultures to Nicaragua early in November. These cultures are not pathogenic and will not cause human disease.

We therefore request a letter from the Embassy of Nicaragua authorizing admittance of these research materials. An early response would be appreciated, so that the authorization can be sent to Dr. Fields prior to her departure in early November.

Thank you for your assistance.

Sincerely yours,

(S)

Robert F. Morris, Ph.D., R.D.
Food Loss Specialist
Agricultural Production Division
Office of Agriculture
Bureau for Science and Technology

cc: R. Skiles, PIP
M. Fields, Univ of Missouri

BEST AVAILABLE DOCUMENT

S&T/AGR/AP:RFMorris:ts:10/13/81

APPENDIX D

recibida Nw 16/81

[illegible]
MANAGUANIC 16/11/81

CONSULNIC/ WASHINGTONG
GLORIA TENNERMANN

REFER. AUTORIZACION PARA INTRODUCIR MATERIALES LABORATORIOS NO PATOGENICOS NI CAUSANTES DE ENFERMEDADES HUMANAS. MINISTERIO SALUD AUTORIZA INTRODUCCION CON SALVEDAD QUE UNA VEZ EN ESTE'' DEBEN SER PRESENTADA A LABORATORIO CENTRA HIGIENE DEL ''MINSA'' PARA ANALISIS RESPECTIVOS.

LUIS M. PEREZ
MINERA
[illegible] NICHIES
[illegible]
[illegible] 11/16
VIA TAT

CON/MIS
No.291-81

APPENDIX E

EMBAJADA DE NICARAGUA
WASHINGTON, D. C.

Noviembre 16, 1981

Robert F. Morris, Ph.D, R.D.
Food Loss Specialist
Agricultural Production Div.
Office of Agriculture
Bureau for Science & Technology
Rm. 413- SA18
AGENCY FOR INT'L DEVELOPMENT
Washington,D.C. 20523

Estimado Dr. Morris:

Hemos recibido su nota del 13 de octubre de 1981 informándonos del próximo viaje del Dr. Marion Fields, de la Universidad de Missouri, quien llevará consigo bacterias no patogénicas ni causantes de enfermedades de acuerdo a solicitud del Director del Laboratorio de Alimentos del Ministerio de Industria, Dr. Gonzalo Partz.

Como notará según copia adjunta de TELEX recibido el 16 noviembre de 1981 el Ministerio de Salud (MINSA) autoriza la introducción de estas bacterias con la salvedad que una vez en Nicaragua éstas sean presentadas al laboratorio Central de Higiene de dicho Ministerio para efectuar los respectivos análisis.

Sin más a que referirme hago propicia la ocasión para reiterarle nuestros más sinceros saludos.

Sinceramente,

Gloria Tunnermann L.
CONSUL

cc: Luis M. Perez, MINEX
Mario De Franco, Embajada

adj: copia de TELEX

APPENDIX F

METHODS FOR MEASURING PROTEIN QUALITY AND AMINO ACIDS

1. Moisture Determination

Determine moisture of the material (AOAC, 14.004, 1965.) In a cooled, weighed dish (with cover) previously heated to 130°C±3°C, accurately weigh about 2 g of a well mixed sample. Uncover sample and dry dish, cover and heat contents 1 hour in oven provided with an opening for ventilation and maintained at 130°C±3°C (with oven at 130°C.) Cover dish still in oven, transfer to desiccator and weigh soon after reaching room temperature. Residue is total solids and loss in weight as moisture.

Calculations

1) Weight of sample plus weight of dish before heating

$$- \frac{\text{weight of dish}}{\text{weight of sample before heating}}$$

2) Weight of sample plus weight of dish after heating

$$- \frac{\text{weight of dish}}{\text{weight of sample after heating}}$$

3) Weight of sample before heating

$$- \frac{\text{weight of sample after heating}}{\text{weight of water loss}}$$

4)

$$\frac{\text{Weight of water loss}}{\text{Weight of sample before heating}} \times 100 = \%\ \text{moisture on wet basis}$$

Moisture content of the sample is necessary so that the amount of N can be adjusted to the same amount in all samples before the RNV test is made. The growth of the protozoan will then reflect the amino acid balance rather than the amount of protein present.

2. Micro-Kjeldahl Method for Determining Nitrogen
(AOAC, 9th edition, 1960, page 643.)

I. Reagents
1) Sulfuric acid - Sp. gr. 1.84, Nitrogen free.
2) Mercuric Oxide, Nitrogen free.
3) Potassium sulfate, Nitrogen free.
4) Sodium hydroxide-sodium thiosulfate solution. Dissolve 60 g NaOH and 5 g $Na_2S_2O_3 5\ H_2O$ to 100 ml of 50% NaOH solution.
5) Boric Acid solution-saturated solution.
6) Indicator solution-a) methyl red-methylene blue. Mix 2 parts of 0.2% alcoholic methylene red with 1 part 0.2% alcoholic blue solution or mix 1 part 0.2% alcoholic methylene red solution with 5 parts 0.2% alcoholic bromocresol green solution.

7. Hydrochloric Acid. 0.02 N To standardize acid, accurately weigh enough standard $Na_2B_4O_7.H_2O$ (Sodium Borate) to titrate about 40 ml (0.15 g) and transfer to 300 ml flask. Swirl gently until samples dissolve. Add 4 drops of Methylene red and titrate with solution that is being standardized to equivalence point as indicated by the reference solution.

$$\text{Normality} = \frac{g\ Na_2Br_4O_7.10\ H_2O \times 1000}{\text{ml acid} \times 190.72}$$

II. Apparatus

1) Digestion rack. Use rack with either gas or electric heaters which will supply enough heat to 30 ml flask to cause 15 ml H_2O at 25°C to come to a rolling boil in not less than 2 or more than 3 minutes.
2) Distillation apparatus. Use one-piece.
3) Digestion flasks-use 30 ml regular Kjeldahl flasks.

III. Determination

1) Weigh sample requiring 3-10 ml 0.01 or 0.02 N HCL and transfer to 30 ml digestion flask. Weight should not be greater than 65 mg dry organic matter.
2) Use charging tube for dry solids to add sample to digestion flask.
3) Add 1.0 $\pm$ 0.1 g K_2SO_4, 40 $\pm$ 10 HgO, and 2.0 $\pm$ 0.1 ml H_2SO_4. If sample weight is greater than 15 mg, add 0.1 ml H_2SO_4 for each 10 mg dry organic matter greater than 15 mg.
4) If boiling time for digestion rack heaters is 2-2.5 minutes, digest for 1 hour after all water is distilled and acid comes to a true boil. If heating time is 2.5 - 3.0 minutes, digest 1.5 hour.
5) Cool, add minimum quantity of water to dissolve solids, cool, place a thin film of vaseline on rim of flask.
6) Transfer digest and boiling chips to distillation apparatus and rinse flask 5 to 6 times with 1-2 ml portions of water.
7) Place 125 ml Erlenmeyer flask containing 5 ml saturated boric acid (H_3BO_3) solution and 2-4 drops indicator under condenser with tip extending below surface of solution.
8) Add 8-10 ml NaOH-$Na_2S_2O_3$ solution to still.
9) Collect about 15 ml distillate, and dilute to about 50 ml.
10) Titrate to gray end point or first appearance of violet.
11) Make determination and calculate % N.
12)

$$\%N = \frac{(\text{ml HCL} - \text{ml blank}) \times \text{normality of HCL} \times 14.008 \times 100}{\text{mg sample}}$$

IV. Calculation Explanation

ml HCL X N HCl = mEq HCl, which is equivalent to mEq NH_3.
The mEq NH_3 is equivalent to mM NH_3. The NH_3 here represents the N value that we are concerned with. Therefore, mM N X 14.008 mg/mM = mg N, and mg N/mg sample = %N in the sample.

2. Assay Procedure for Determining Relative Nutritive Value of Proteins

The procedure used in this assay follows that of Stott and Smith (1963, Brit. J. Nutr. 17:227.)

1) The test material is ground in a Wiley Mill through a 1-mm screen and mixed well. A sample is weighed precisely so that the nitrogen content is 15 mg N and transferred to a 100-ml beaker. Extract 3X with diethyl ether at room temperature and once with denatured ethyl alcohol and finally extract with either to remove the residual alcohol. Air dry at room temperature. Casein is not extracted. Add 30 ml of water so that the pH can be adjusted to 8.2. After adjusting the pH bring bolume to 40 ml. By adding 40 ml to the 15 mg, N 4 ml will contain 3 mg/4 ml.
2) Stock solutions A, B, C, and D-all 100 times stronger in the final medium are prepared as shown in Table 3. Solution E, which is 5X stronger than the assay is prepared and adjusted to pH 8.2.
3) Into each of a series of screw-capped tubes pipet (a) 2 ml of Solution E (b) 4 ml of the test suspension (c) 2 ml of distilled water. Autoclave tubes at 121°C for 10 minutes.
4) Autoclave at 121°C for 10 minutes stock Solution A (Table 3) diluted 10X and a solution of 15% glucose (w/v). The glucose concentration is 10X stronger than the final medium.
5) Add 1 ml of diluted Solution A and 1 ml of the glucose to each tube after cooling.
6) Use three replicate tubes per sample.
7) Add three drops of a 3-day broth culture of *T. pyriformis* W to each tube. Incubate at 25°C for 4 days. The screw caps are left loose during this period and the tubes inclined at 15° to the horizontal to provide sufficient aeration for rapid growth of *T. pyriformis* W.
8) One ml of the *T. pyriformis* W culture solution used as the inoculum is added to 1 ml of the preserving fluid (90 ml water, 20 ml 36% w/v formaldehyde and 10 ml stock solution D.) This is done at the time of inoculation of the tubes of the test material. The log count of the inoculum is used in the formula to calculate % RNV.
9) After 4 days of incubation, the test culture and the casein (control culture) are shaken for about 30 seconds on a Vortex-Genie test tube shaker. One ml of the culture are transferred to screw-capped culture tubes containing 1 ml of the preserving fluid.
10) The protozoa are counted in a double-cell lumecyte hemocytometer (Propper Mfg. Co., Inc.). The larger squares (each containing 16 small squares) are 1 mm by 1 mm. The mean number (one can count the 8 large squares as shown in Figure 1) per tube. Since there are 3 replicate tubes per sample, at total of 24 square mm^2 are counted is determined.

11) The mean number of protozoa are multiplied by 2 (dilution factor due to the addition of 1 ml of the sample in 1 ml of the preserving fluid) and by 10^4 protozoans/ml.

12) Review of Calculations

a) Production of Inoculum
Ten mls of Tetrahymena broth (5 g proteose-peptone, 5 g tryptone, 0.2 g dipotassium phosphate brought to 1 Liter with pH adjusted to 7.2; cotton-plugged culture tubes with 5.0 ml per tube) are used; incubate three days at room temperature.

Always check the inoculum for bacterial contamination! It cannot be used if contaminated!

b) Inoculum Count
One ml of protozoan culture is added to 1 ml of preserving fluid to give 1:2 dilution.

c) Inoculation of Tubes for Treatment
Three drops are used as the inoculum in a volume of 10 ml of casein and test protein.

d) Slide Factor and Dilution Factor
Size of square counted 1 mm X 1 mm X 0.1 mm = 0.1 mm^3.
1 ml = 1000 mm^3 = .001 mm^3
.001 X 0.1 = 0.0001 or 10^4
The mean count is multiplied by 2 (dilution factor) and times 10^4 (slide factor.)

e) Count of Tetrahymena after Incubation for 3 Days at Room Temperature
Three drops (measured by plastic 1 ml-pippitt divided into 0.1 units) were measured and found to be 0.1 ml.
The 0.1 ml is diluted ten fold when put into 10 mls of media. This gives a dilution factor of 0.1 in 10 ml or 0.01 or 10^2.

f) 2X Factors Drops Out
The factor for the inoculum count and for the treatment count was derived from a 1:2 dilution was 2, but since both have the same factor one can drop out this factor for both the inoculum count and treatment count.

g) Factors for -
Inoculum count (mean per square mm X 10^4.)
Test protein count mean per square mm X 10^4 X 10^2. The 10^2 factor (0.1 ml of inoculum in 10 ml media.)
Example: Mean count for inoculum was 8 protozoans per square mm.
8 X 10^4 = 80,000 protozoans
Mean count for treatment was 10 protozoan per square mm.
10 X 10^4 X 10^2 = 10,000,000
Casein 15 X 10^4 X 10^2 = 15,000,000
% RNV

$$\text{RNV\%} = \frac{\text{Log (count for test protein)} - \text{log (count inoculum)}}{\text{Log (count for casein)} - \text{log (count inoculum)}}$$

$$= \frac{\text{Log (10,000,000)} - \text{log (80,000)}}{\text{Log (15,000,000)} - \text{log (80,000)}}$$

$$= \frac{7.0000 - 4.90309}{7.17609 - 4.90309} = \frac{2.09691}{2.27300} \times 100 = 92\%$$

IV. Acknowledgements

The assistance of Dr. Gonzalo Pertz and his staff in all phases of the seminar and class activities in Nicaragua is gratefully acknowledged.

V. Evaluation of Trip

In a discussion held with Director Pertz, it was felt that the entire time spent in Nicaragua was very useful to LABAL, various govermental agencies and to Nicaraguan industry. It is believed that Dr. Baldwin's trip will also add to the the usefulness of LABAL efforts on evaluating Nicaraguan foods.

Table 3

Composition of stock solutions and solution E

Stock solution A (100 times final strength)	(mg/200 ml)
Calcium pantothenate	12.5
Nicotinamide	12.5
Pyridoxine hydrochloride	125.0
Pyridoxal hydrochloride	12.5
Pyridoxamine hydrochloride	12.5
Riboflavin	12.5
Folic acid	1.25
Thiamine hydrochloride	125.0
Inositol	12.5
Choline chloride	125.0
p-Aminobenzoic acid	12.5
Biotin	1.25
DL-α-lipoic acid	0.4

Stock solution B (100 times final strength)	(g/200 ml)
$MgSO_4 \cdot 7H_2O$	2.8
$Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$	1.25
$MnCl_2 \cdot 4H_2O$	0.025
$ZnCl_2$	0.0025

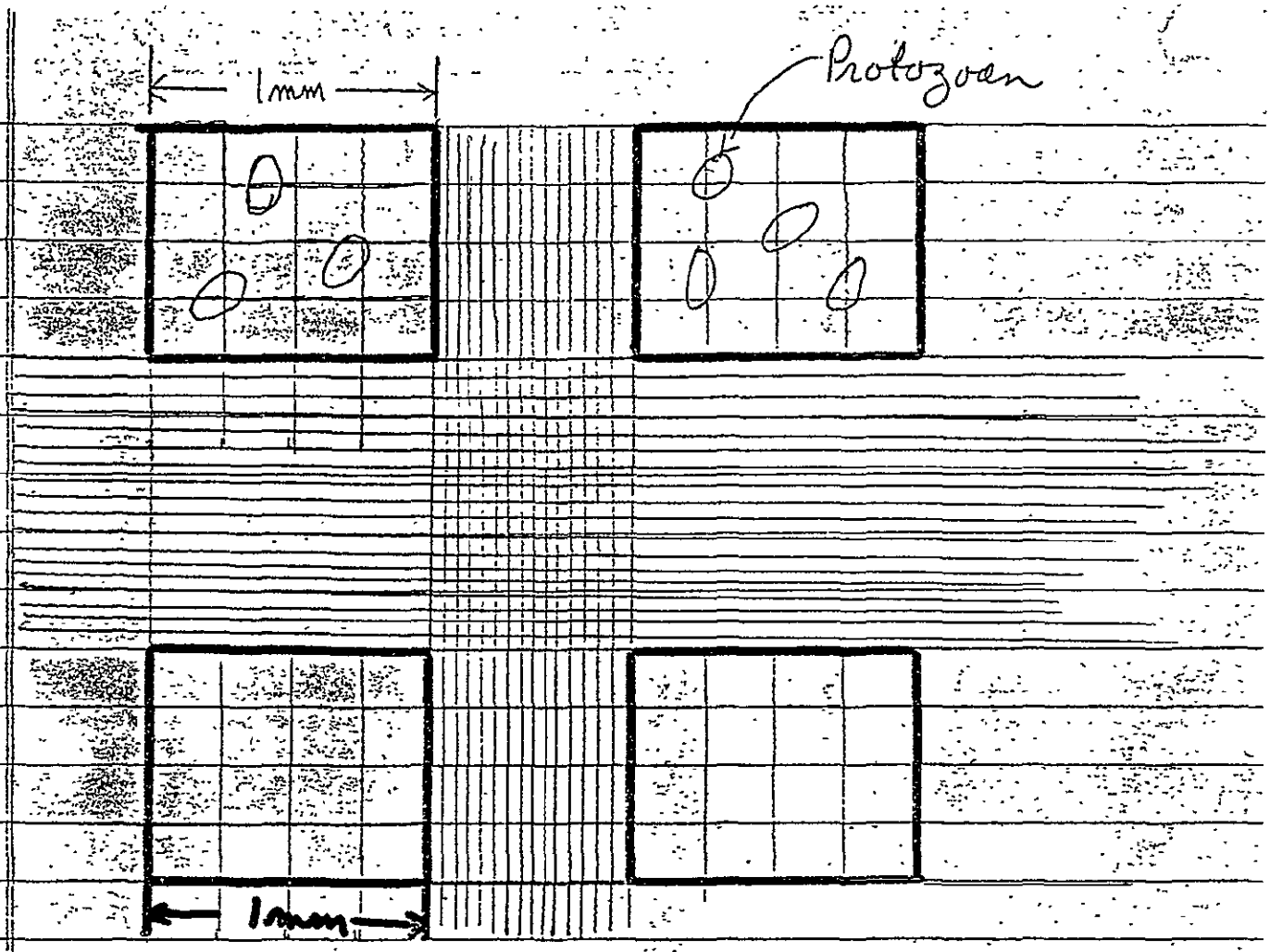
Stock solution C (100 times final strength)	(mg/200 ml)
$CaCl_2 \cdot 2H_2O$	600
$CuCl_2 \cdot 2H_2O$	60
$FeCl_3 \cdot 6H_2O$	15

Stock solution D (100 times final strength)	(g/200 ml)
KH_2PO_4	3.5
H_2HPO_4	3.5

Solution E (5 times final strength)

(The appropriate weights of the components listed below are dissolved in approximately 10 ml distilled water, 1 ml stock solutions B, C, D added and the whole made up to 20 ml)

	mg/20 ml solution E
Guanylic acid (sodium salt)	15
Adenosine-2' (3')-phosphoric acid monohydrate	10
Cytidylic acid	12.5
Uracil	5



Counted by = 10X 12.5 125X

10 eye scales = 1.0 mm

Figure 1

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Bacto-Methionine Assay Medium

Bacto - Dextrose	50 g	DL-Isoleucine	0.5 g
Sodium Acetate	40 g	Adenine Sulfate	20 mg
Ammonium Chloride	6 g	Guanine Hydrochloride	20 mg
DL-Alanine	0.4 g	Uracil	20 mg
L-Arginine Hydrochloride	0.484 g	Xanthine	20 mg
Bacto - Asparagine	0.3 g	Thiamine Hydrochloride	1 mg
L-Aspartic Acid	0.2 g	Pyridoxine Hydrochloride	2 mg
L-Cystine	0.1 g	Pyridoxamine Hydrochloride	600 mcg
L-Glutamic Acid	0.6 g	Pyridoxal Hydrochloride	600 mcg
Glycine	0.2 g	Calcium Pantothenate	1 mg
L-Histidine Hydrochloride	0.124 g	Riboflavin	1 mg
DL-Phenylalanine	0.3 g	Nicotinic Acid	2 mg
L-Proline	0.2 g	p-Aminobenzoic Acid	200 mcg
DL-Serine	0.1 g	Biotin	2 mcg
DL-Threonine	0.4 g	Folic Acid	20 mcg
DL-Tryptophane	30 mg	Monopotassium Phosphate	1.2 g
L-Tyrosine	0.2 g	Dipotassium Phosphate	1.2 g
DL-Valine	0.5 g	Magnesium Sulfate	0.4 g
L-Lysine	0.5 g	Ferrous Sulfate	20 mg
DL-Leucine	0.5 g	Manganese Sulfate	40 mg
		Sodium Chloride	20 mg

Bacto - Methionine Assay Medium prepared according to the formula given by Steel, Sauberlich, Reynolds and Baumann[1] is a complete dehydrated medium for the assay of DL-Methionine. It is free from Methionine but contains all the other growth factors and amino acids necessary for the growth of *Pediococcus cerevisiae* ATCC 8042. The addition of DL-Methionine in specified increasing concentrations gives a growth response by *P. cerevisiae* 8042 which may be measured acidimetrically or turbidimetrically.

The following procedure is recommended for the use of Bacto - Methionine Assay Medium:

Stock cultures of the test organism, *P. cerevisiae* 8042 are prepared by stab inoculation of Bacto - Lactobacilli Agar AOAC or Bacto - Micro Assay Culture Agar. After 24-48 hours incubation at 35-37 C, the tubes are stored in the refrigerator. Transplants are made at monthly intervals in triplicate.

Inoculum for assay is prepared by subculturing from a stock culture to 10 ml Bacto - Lactobacilli Broth AOAC or Bacto - Micro Inoculum Broth. After 16-24 hours incubation at 35-37 C, the cells are centrifuged under aseptic conditions and the supernatant liquid decanted. The cells are resuspended in 10 ml sterile 0.85% NaCl solution. The cell suspension is then diluted 1:20 with sterile 0.85% NaCl. One drop of this latter suspension is used to inoculate each of the assay tubes (10 ml).

It is essential that a standard curve be constructed each time an assay is run, since conditions of autoclaving, temperature of incubation, etc., which influence the standard curve readings, cannot be duplicated exactly from time to time. A standard curve is obtained by using DL-Methionine at levels of 0.0, 6, 12, 18, 24, 30, 36, 48 and 60 mcg per assay tube (10 ml).

The concentrations of Methionine required for the preparation of the standard curve may be prepared by dissolving 1.2 g DL-Methionine in 1000 ml distilled water. This is the stock solution (1200 mcg per ml). Dilute the stock solution by adding 1 ml to 99 ml distilled water. Use 0.0, 0.5, 1, 1.5, 2, 2.5, 3, 4 and 5 ml per tube. The stock solution is prepared fresh daily.

Bacto - Methionine Assay Medium may be used for both turbidimetric and acidimetric analyses. Turbidimetric readings should be made after 16-20 hours incubation at 35-37 C. Acidimetric determinations are made after 72 hours incubation. The most effective assay range, using Bacto - Methionine Assay Medium, has been found to be between 6 and 48 mcg DL-Methionine.

To rehydrate the medium, suspend 10.5 g in 100 ml distilled water and heat to boiling for 2-3 minutes. The slight precipitate which forms should be evenly distributed by shaking. Five (5) ml of the medium is added to each tube in the preparation of the tubes for the standard curve and to each tube containing material under assay. For the assay, each tube must contain 5 ml of rehydrated medium, increasing amounts of the standard or the unknown and sufficient distilled water to give a total volume of 10 ml per tube. The tubes are autoclaved for 10 minutes at 15 lbs pressure (121 C). Oversterilization of the medium will give unsatisfactory results.

Final reaction of the medium will be pH 6.8 ± 0.2 at 25 C.

One hundred g of Bacto - Methionine Assay Medium will make 1.9 liters of final medium.

PACKAGING
0423-15-2* 100 g

*Store at 2 - 8 C.

REFERENCE
[1] J. Biol. Chem., 177:533, 1949

BEST AVAILABLE DOCUMENT

BACTO - LYSINE ASSAY MEDIUM

Code 0422

Bacto - Dextrose	50 g	DL-Methionine	0.2 g
Sodium Acetate	40 g	Adenine Sulfate	20 mg
Ammonium Chloride	6 g	Guanine Hydrochloride	20 mg
DL-Alanine	0.4 g	Uracil	20 mg
L-Arginine Hydrochloride	0.484 g	Xanthine	20 mg
Bacto - Asparagine	0.8 g	Thiamine Hydrochloride	1 mg
L-Aspartic Acid	0.2 g	Pyridoxine Hydrochloride	2 mg
L-Cystine, Difco	0.1 g	Pyridoxamine Hydrochloride	600 mcg
L-Glutamic Acid	0.6 g	Pyridoxal Hydrochloride	600 mcg
Glycine	0.2 g	Calcium Pantothenate	1 mg
L-Histidine Hydrochloride	0.124 g	Riboflavin	1 mg
DL-Phenylalanine	0.2 g	Nicotinic Acid	2 mg
L-Proline	0.2 g	p-Aminobenzoic Acid	200 mcg
DL-Serine	0.1 g	Biotin	2 mcg
DL-Threonine	0.4 g	Folic Acid	20 mcg
DL-Tryptophane	80 mg	Monopotassium Phosphate	1.2 g
L-Tyrosine	0.2 g	Dipotassium Phosphate	1.2 g
DL-Valine	0.5 g	Magnesium Sulfate	0.4 g
DL-Leucine	0.5 g	Ferrous Sulfate	20 mg
DL-Isoleucine	0.5 g	Manganese Sulfate	40 mg
		Sodium Chloride	20 mg

Bacto - Lysine Assay Medium prepared according to the formula given by Steel, Sauberlich, Reynolds and Baumann[1] is a complete dehydrated medium for the assay of L-Lysine. It is free from Lysine but contains all the other growth factors and amino acids necessary for the growth of *Pediococcus cerevisiae* ATCC 8042. The addition of L-Lysine in specified increasing concentrations gives a linear growth response by *P. cerevisiae* 8042, which may be measured acidimetrically or turbidimetrically.

The following procedure is recommended for the use of Bacto - Lysine Assay Medium:

Stock cultures of the test organism, *P. cerevisiae* 8042, are prepared by stab inoculation of Bacto - Lactobacilli Agar AOAC or Bacto - Micro Assay Culture Agar. After 24-48 hours incubation at 35-37 C, the tubes are stored in the refrigerator. Transplants are made at monthly intervals in triplicate.

Inoculum for assay is prepared by subculturing from a stock culture to 10 ml Bacto - Lactobacilli Broth AOAC or Bacto - Micro Inoculum Broth. After 16-24 hours incubation at 35-37 C, the cells are centrifuged under aseptic conditions and the supernatant liquid decanted. The cell suspension is then diluted 1:20 with sterile 0.85% NaCl. One drop of this latter suspension is used to inoculate each of the assay tubes (10 ml).

It is essential that a standard curve be constructed each time an assay is run, since conditions of autoclaving, temperature of incubation, etc., which influence the standard curve readings, cannot be duplicated exactly from time to time. A standard curve is obtained by using L-Lysine at levels of 0.0, 30, 60, 90, 120, 150, 180, 240 and 300 mcg per assay tube (10 ml).

The concentration of Lysine required for the preparation of the standard curve may be prepared by dissolving 6.0 g of L-Lysine in 1000 ml distilled water. This is the stock solution (6000 mcg per ml). Dilute the stock solution by adding 1 ml to 99 ml distilled water. Use 0.0, 0.5, 1, 1.5, 2, 2.5, 3, 4 and 5 ml per tube. The stock solution is prepared fresh daily.

Bacto - Lysine Assay Medium may be used for both turbidimetric and acidimetric analyses. Turbidimetric readings should be made after 16-20 hours incubation at 35-37 C. Acidimetric determinations are made after 72 hours incubation. The most effective assay range, using Bacto - Lysine Assay Medium, has been found to be between 30 mcg and 240 mcg of L-Lysine.

To rehydrate the medium, suspend 10.5 g in 100 ml distilled water and heat to boiling for 2-3 minutes. The slight precipitate which forms should be evenly distributed by shaking. Five (5) ml of the medium is added to each tube in the preparation of the tubes for the standard curve and to each tube containing material under assay. For the assay, each tube must contain 5 ml of rehydrated medium, increasing amounts of the standard or the unknown and sufficient distilled water to give a total volume of 10 ml per tube. The tubes are autoclaved for 10 minutes at 15 lbs pressure (121 C). Oversterilization of the medium will give unsatisfactory results.

Final reaction of the medium will be pH 6.8 ± 0.2 at 25 C.

One hundred g of Bacto - Lysine Assay Medium will make 1.9 liters of final medium.

BACTO - TRYPTOPHAN ASSAY MEDIUM

Code 0327

Bacto - Vitamin Free Casamino Acids	12 g	Riboflavin	400 mcg
Bacto - Dextrose	40 g	p-Aminobenzoic Acid	200 mcg
Sodium Acetate	20 g	Niacin	200 mcg
L-Cystine	0.2 g	Biotin	0.8 mcg
Adenine Sulfate	20 mg	Dipotassium Phosphate	1 g
Guanine Hydrochloride	20 mg	Monopotassium Phosphate	1 g
Uracil	20 mg	Magnesium Sulfate	0.4 g
Thiamine Hydrochloride	200 mcg	Sodium Chloride	20 mg
Calcium Pantothenate	200 mcg	Ferrous Sulfate	20 mg
Pyridoxine Hydrochloride	400 mcg	Manganese Sulfate	20 mg

Bacto - Tryptophan Assay Medium is a complete dehydrated medium for the assay of L-Tryptophan or DL-Tryptophan. It is free from Tryptophan but contains all the other factors necessary for the growth of *Lactobacillus plantarum* ATCC 8014. The addition of Tryptophan in certain concentrations gives a linear growth response by *L. plantarum* which may be measured acidimetrically or turbidimetrically. Bacto - Tryptophan Assay Medium is prepared according to the formula described by Greene and Black[1].

The following procedure is recommended for the assay of Tryptophan using the dehydrated medium:

Stock cultures of *L. plantarum* ATCC 8014 are prepared by stab inoculation of Bacto - Lactobacilli Agar AOAC or Bacto - Micro Assay Culture Agar. Following incubation at 35 - 37 C for 24 - 48 hours, the tubes are stored in the refrigerator. Transplants are made at monthly intervals. Inoculum for assay is prepared by subculturing from a stock culture of *L. plantarum* ATCC 8014 into a tube containing 10 ml of Bacto - Lactobacilli Broth AOAC or Bacto - Micro Inoculum Broth. After 24 hours incubation at 35 - 37 C, the cells are centrifuged under aseptic conditions and the supernatant liquid decanted. The cells are resuspended in 10 ml sterile 0.85% NaCl. The cell suspension is then diluted 1:100 with sterile 0.85% NaCl. One drop of this latter suspension is then used to inoculate each of the assay tubes.

It is essential that a standard curve be constructed each time an assay is run, since conditions of autoclaving, temperature of incubation, etc., which influence the standard curve readings, cannot be duplicated exactly from time to time. A standard curve is obtained by using L-Tryptophan at levels of 0.0, 2, 4, 6, 8, 10 and 12 mcg per assay tube (10 ml). DL-Tryptophan may be used at levels of 0.0, 4, 8, 12, 16, 20 and 24 mcg.

The concentrations of Tryptophan required for the preparation of the standard curve may be prepared by dissolving 0.1 g of L-Tryptophan (0.2 g DL-Tryptophan) in 1000 ml of distilled water giving a stock solution of 100 mcg of L-Tryptophan per ml. Dilute this stock solution by adding 4 ml to 96 ml distilled water. Use 0.0, 0.5, 1, 1.5, 2, 3 and 5 ml per tube. This stock solution is prepared fresh daily.

Following inoculation, tubes are incubated at 35 - 37 C for 72 hours for the acidimetric assay and 16 - 20 hours for the turbidimetric assay.

To rehydrate the medium, suspend 7.5 g in 100 ml distilled water and heat to boiling for 2 - 3 minutes. The slight precipitate which forms should be evenly distributed by shaking. Five (5) ml of the medium is added to each tube in the preparation of the tubes for the standard curve and to each tube containing material under assay. For the assay, each tube must contain 5 ml of rehydrated medium, increasing amounts of the standard or the unknown and sufficient distilled water to give a total volume of 10 ml per tube. The tubes are sterilized by autoclaving for 10 minutes at 15 lbs pressure (121 C). Oversterilization of the medium will give unsatisfactory results.

Final reaction of the medium will be pH 6.8±0.2 at 25 C.

One hundred g of Bacto - Tryptophan Assay Medium will make 2.6 liters of final medium.

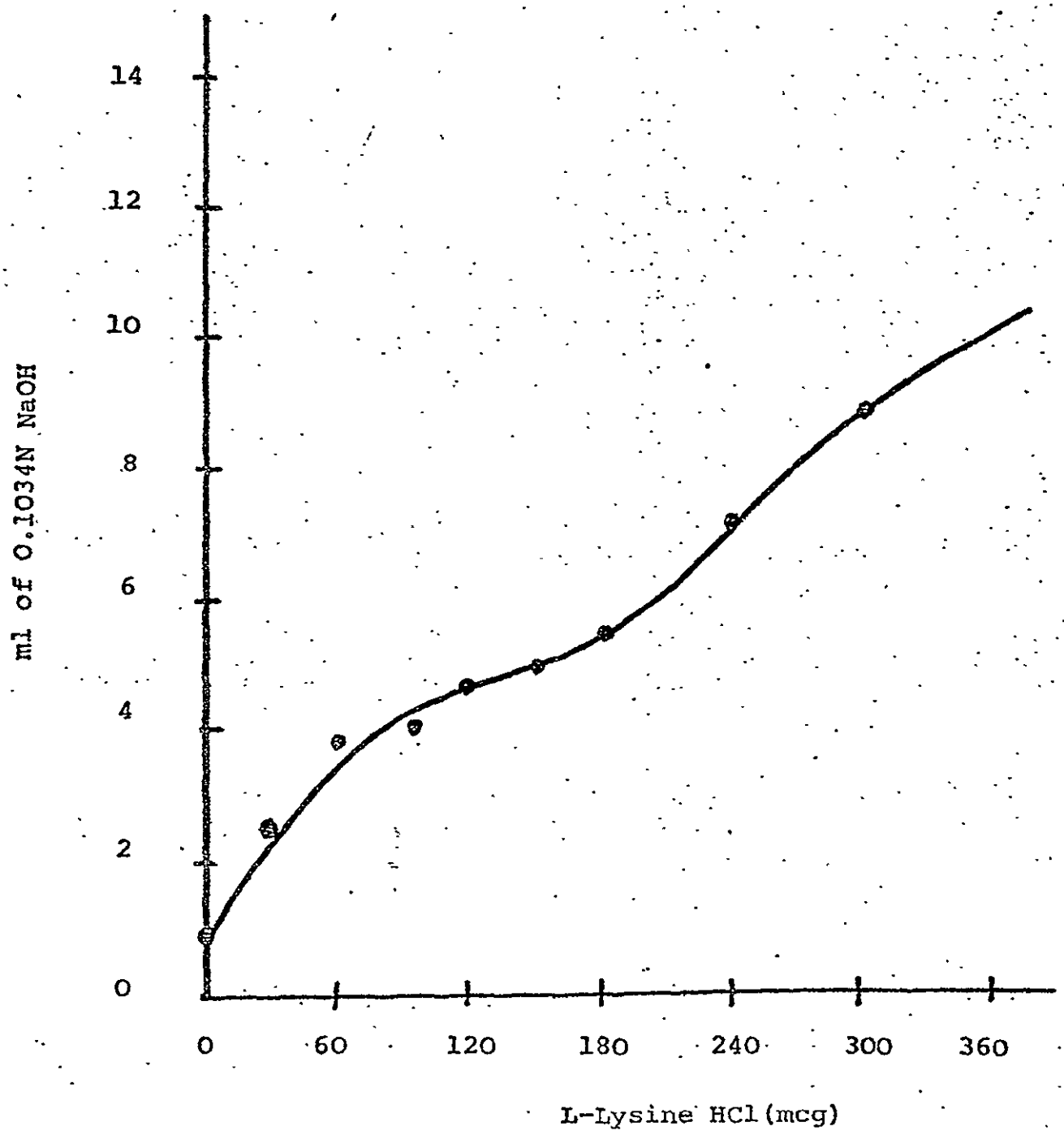
PACKAGING

0327-15-9* 100 g

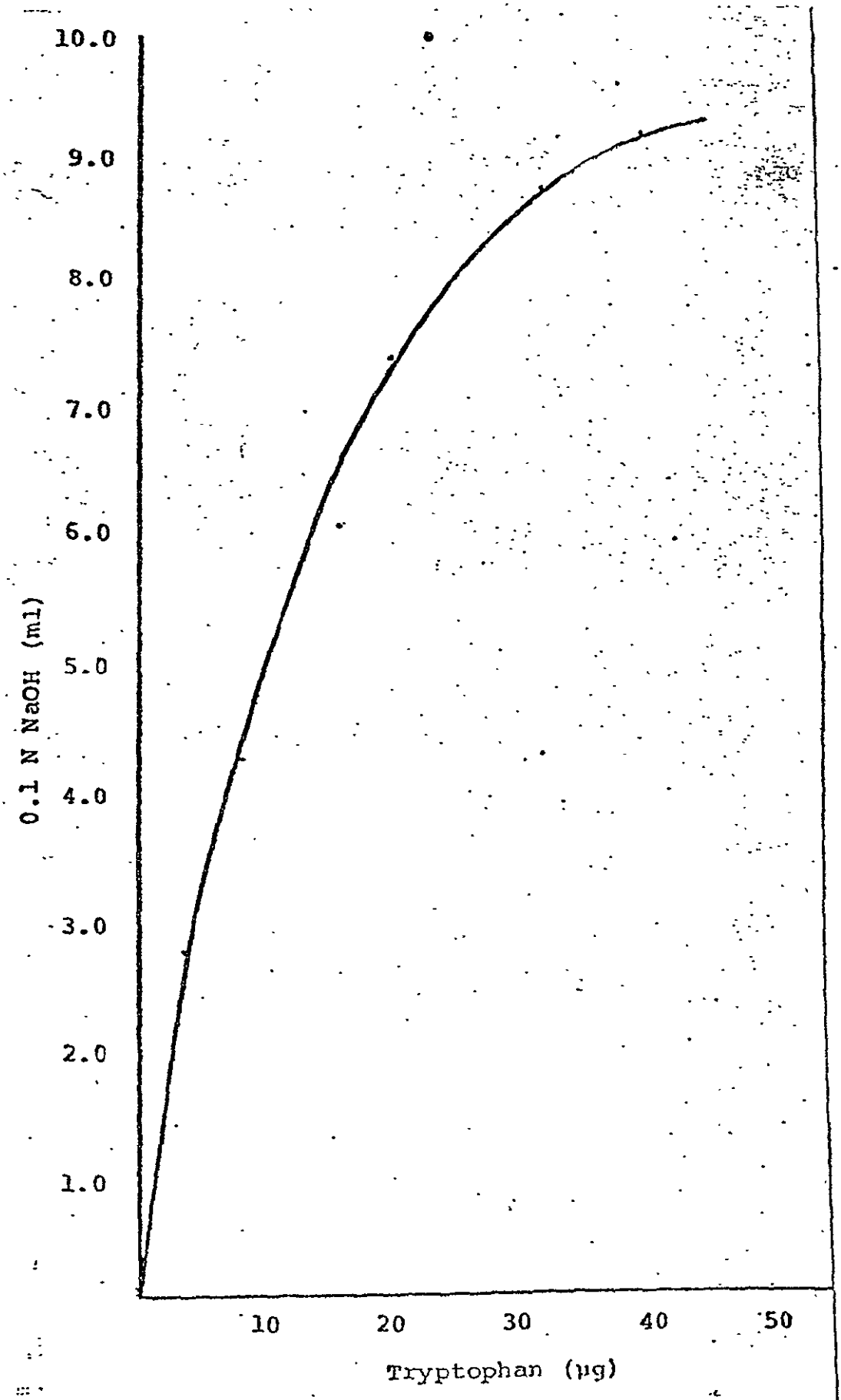
*Store at 2 - 8 C.

REFERENCE

[1] J. Biol. Chem., 155:1,1944



Standard Curve for Lysine



Standard curve for tryptophan.

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Appendix G

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