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Puerto Rico Nuclear Center
Food Preservation by Radiation
Technical Assistance Program
for
Instituto Centroamericano de Investigación y Tecnología Industrial

FINAL REPORT

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PUERTO RICO NUCLEAR CENTER
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FINAL REPORT

TECHNICAL ASSISTANCE PROGRAM IN FOOD PRESERVATION BY RADIATION FOR INSTITUTO CENTROAMERICANO DE INVESTIGACION Y TECNOLOGIA INDUSTRIAL (ICAITI)

PUERTO RICO NUCLEAR CENTER

San Juan, Puerto Rico

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I. Early Stages of Program Planning

In February 1968, the Puerto Rico Nuclear Center was approached by the US Agency for International Development (AID) through the US AEC's Division of International Affairs about the possibility of providing technical assistance in the field of food and product irradiation to the Instituto Centroamericano de Investigación y Tecnología Industrial (ICAITI) in Guatemala. The Nuclear Center responded promptly and favorably to the request, primarily because it fell within the major objectives of PRNC which include offering training and research opportunities in the peaceful applications of nuclear energy for Latin America.

Upon receiving an affirmative commitment from AID to provide support for the technical assistance requested, ICAITI had originally planned to recruit an expert in the field to spend six months in residence training the staff and developing research programs. Dr. H. D. Graham, then a part-time employee of PRNC, was approached on this matter, but he was unable to make himself available for this assignment. As an alternative, PRNC agreed to formulate a program which would require a team of experts for the training of ICAITI staff. A proposal for the program was outlined by Dr. R. A. Luse during the latter part of February and forwarded to AID through AEC. The proposal received approval of ICAITI and AID's Regional Office for Central America and Panama Affairs (ROCAP). The proposed program encompassed the use of several experts on the PRNC staff and also included a training period for ICAITI personnel using the PRNC irradiation facilities and laboratories and follow-up consultation visits in Guatemala by PRNC staff.

To meet the needs originally set forth by ICAITI, the program proposed to accomplish the following objectives:

1. To plan and design new gamma radiation projects - as part of a new ICAITI Gamma Irradiation Program - taking into consideration the needs of the CACM countries, and indicating the necessary specific steps to be taken for project implementation.

2. To perform applied technology work, which will transfer applied technology experience accumulated in the U.S. - insofar as applicable - to the conditions characteristic of Central America, and also contribute to the upgrading of ICAITI's personnel in the operation of available equipment (Cobalt-60 Gamma Radiation Source) in order to increase its utilization. Specifically, this work is to include:

   a) Irradiation of raw tropical fruit for shelf life extension;

   b) Irradiation of canned fruit;

   c) Irradiation of potatoes, onions, etc., to inhibit sprouting;

   d) Disinfestation of grains by irradiation;

   e) Exploration of possibilities of polymerization of cellulose-resins; and

   f) Exploration of possibilities of polymerization of vegetable fiber-resins. Likewise explore the possibilities of application to other substances as may be requested by ICAITI.

3. Based on the results of the above work, to assist ICAITI in the preparation of a Gamma Irradiation Program best suited to the needs of the CACM countries, including a detailed list - complete with specifications, costs, etc. - of ICAITI's minimum additional equipment requirements in this field.

The program was to be carried out as follows:

Phase 1. Survey of the proposed new projects, to assign project priorities, to make final experimental designs, and to co-ordinate efforts of ICAITI and PRNC staff. This is essentially Objective 1. To be carried out by a PRNC staff member during a 2 week stay in Guatemala (May 19 through June 1, 1968).

Phase 2. Laboratory training of two (or more) ICAITI staff members in those aspects essential to the proposed research. This will include a) analytical techniques for determining shelf life extension and loss of food quality after irradiation, b) irradiation dosimetry techniques, and c) radiation polymerization techniques. Trainees will work in close cooperation with PRNC scientists and technicians doing this work. In addition, lectures and demonstrations of procedures for personnel protection and monitoring can be provided if desired. This phase initiates Objective 2. To be carried out by two (or more) ICAITI staff members working at PRNC, Mayaguez, for seven weeks, during the period June 1 through July 20, 1968.

Phase 3. Establishment at ICAITI of techniques learned and research initiated during training at PRNC. This continues Objective 2. To be carried out by a PRNC staff member during a 1 week period in Guatemala after the ICAITI trainees have returned, e.g. July 20 through 27.

Phase 4. Continuation with PRNC assistance of new projects, a further part of Objective 2. To be carried out by a PRNC staff member during a two week stay in Guatemala (July 24-August 7) overlapping the stay of the other PRNC staff member, so that there is continuity of PRNC assistance.

Phase 5. Continuation by ICAITI staff of new projects. On-site PRNC assistance does not seem necessary during this time, as by early August ICAITI staff will have been in direct contact with PRNC staff since mid-May (12 weeks).

Phase 6. Review of ICAITI research, to permit preparation of a Gamma Irradiation Program suitable for the CACM countries and a list of equipment needs for an expanded ICAITI program in food and product irradiation. This phase covers Objective 3 and will conclude the formal part of this technical assistance. This phase will consist, first, of a two-week visit by a PRNC staff member to Guatemala (August 25-September 6) and, second, of a one-week visit by another PRNC staff member in early November (November 3-9).

The above phases represented a total PRNC involvement of 13.6 man-weeks, or 3 months' work time. This training period might be considered as equivalent to nearly 6 months' time spent continuously on-site by a single expert in residense at ICAITI since the group training at PRNC would be particularly effective, involving several persons, and since much of the actual research could be carried out by ICAITI personnel without day-to-day supervision at the latter part of the training period.

The program was scheduled to start immediately. However, PRNC was informed that a temporary shortage of technical personnel precluded immediate implementation of the program and ICAITI requested that the program be started toward the end of 1968. Through discussions between Dr. F. Aguirre, Deputy Director of ICAITI and Dr. H. J. Gomberg, Director of PRNC, a change in time schedule was considered necessary. Consequently a revised schedule was outlined as follows:

Phase 1. (1 week in early October, 1968): Visit by a PRNC staff member to ICAITI, to correlate project planning with ICAITI staff and with Dr. Bussel, an advisor from California.

Phase 2. (4-6 weeks, early November to mid-December, 1968): Laboratory training of ICAITI staff members at PRNC, to cover the objectives stated in

our February proposal to AID, and/or others which may have developed since that time.

Phase 3. (1 week in early January, 1969): Visit of a PRNC staff member to assist in establishing at ICAITI the techniques and research projects begun at PRNC.

Phase 4. (1 week in spring, 1969): Visit of a PRNC staff member to ICAITI to review research and to assist in preparing a Gamma Irradiation Program for CACM countries.

The above represented a modification of the original proposal and ICAITI was advised to notify PRNC if there was any disagreement.

The AID-AEC Participating Agency Service Agreement for the program was executed on September 30, 1968, authorizing PRNC to provide technical assistance to ICAITI on food irradiation studies during FY-1969 with AID funding of $12,000. PRNC promptly proceeded with the program which was directed toward starting the training of ICAITI staff at PRNC in early November 1968.

II. Coordination of Final Program Planning

On October 6-10, 1968, Dr. F. K. S. Koo traveled to ICAITI to coordinate the final plannings of the program and make arrangements for the first stage training.

He was briefed by Dr. Aguirre, Dr. Pedro Solé and Mr. Carlos Rolz, on ICAITI's major interests and needs in food preservation investigations. Discussions and consultations on the subject were subsequently made and finally a tentative schedule for ICAITI staff training at PRNC and for PRNC staff visits to ICAITI was outlined. This working plan included a 6-week research training for two ICAITI staff members at PRNC in highly specialized fields

of food science and consultation visits to ICAITI by three PRNC scientists at later dates.

In the new plan for program implementation, several revisions were made, mainly for the following reasons:

1. New emphasis and interest of ICAITI on research subject matter--ICAITI wishes to concentrate its effort on fresh fruit preservation studies with particular emphasis on comparing various methods of preservation, such as irradiation, gas storage, etc., and also on the biochemical nature of fruit ripening process, fruit flavor, etc.
2. Consideration of the experience of ICAITI staff, participating in training at PRNC--Instead of sending junior staff, ICAITI assigns two senior staff members for the training. Since these trainees have advanced degrees in chemical engineering and have done preservation experiments with tropical fruits using irradiation and freeze-drying methods, it seems routine technical orientation for them is not needed. At PRNC they wish to learn new, highly specialized analytical methods and techniques including infra-red spectrophotometry, gas liquid chromatography, enzymological methods and other techniques used in food irradiation studies. This training period at PRNC would serve as the initial phase of their research program rather than technical training. On returning to their home institution, they would be able to continue the research initiated at PRNC.
3. Changes in PRNC staff involvement and visitation schedule as a result of ICAITI's new emphasis and interest--To provide a type of training needed by ICAITI staff, Dr. H. D. Graham, Dr. S. N. Deshpande and Mr. J. Cuevas-Ruiz are assigned to the program serving as scientific

and technical experts. They would devote their time not only during the training period at PRNC but also on visits to ICAITI. Their visits will be scheduled to avoid conflicts with other obligations as well as to meet ICAITI's requirement. Dr. F. K. S. Koo would serve as the coordinator of the technical assistance program.

The budget breakdown was revised to accomodate requirements arising from changes in staff involvement in the program and the duration for training and visits, etc. Also the living allowance for ICAITI trainees in Puerto Rico had to be increased as there was no allocation of funds in ICAITI's budget for staff training in this case.

III. Final Plan for Program Implementation

The schedule for ICAITI staff training at PRNC and for PRNC staff visits at ICAITI was finalized as follows:

1. Training for ICAITI staff at PRNC

   a) Dr. Solé - 3 weeks (November 1-21)

   b) Mr. Rolz - 3 weeks (November 15 - December 5)

Research emphasis will be placed on the radiation effect on papaya shelf-life, respiration, pectic enzyme activity and flavor. Comparisons will be made of fruits under different storage conditions, such as in air, nitrogen, etc. This work will be carried out as an initial phase of a research project rather than technical training.

2. PRNC staff visits to ICAITI

   a) Dr. Deshpande - 2 weeks (beginning in the first part of January, 1969)

      To assist ICAITI staff to establish a program on papaya flavor

study initiated at PRNC and to set up a new program on studies of radiation effect on pectin and the enzymes involved.

b) Dr. Graham - 2-3 days (beginning or middle of February)
To review research initiated at ICAITI and to provide consultation on developing new research programs.

c) Mr. Cuevas - 2 weeks (in March at the beginning of mango season)
To provide consultation on mango irradiation study including shelf-life extension, biochemical and respiration analyses, etc.

d) Dr. Graham - 1 week (end of May)
To review research progress and to assist in preparing a gamma irradiation program for CACM countries.
(The above schedule was not strictly followed due to the necessity of making changes in the dates of PRNC staff consultation visits as requested by ICAITI)

IV. ICAITI Staff Training at PRNC

Training of the ICAITI staff at the PRNC irradiation installation and laboratories constituted an important part of the program. PRNC staff members involved in the scientific and technical training included Dr. Deshpande, Mr. Cuevas and Dr. Graham. On November 1, Dr. Solé arrived in Puerto Rico to begin a 3-week training and on November 17 Mr. Rolz also arrived for the same purpose. As indicated earlier in Section II, the training items were carefully selected to suit the trainee's interest, needs and background. The specialized training in methods and techniques includes:

1. Characterization of the volatile flavoring constituents--The ICAITI personnel, Dr. Solé and Mr. Rolz, were given a brief orientation in

basic gas liquid chromatography. Some work on the characterization of the volatile components responsible for the flavor of mango was done at PRNC in earlier years using the techniques of gas liquid chromatography. The indications were that carbonyl compounds were predominantly found among the volatiles recovered. In order to repeat this work, ripe papaya fruits were macerated and the macerate was subjected to extraction by means of a complex assembly for isolation of the volatile flavoring compounds. This assembly utilized the principle of flash evaporation and vaporization from a continuous thin heated film. Water and vaporized flavoring constituents were recovered by condensation in a series of cold traps cooled by liquid nitrogen and dry ice and acetone. The condensates from these traps were subjected to analysis by means of gas liquid chromatography. Chromosorb W columns coated with carbowax 1500 and di-isodecyl phthalate were used for separation of the volatiles. Judging from the retention times for the standard carbonyl compounds, there was again a very strong evidence for the preponderance of the carbonyl compounds in the condensates, followed by traces of esters and organic acids.

In order to confirm the occurrence of carbonyl compounds, 2,4, dinitrophenylhydrazine derivatives were made from the condensates and subjected to separation by means of thin layer chromatography. Even though there was the formation of hydrazine derivatives, indicated by characteristic precipitates, the resolution of the individual aldehydes, and ketones both by gas liquid chromatography, and thin layer chromatography was poor. However, the basic process of isolation and characterization of the flavor could be demonstrated.

2. Characterization of the structural polysaccharides--Mainly the pectic constituents are responsible for the integrity of the tissues and govern the texture and firmness of fruits. Ionizing radiation and the activity of the pectic enzymes in post irradiation storage are the factors responsible for degradation of the cell wall constituents and result in loss of texture. In order to study these factors it is necessary to determine the total pectic content, and characterize the pectic constituents with respect to their alkoxyl content and molecular weights. The pectic constituents were extracted from the fruits by homogenization in a Waring blender with 95% ethyl alcohol. The alcohol insoluble residue containing the pectic constituents, cellulose and hemicellulose, was further extracted with 0.5% ethylene diamine tetra acetic acid (EDTA) and the protopectin was extracted by hydrolysis with 0.05N HCl. The pectic constituents from these extracts were precipitated by four volumes of 95% ethyl alcohol, the precipitate was separated by centrifuging, dried in a vacuum oven and ground to a fine powder by means of a Wiley mill. The following methods were used for the characterization of the pectic constituents.

a) The McCready and McComb colorimetric method for the determination of the total uronide content. The pectic substances were dissolved in water to yield a 0.5% solution, the metal ions were sequestered with 0.5% EDTA, and an enzyme preparation pectinol supplied by Rohm and Haas Company was used to hydrolyze the pectic constituents. The galacturonic acid resulting from the enzymatic hydrolysis was determined by the carbazole reaction colorimetrically.

b) The alkoxyl content was determined by deesterification of the methoxyl groups by alkali, and the methyl alcohol liberated was distilled out by a microassembly. The methyl alcohol recovered in the condensate was oxidized to formaldehyde by $KMnO_4$, and reacted with 4,5, dihydroxy - 2,7, naphthalene disulphonic acid. The concentration of the methyl alcohol in the condensate was determined by measuring the intensity of the colour complex.

c) The weight average molecular weight ($\bar{M}w$) was determined by viscometric techniques. The Ostwald Fenske viscometer was used to determine the specific viscosities of pectin solutions. The ratio of specific viscosities to concentration (nsp/c) was plotted against a series of concentrations of these solutions.

The intrinsic viscosity ni was determined by extrapolation. ($\bar{M}w$) values were calculated by using the equation $ni = \log 1.4 \times 10^{-6} (\bar{M}w)^{1.34}$.

d) The number average molecular weight ($\bar{M}n$) was determined by measurement of the reducing power of the aldehyde group. The alkaline copper method was used for this determination.

It was not possible to characterize the cellulose and hemicellulose residues at this stage but a method was outlined for this while on the technical assistance trip.

3. Study of the kinetics of pectic enzymes--Two pectic enzymes--pectin methylesterase and pectin polygalacturonase--seem to be involved in post irradiation storage of fruits. There was some evidence of an increase in the pectin methylesterase activity in fruits stored after irradiation. In order to study the kinetics of this increase

in the enzyme activity, irradiated papaya fruits were macerated. A 10 g sample of the pulp was mixed with 10% of its weight of NaCl and suspended in 200 ml of borate-phosphate buffer at pH 8.2 by blending. The precipitate was separated by centrifuging and filtration and transferred to a dialysis tubing. It was dialyzed against distilled water until it was free of electrolytes, resuspended in the borate-phosphate buffer and dialyzed again. At this stage, attempts were made to concentrate the homogenate by lyophilization but a loss in the enzyme activity was noticed. In the subsequent trials, therefore, both the second dialysis step and lyophilization process were eliminated. The protein content of this preparation was determined by the micro-Kjeldahl method. In order to assay the activity of this enzyme, to a 50 ml aliquot of the substrate solution, consisting of 0.25% pectinic acid, a 10 ml portion of the homogenate was added. The pH of the reaction mixture was adjusted to 7.2 and the activating concentration of NaCl was adjusted to 0.1M. The mixture was kept agitated by means of a magnetic stirrer and the temperature was maintained by a circulated water bath. The carboxyl groups liberated as a result of enzyme-mediated deesterification were determined by neutralization with 0.01N NaOH. A Fisher automatic titrator with calomel and glass reference electrodes was used for the assay of enzyme action. Micromoles of carboxyl groups produced per gram of the enzyme protein were plotted against one minute time intervals. The results indicated a distinct increase in the activity of the enzyme with increasing radiation doses compared with the unirradiated controls.

Study of the kinetics of pectin polygalacturonase was complicated by the elusive nature of this enzyme. A 10 ml extract of the enzyme preparation was added to 0.25% pectic acid solution and was kept agitated at a constant speed at 25°C. One milliliter aliquot was withdrawn from this enzyme substrate mixture at one minute intervals and added to a series of graduated centrifuge tubes containing 2 ml of 3,5, dinitrosalicylic acid reagent. The alkalinity of the reagent arrested the enzyme action immediately. The increase in the colour intensity caused by liberation of the aldehyde groups set free by the enzyme was measured colorimetrically. The results were not consistent, and this was perhaps due to the presence of enzyme inhibitors reported in the literature. However, the trials served the purpose of training in methods of enzyme assay.

4. Irradiation and determination of respiration in papaya fruits-- Selection of papaya fruits and irradiation procedures used at PRNC were first demonstrated to the ICAITI staff and then practiced by them. For respiration study, an infra-red CO_2 analyzer was installed and calibration and standardization of the instrument were carried out. The fruits were picked at the Isabela Experiment Substation and selected for the study. One fruit was exposed to 25 krads of gamma radiation and the other served as control. Both fruits were held in a respiration chamber and the respiration was measured for a period of time before rotting. Repeated runs were made. It was found that the respiration rate for the green fruit was low and steady initially, followed by a rapid rise during the early ripening period. The ripe and overripe fruit showed a slowly rising rate

until the peel became completely brown and serious fungal infection took place. At this stage a final rise occurred. These results were in good agreement with the findings reported by other workers in the respiration studies of tropical fruits. The study with the irradiated fruit was not satisfactory as the fruit in storage rotted prematurely. The period of rapid increase in carbon dioxide liberation is called the 'climacteric' of the fruits. During this stage the tissues are passing from a low level of metabolic activity to a higher one. Studies of climacteric pattern in irradiated fruits as compared with that of the controls provide information on the radiation effect on the ripening process of the fruits.

V. PRNC Staff Consultation Visits to ICAITI

1. Mr. J. Cuevas-Ruiz, May 13-25, 1969

The follow-up consultation visit to ICAITI by the PRNC specialists was considered as one of the essential steps in the implementation of the program. Mr. Cuevas was the first one traveling to ICAITI to assist its staff in organizing new research programs and to provide consultations on studies of shelf life extension and respiration measurement in tropical fruits using infra-red spectrophotometric techniques.

The first two days were solely devoted to discussions with Dr. Solé and Eng. Rolz of the past accomplishment and the present status of the food irradiation program at ICAITI, outlining experimental procedures and the working schedule, preparing laboratory needs and testing instruments.

The major studies conducted during the 2-week period were on the respiration measurement of mango fruits. The fruits in the mature-green stage were obtained from the market. In the laboratory the fruits were selected and weighed, and then were placed in a respiration chamber (4-liter size beaker). The amount of fruit needed for obtaining a good respiration rate measurement was above 1 kg. A flow rate of 300 cc/min was maintained during the experiment by using a rotameter. Every 2 1/2 hours a sample of CO_2 evolved from the fruits was taken by the sample cell and this was placed in a Perkin-Elmer 337 Grating Infra-red Spectrophotometer where the characteristic CO_2 peak was determined. By using the air in the reference cell the percentage of CO_2 evolved by the fruit was directly recorded on the graph. Some inconvenience was encountered because the respiration experiment was done in one laboratory and the infra-red spectrophotometric measurement was made in the instrument room.

After many trials and readjustments, it was possible to measure the respiration pattern of the fruit and to calculate the climacteric peak. Preliminary results with non-irradiated mangoes indicate a respiratory pattern consistent with that reported in the literature for other varieties of mangoes. Study plans were also outlined for papayas and avocados.

At PRNC, it was found that the proper selection of fruits as to the age, maturity, etc., in addition to selecting fruits free of disease infection and mechanical damage for irradiation are of vital importance. Also the fruits should be irradiated within 24 hours after harvest. These points were made known to the staff there and

followed strictly in practice. The fruits used in the experiments at ICAITI were obtained from the market where the fruits of different varieties, maturity, etc., were all kept together. This made it extremely difficult in the pre-irradiation fruit selection and varietal separation.

Mr. Cuevas was also requested to design a thesis problem for and provide instructions to Julio César Corado, a chemical engineering student from the National University of San Carlos, Guatemala City, assigned to do thesis research under the supervision of Dr. Solé. The study consisted of a series of experiments to measure the respiration pattern of mango fruits following gamma irradiation by infra-red spectrophotometry. The doses used were 25, 50, and 75 kr. The findings of this work will be reported elsewhere as the bulk of this investigation was undertaken under a separate support.

2. Dr. S. N. Deshpande, August 4-15, 1969

During his visit, Dr. Deshpande placed major emphasis on the research projects originally initiated at PRNC and subsequently continued at ICAITI. The ripening condition of fruits can be determined by measuring their respiration patterns using infra-red spectrophotometry. Also the objective evaluation of ripening can be done by use of firmness pressure gauges. Suggestions were made to include the following biochemical assays on the fruits subjected to the irradiation treatments, in order to complement the respiration and firmness determinations, for evaluation of maturity: a) determination of sugar/acid ratios, and b) determination of carotene as an index of maturity. The second project emphasized was the study of the kinetics

of the action of pectic enzymes, which seem to be activated in post-irradiation storage of fruits. The techniques were standardized for isolation and assay of the activity of pectin polygalacturonase and pectin methylesterase. Techniques were also standardized for the characterization of the pectic constituents, the substrates of these enzymes. Techniques for the determination of protein content of the enzyme preparations by means of the micro-Kjeldahl technique were also standardized.

In addition, techniques were partially developed for the standardization of non-pectic structural polysaccharides such as cellulose and hemicellulose. It was suggested that cellulosecupriethylenediamine and cellulose nitrate derivatives should be made for the determination of their molecular weights by viscometric techniques and techniques of molecular sieve chromatography. So also colorimetric methods based on the anthrone reaction and chromic acid oxidations were suggested for determination of sugars and total carbohydrates.

A new trial was outlined involving irradiation of bananas, guavas, and other local annonaceous fruits. The doses suggested were 25, 50 and 75 kr. It consisted of the foregoing parameters of respiratory studies and the determination of the kinetics of the action of the two pectic enzymes. Two graduate students from the University of San Carlos were assigned to continue these studies for their thesis research. Results will be reported under a separate program.

ICAITI has recently received added support from AID to continue food preservation and related studies. In addition, the local

coffee growers have also been supporting a project to study the feasibility of enzymatic depulping of coffee berries, and the consequent effects of these treatments on the flavour of roasted coffee beans. Irradiation of coffee berries prior to enzymatic depulping can, perhaps, increase the efficiency of depulping. ICAITI has shown interest in future collaboration with PRNC in order to continue research on the projects supported by AID, and other projects such as depulping of coffee by irradiation.

3. Dr. O. H. Wheeler, January 10-15, 1970

The main objectives of Dr. Wheeler's visit were to review and discuss with the ICAITI staff the needs of their research programs for future development and to outline a workable program for gamma irradiation studies for the CACM countries. Dr. Wheeler observed that in the last few years ICAITI has added new buildings, acquired scientific equipment such as IR, UV, gas chromatography, electrophoresis, atomic absorption spectroscopy and received research funds, and the general conditions have improved considerably. The research funds are for specific projects and as a result, the Institute has had to define its fields of research. The Applied Research Division is now headed by Mr. Rolz. The former head, Dr. Solé, is now production manager for Ducal, a food packing plant (Grace subsidiary). The Division has three professionals and three technicians.

The Institute receives AID funds as well as supports from OAS for research related to the industrial needs of Central America. The research at present covers the preservation of tropical fruits (including irradiation preservation) and the determination of the

optimum conditions of obtaining a high protein cotton cake with maximum recovery of cotton seed oil by pressing. The project will receive support from a botanist and chemist from Saint Louis University to initiate work on natural products from Guatemala. The University of Concepción in Chile is also investigating anti-tumor compounds in plants; and the Polytechnic of Ecuador, plant insecticides, under the same scheme. Dr. Wheeler suggested natural product organic chemists (Djerassi, Barton, Cava) who could assist in determining the chemical structure of any new products isolated. A certain amount of funds has been used to support workshops in food processing and marketing at ICAITI, and to train students at the University of California at Davis. The funds have also been used to set up a microbiological laboratory to offer service to the local food industries. The laboratory is well equipped and includes a 7 liter controlled environment fermenter. Also an industrial extension service in conjunction with the Denver Research Institute and other U.S. organizations will be set up.

Based on his observations and discussions with ICAITI staff Dr. Wheeler outlined in very broad terms the gamma irradiation program for the CACM countries which is incorporated in Section VI (Recommendations) of this Report.

VI. Recommendations

Due to the recent lack of interest in food irradiation in the USA, ICAITI has shown reservations toward the practicability of radiation preservation of food. Lately this Institute has placed more and more emphasis on comparative

studies of different food preservation methods rather than on irradiation preservation alone. In view of this, it is suggested that fruit irradiation should be deemphasized or discontinued. The inhibition of sprouting of potatoes and onions, and the disinfestation of grain by irradiation which were both originally considered, should not be studied for the same reason, and also because of their limited economic potentialities in Central America. On the other hand, ICAITI has shown great interest in enzyme studies related to the ripening of tropical fruits and the depulping of coffee beans by combination treatment with radiation and enzymes. These studies should be initiated or developed further. It appears appropriate at this point to suggest that as a continuation of the present program a new short term technical assistance program be established immediately. This could meet ICAITI's additional needs for the development of new programs such as the one for depulping coffee beans or for acquiring new techniques. For instance, new methods developed at PRNC for amino acid determination from protein hydrolyzates using isotopic dilution techniques could be learned by the ICAITI staff for possible application to food protein quality studies. It is the consensus of the participants of both institutions that before establishing a gamma irradiation program for CACM countries, it would be highly desirable and most profitable scientifically to develop a new short term assistance program to meet the immediate needs of ICAITI.

During Dr. Wheeler's trip to ICAITI, information was provided to the institution on the work of Dr. Benegas in Honduras on Torsalo (Dermatobia hominis), a parasite which attacks cattle, causing spoil of hides, particularly in Nicaragua and Guatemala (information supplied by Dr. D. W. Walker, PRNC). Although ICAITI does not intend entering the area of entomology, the

suggestion for radiation sterilization study should be made here since the problem of Morsalo is serious enough to warrant such attentions.

ICAITI is a technological center established for the benefit of the industrial and economic integration of five Central American countries, namely, Guatemala, El Salvador, Honduras, Nicaragua and Costa Rica. It provides consultation and advice to public institutions as well as private enterprises in solving industrial and technological problems, conducts scientific investigations on the utilization of regional raw materials and promotes application of new technology. Because of its very nature, ICAITI has great flexibility of venturing into new areas of inquiries other than food irradiation.

ICAITI has well-equipped pilot plant laboratories for the study of cotton fibers, for the manufacture of paper and board from vegetable fibers, and for leather tanning. The Applied Research Division should initiate a program on the cross-linking of cotton fibers with a monomer, such as styrene or methyl methacrylate, using gamma radiation. Woven cloth is only manufactured for the local Common Market, but cotton fiber is an important Central American export. The possibility of using radiation polymerization to make fiber board, and of accelerating the tanning of leather should be explored also. Corn stalk fiber has been used to make good quality wrapping paper at ICAITI and is superior to cane bagasse and cotton stalk fiber. Shoe leather, soles and uppers, but not kid leather is produced in Central America.

To implement a gamma irradiation program of such a complex and broad nature as mentioned above, one needs a concerted effort contributed by various well-established research and development institutions and private enterprises in the CACM countries including ICAITI, the nucleus and prime mover of the projected program.

ICAITI has a small gamma source obtained from the 'Atoms in Action' Exhibit several years ago, freezing and drying equipment, a food technology laboratory and a chemistry laboratory adequately equipped for routine analyses and other special laboratories for studying industrial products as mentioned earlier in this Section. The instrument room is equipped with gas chromatographic and infra-red spectrophotometric instruments and others. Therefore, equipment for technical and scientific research programs using the gamma source is adequate, except for techniques involving the use of isotopes. The analysis of the essential aminoacids in cotton cake protein for human consumption can be best carried out by isotope dilution using labeled aminoacids. The development of the physiological studies of the ripening of fruits would also be greatly aided by the use of labeled compounds (carbon dioxide, ethylene, ascorbic acid). Radiochemical counting equipment would also allow the use of tracers in the study of the uptake of fertilizers in soil, and in mineral separations. Also if the studies for the isolation and molecular characterization of the pectic enzymes, fruit flavoring constituents, etc., are to be initiated or intensified, other analytical instruments may be necessarily acquired.

In the form of a summarized recommendation, the gamma irradiation program for the CACM countries is presented as follows:

Gamma irradiation program

Objective--Application of nuclear science and technology to the improvement of industrial products, food and agriculture.

Scope--The following subjects are to be investigated:

a) Cross-linking by irradiation of cotton fibers with monomers.

b) Radiation polymerization as a process of making fiber board and other products.

c) Acceleration of tanning process in leather by radiation treatment.

d) Depulping of coffee beans using combination treatment with radiation and enzymes.

e) Enzyme studies related to fruit ripening.

f) Control of insects (Torsalo) by irradiation sterilization.

Organization--ICAITI serves as the program's principal investigator and also as the coordinator of the program, with the support of a team of institutions of excellence in special fields selected from various CACM countries.

Equipment required--It is recommended that the following items of equipment be furnished to ICAITI to strengthen and extend the Gamma Irradiation Program:

a)	1 Unilux Bench-top Liquid Scintillating System model 6850, Nuclear Chicago Corp., Des Plaines, Illinois	$ 7,300
b)	1 Decade Scaler-Timer, model 8765, Nuclear Chicago	1,100
c)	1 Manual Planchet Sample Changer, model 1123, Nuclear Chicago	500
d)	3 Geiger Tubes, Thin Window, model 108, Nuclear Chicago	200
e)	1 Fume Hood, Six-Foot, Superstructure, model 93-602, Fisher Scientific Co., New York	700
f)	20 Virgin Lead Bricks, 2" x 4" x 8", #947-0024, Baird Atomic, Inc., Atomic Accessories Division, Cambridge, Mass.	200

g)	1 Flavor component extraction apparatus (to be fabricated)	$ 600
h)	1 Constant low temperature room (to be constructed, cost of insulation material, refrigeration, labor, etc.)	1,400
	Total cost, including transportation	$12,000

VII. Conclusions

The principal aims of this technical assistance program were to transfer knowledge and experience in food irradiation science and technology to the ICAITI staff from a team of experts on the PRNC staff, to assist ICAITI in developing new research programs of its interest and to formulate a gamma irradiation program best suited to the needs of the CACM countries. These objectives and requirements were fulfilled through the joint effort of the staffs of both institutions who participated in the program.

Based on our experience with this program, we sincerely believe that a technical assistance program such as this one with two major phases of operation--in residence training and follow-through visitation, is the most effective approach to a problem of this nature.

The training period for the ICAITI staff was solely devoted to the advanced analytical techniques and methods regularly used at PRNC. The training was so designed that learning of these techniques would serve as the initial phase of the new research program; on returning to their home institution, these scientists would be able to continue the research initiated at PRNC. Although the material used in the training was limited in variety,

the experimental trials with this material were carried out using the techniques and methods which can be readily applied to other food items. In the training process, discussions and exchange of ideas among scientists of both institutions were regularly practiced.

The impact of the in-residence training of ICAITI staff at PRNC was further enhanced by the follow-up visits to ICAITI by the PRNC staff. During these visits, discussions were made possible concerning the problems arising from research done at ICAITI with the experience gained at PRNC. In developing new research programs, not only could the ICAITI staff receive help from the PRNC experts but also the graduate students who were initiating their thesis research at ICAITI could receive advise and supervision directly from our staff. Contributing to the training of graduate students who will be the future scientists is a very important additional benefit of this program.

Based on the results and experience obtained and observations made during the program period together with the interest and needs of ICAITI in particular and the CACM countries in general, we have formulated a gamma irradiation program for the CACM countries. The new program can be implemented in full or in part depending on the extent of support it may obtain. In the implementation of the new program, we believe the most important elements for success are the capability, desire and determination to accomplish and surpass, which fortunately are the very characters found at ICAITI.