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MEASUREMENT OF ANTIOXIDATIVE EFFECT IN MODEL SYSTEM

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ABSTRACT

Three different methods to evaluate antioxidative effect using assay systems consisting of emulsified linoleic acid are described. Oxygen consumption was measured by a polarographic technique using hemin to accelerate the lipid oxidation. The formation of conjugated diene compounds during oxidation at 37°C was determined by spectrophotometric measurement of absorption at 234 nm. A sensitive gas chromatographic procedure was used for direct recording of the development of different volatile compounds by analyzing the headspace gas over the reaction medium. In the latter case denatured horseradish peroxidase was used as the catalyst. The advantages and limitations of the different methods are discussed.

INTRODUCTION

Lipid oxidation is a common deteriorative reaction in foods, even in those with low fat content. The oxidation can be catalyzed in many ways *e.g.* by lipoxygenase, hematin compounds and trace metals. Other food components like tocopherols, certain amino acids, plant phenols, and browning reaction products are able to act as antioxidants in food. In order to measure the effect of these types of naturally occurring or process induced antioxidants, simple and reliable methods for the assessment of antioxidative effect are required. Such measurements are preferably done in well defined model systems using pure fats, fatty acids or esters of fatty acids as substrates. The lipid oxidation can be measured in many different ways, *e.g.* by recording the oxygen consumption, by measuring the formation of primary lipid oxidation products such as hydroperoxides, or secondary products such as volatile flavor compounds. The oxidation products may be rather unstable.

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The hydroperoxides formed may for example, disappear by further reactions, the velocity of which is influenced by the presence of catalysts. As oxidizing lipids constitute such a dynamic system, it is often advisable to use more than one method of measuring when evaluating the effects of antioxidants.

Measurements of secondary products are often made by rather unspecific methods, for example by the TBA (thiobarbituric acid) reaction. The contribution of the volatile compounds, derived from lipid oxidation, to the flavor of a food is, however, complex due to the fact that many such compounds, differing in odor strength and character, are formed from the individual fatty acids which in turn are oxidized at varying speeds. Further reactions of the volatile compounds may also occur. Hence, measurements directly on separated odor compounds during lipid oxidation may be an alternative way of studying lipid oxidation and measuring the effect of antioxidants. This provides the possibility of using individual volatile compounds or specific combinations of volatile compounds as indicators of lipid oxidation. Good correlation is, for example, reported between rancid odor and concentration of hexanal in explosion-puffed potatoes (Konstance *et al.* 1978) as well as in other low fat, dehydrated foods (Fritsch and Gale 1977). In vegetable oils and in potato chips, significant correlations between the amount of pentane detected in headspace and the rancidity described by a trained panel is reported (Warner *et al.* 1974). The concentration of pentane has also been found to correlate with taste panel scores for nut-containing products (Bigalli 1977).

In the present investigation, model systems consisting of emulgated linoleic acid as the substrate and, in some cases, hematin compounds as the catalyst were studied. Antioxidative effect was evaluated by three different methods. Oxygen consumption was recorded by a polarographic method, the formation of conjugated diene compounds (linoleic acid hydroperoxides) was measured by spectrophotometry, and the formation of particular volatile compounds was measured by a gas chromatographic procedure based on precolumn concentration technique.

MATERIALS AND METHODS

In all measuring systems the substrate consisted of linoleic acid (>99%, Hormel Inst., USA, or Nu Chek-Prep. Inc., USA) emulsified with the aid of an equal amount of Tween 20 in 0.1 M potassium phos-

phate buffer. The linoleic acid concentration and the pH of the buffer differed somewhat between the measuring methods. The degree of oxidation of the linoleic acid emulsion was checked prior to use by spectrophotometric measurement of diene absorption at 234 nm (Hitachi 124, Japan). Where not otherwise stated 0.2 ml linoleic acid emulsion was solubilized in 2 ml of absolute methanol. Then 6 ml of 60% methanol in water were added and the absorption at 234 nm was measured (Surrey 1964).

The following were used as antioxidants: BHA (Butylated Hydroxy Anisole), n-propyl gallate and browning reaction products obtained by refluxing 5 mmol amino acid (L-histidine monohydrochloride monohydrate or L-arginine monohydrochloride) and 10 mmol sugar (D-glucose or D-xylose) in 5 ml potassium phosphate buffer (pH 7.0) for 5 h. BHA was dissolved in absolute ethanol and n-propyl gallate in water.

Polarographic Measurement of Oxygen Consumption

Oxygen consumption was measured by a polarographic procedure previously used for measuring lipoxygenase activity as described by Svensson and Eriksson (1972). The substrate used was 10 mM linoleic acid emulsion pH 6.5. The oxidation took place in a thermostatically controlled vessel (volume: approximately 6.5 ml; temperature: 25°C) equipped with a P_{O_2}-electrode, type E 5046, connected to an Acid-Base Analyzer type PHM 71 equipped with a P_{O_2} Module type PHA 930 (Radiometer, Denmark). The reaction vessel was completely filled with air saturated emulsion containing the antioxidant and 0.5 ml of 10 μM hemin in a mixture of 0.02 M potassium phosphate buffer pH 7.0 and 95% ethanol (1:1) was injected into the vessel to start the oxidation, and the decrease in oxygen partial pressure was recorded. (The hemin solution was stable for at least one month in the refrigerator). Controls, with no added antioxidant, were run in the same way, and the antioxidative effect (A.E. (1)) was calculated according to the following equation:

$$\text{A.E.(1)} = \frac{T_a - T_c}{T_c}, \text{ where} \qquad (1)$$

T_a = time elapsed for 50% reduction of the available gaseous oxygen in the sample containing the antioxidative addition.
T_c = corresponding time interval in the control.

Spectrophotometric Measurement of Absorption Increase at 234 nm

The antioxidant was mixed with 2 ml 10 mM linoleic acid emulsion pH 6.5 in test tubes. The tubes were placed in darkness at 37°C to accelerate the oxidation. Controls without antioxidant were run parallel. Both before starting the incubation and after 15–20 h, 0.2 ml of the substrate-antioxidant mixture withdrawn and the absorption at 234 nm was measured as described above. The antioxidative effect (A.E. (2)) was calculated according to the following equation:

$$\text{A.E. (2)} = \frac{\Delta A_{234}(C) - \Delta A_{234}}{\Delta A_{234}(C)} \text{ where} \qquad (2)$$

ΔA_{234} = increase of absorption at 234 nm during the incubation time

ΔA_{234} (C) = the corresponding increase in the control.

Gas Chromatographic Measurement of Volatile Compounds

The substrate used in this system was 3 mM linoleic acid emulsion, pH 6.8. The catalyst was horseradish peroxidase first purified to RZ > 3 (RZ = absorbance ratio $A_{403\ nm}/A_{275\ nm}$), and then activated by heat treatment at 120°C for 7 min in 0.01 M phosphate buffer pH 7.0 by a previously described procedure (Eriksson *et al.* 1971). Peroxidase was chosen as the catalyst in this system owing to its better long term stability regarding the catalytic activity as compared with hemin. The catalyst was always activated the day before use, while the substrate was prepared immediately before use. The incubation was carried out with 400 ml of the linoleic acid emulsion in a one-liter flask immersed in a water bath thermostated at 20°C. The flask was purged with air which was first purified by being passed through a molecular sieve (No. 5) and activated charcoal; the flow rate was 10 ml/min. The incubation flask was kept closed with a stainless steel lid tightened against the neck by an o-ring and a clamp. Tubes for controlled inlet and outlet of the air stream as well as for leak-proof addition of the catalyst and withdrawal of the samples went through the lid. At the bottom of the incubation flask was a glass side arm for leak-proof insertion of an oxygen electrode beneath the emulsion surface. The emulsion was agitated by a magnetic stirrer. After liquid — gas phase equilibration and zero check headspace sampling, as described below, the oxygenation reaction was

started by adding 3 mg of heat activated horseradish peroxidase in 1 ml of phosphate buffer. The reaction was followed by recording the oxygen tension in the emulsion, the absorption increase at 234 nm and by gas chromatographic analysis of the headspace gas over the emulsion. The oxygen tension in the medium was checked by continuous polarographic measurements using the equipment described above.

The absorption increase at 234 nm was followed spectrophotometrically by taking out 0.2 ml aliquots of substrate emulsion every 5 min for 30 min and then every 30 min, or whenever possible, until the experiment ceased. The aliquots were in this case immediately diluted to 5.2 ml in a mixture of equal volumes of 0.2 M potassium borate pH 9.0 and 95% ethanol.

The headspace sampling was performed according to a previously described, slightly modified, pre-column concentration technique (von Sydow *et al.* 1970). The air stream through the incubation flask was allowed to pass through a trap, cooled by a mixture of solid carbon dioxide and ethanol, in the pre-column concentration assessory whereby the volatile compounds in the headspace, derived from the lipid oxidation, were condensed. On each occasion 300 ml headspace gas was sampled before and 600 ml after the addition of the catalyst. The results obtained with the smaller sample were recalculated to be comparable with those of the larger one. The condensed material was injected on the gas chromatographic column by replacing the carbon dioxide-ethanol cooling mixture surrounding the trap by an oil bath held at 140° C.

Gas chromatography was performed in a Perkin-Elmer 900 instrument provided with a flame ionization detector (FID) and the pre-column concentration assessory. The open tubular column used, consisted of a 0.76 mm I.D. × 181 m stainless steel tube coated with SF96/Igepal CO 880 (95/5). The oven temperature was programmed 20–140° C at 2° C/min after an initial isothermal period of 3 min. The injector temperature was 110° C and that of the detector 170° C; the nitrogen gas flow was 12 ml/min. The FID-signal was fed into an Infotronics CRS-101 A electronic integrator provided with digital printout equipment.

For the conversion of integrator values into ppm n-hexanal in the emulsion at 20° C, standard lines were constructed by analyzing headspace samples equilibrated with linoleic acid emulsion containing known amounts of n-hexanal (Fluka, Switzerland) purified to 99.99% by preparative gas chromatography. For other compounds the integrator values were used directly.

RESULTS

Polarographic Measurement of Oxygen Consumption

The oxygen consumption in the absence of antioxidant and at various concentrations of BHA is shown in Fig. 1. It can be seen that the antioxidative effect is measurable only within certain limits of BHA concentrations. In run G, where the concentration of BHA is 8.0 mmol per mol linoleic acid, it takes 1840 s to consume 50% of the available oxygen, which must be regarded as the upper limit of the method. In practice, the time for consumption of 50% of the oxygen ought not to exceed 500-600 s.

There is no linear relationship between antioxidant concentration and antioxidative effect as measured by this method, as shown in Fig. 2, where the antioxidative effect calculated from the runs shown in Fig. 1 is plotted versus the concentration of BHA. From these results, it can be concluded that although the value of the antioxidative effect theoretically can vary from 0 to infinity, in practice it seldom gets higher than 10, although values as high as about 25 can be reached.

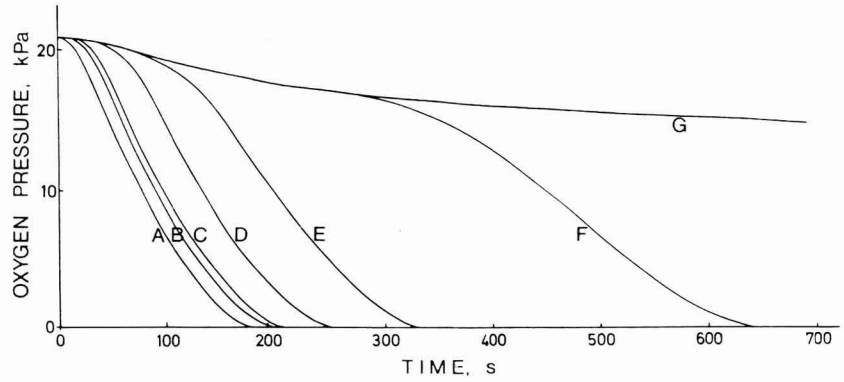

FIG. 1. CHANGES IN OXYGEN TENSION DURING OXIDATION AT 25°C OF LINOLEIC ACID CATALYZED BY HEMIN AND IN THE PRESENCE OF VARYING AMOUNTS OF BHA

Curve	mmol BHA/mol Linoleic Acid
A	0
B	0.25
C	0.5
D	1.0
E	2.0
F	4.0
G	8.0

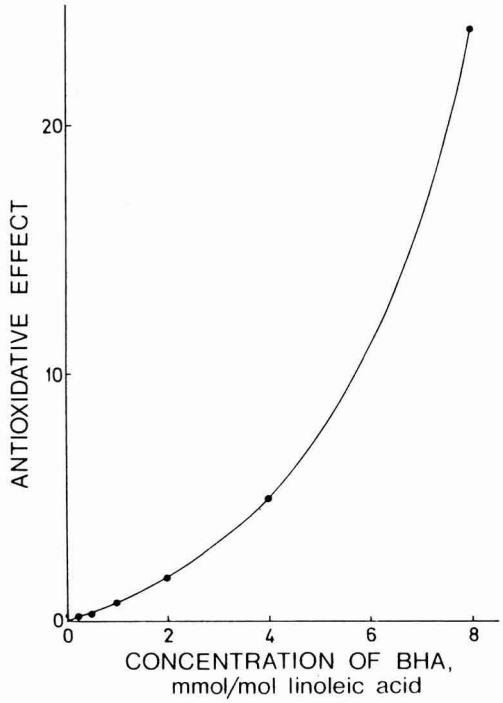

FIG. 2. THE INFLUENCE OF THE CONCENTRATION OF BHA ON THE ANTIOXIDATIVE EFFECT AS MEASURED BY POLAROGRAPHIC TECHNIQUE

The antioxidative effect is calculated according to Equation 1 from the runs shown in Fig. 1.

Figure 3 shows the influence of the initial degree of oxidation of the linoleic acid emulsion on the oxidation rate. Linoleic acid emulsion containing no antioxidant is compared with linoleic acid containing 1.0 mmol BHA per mol linoleic acid at various initial absorptions at 234 nm. As can be seen, the time elapsed for 50% reduction of available oxygen is considerably decreased when the initial diene absorption is increased up to 0.5-0.6 especially when the antioxidant is present in the linoleic acid emulsion. Consequently, the antioxidative effect is also influenced by the degree of oxidation of the substrate. Using data from Fig. 3 the antioxidative effect of 1.0 mmol BHA per mol linoleic acid calculated by using equation 1 is found to decrease from 12.3 at $A_{234\ nm} = 0.03$ to 0.9 at $A_{234\ nm} = 0.2$.

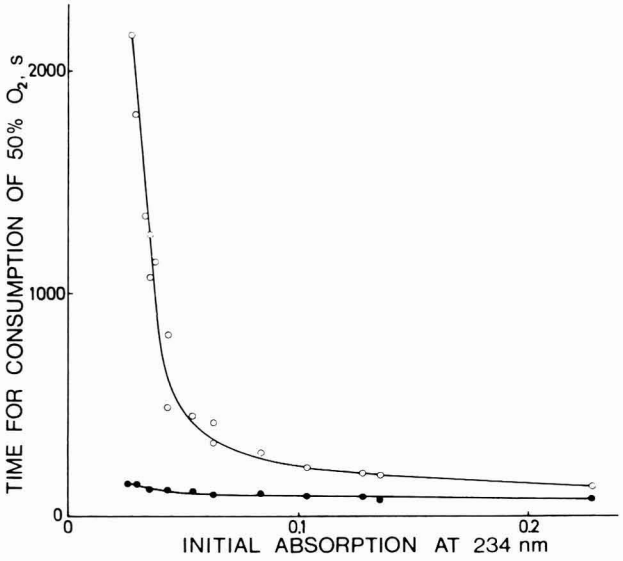

FIG. 3. THE INFLUENCE OF THE INITIAL STATE OF OXIDATION OF LINOLEIC ACID ON THE TIME FOR CONSUMPTION OF 50% OF AVAILABLE DISSOLVED OXYGEN DURING HEMIN CATALYZED OXIDATION AT 25°C WITH NO ADDED ANTIOXIDANT (●——●) AND WITH 1.0 MMOL BHA/MOL LINOLEIC ACID (○——○).

The initial state of oxidation is expressed as absorption at 234 nm. 0.2 ml of the linoleic acid emulsion were solubilized in 2 ml of absolute methanol. Then 6 ml of 60% methanol in water were added and the absorption at 234 nm was measured.

The absorption at 234 nm of freshly made linoleic acid emulsion was normally 0.03. After standing in darkness at room temperature for 5 h the conjugated diene absorption had increased to 0.06 and after 10 h the absorption had increased to 0.12. In darkness at 5°C the absorption increased to 0.05 in 24 h and to 0.10 in 48 h. The measure of antioxidative effect can, then, change considerably during one day, especially when the initial concentration of conjugated dienes is low. Therefore, it is important to know the degree of oxidation of the linoleic acid used. When comparisons between the antioxidative effect of several antioxidants are to be made, the oxidation rate should change as little as possible during the experimental period. The conjugated diene absorption of linoleic acid used for this purpose must consequently not be too low. When, on the other hand, single substances are to be tested

and high sensitivity is needed the linoleic acid emulsion ought to be as fresh as possible.

Spectrophotometric Measurement of Absorption Increase at 234 nm

Table 1 describes the rate of formation of conjugated dienes during incubation of linoleic acid emulsion at 37°C in the presence of varying amounts of BHA and browning reaction products made from histidine and glucose. As can be seen, the absorption at 234 nm reaches a maximum after about 20 h when no antioxidant is present. Therefore when using this method for evaluating antioxidative effect, the incubation time should preferably not exceed 20 h. The necessity of measuring the absorption before starting the incubation in cases when the antioxidant absorbs at 234 nm is illustrated in Table 1 by the experiments with high concentrations of browning reaction products.

In Table 2 the results from 15 h of incubation have been recalculated and expressed as antioxidative effect according to equation 2. The

Table 1. Absorption at 234 nm of linoleic acid emulsion incubated at 37°C for various times and containing varying amounts of BHA and histidine-glucose reaction products.[1]

Addition	Time of Incubation				
	0 h	10 h	15 h	20 h	25 h
No Addition	0.03	0.57	0.80	0.88	0.61
0.01 mmol BHA/mol linoleic acid	0.03	0.38	0.62	0.63	0.49
0.1 mmol BHA/mol linoleic acid	0.03	0.19	0.50	0.69	0.46
0.5 mmol BHA/mol linoleic acid	0.03	0.04	0.06	0.07	0.06
2.5 mmol BHA/mol linoleic acid	0.03	0.03	0.04	0.04	0.03
0.01 µl Histidine-glucose /ml linoleic acid	0.03	0.40	0.62	0.82	0.87
0.05 µl Histidine-glucose /ml linoleic acid	0.04	0.28	0.42	0.56	0.72
0.25 µl Histidine-glucose /ml linoleic acid	0.06	0.13	0.16	0.21	0.25
1.25 µl Histidine-glucose /ml linoleic acid	0.16	0.17	0.19	0.20	0.21

[1] 0.2 ml of the linoleic acid emulsion were solubilized in 2 ml of absolute methanol. Then 6 ml of 60% methanol in water were added and the absorption at 234 nm was measured

Table 2. Antioxidative effect of varying amounts of BHA and histidine-glucose reaction products

Antioxidant	Concentration of Antioxidant	Antioxidative Effect[1]
BHA	0.02 mmol/mol linoleic acid	0.23
BHA	0.01 mmol/mol linoleic acid	0.39
BHA	0.5 mmol/mol linoleic acid	0.96
BHA	2.5 mmol/mol linoleic acid	0.99
Histidine-glucose	0.01 μl/ml linoleic acid	0.23
Histidine-glucose	0.05 μl/ml linoleic acid	0.51
Histidine-glucose	0.25 μl/ml linoleic acid	0.87
Histidine-glucose	1.25 μl/ml linoleic acid	0.96

[1] Calculated according to equation 2 using data from Table 1. Incubation time: 15 h

findings that the browning reaction products are antioxidative are consistent with earlier reports based on other measuring methods (Kirigaya *et al.* 1968, 1969).

Gas Chromatographic Measurement of Volatile Compounds

The oxygen tension changes and formation of conjugated diene compounds ($A_{234\ nm}$) during the first hours of the reactions in the absence and presence of n-propyl gallate as the antioxidant are exemplified in Fig. 4.

From the oxygen pressure tracings one can see that in the absence of antioxidant, the initial value, 21 kPa, (corresponding to 21% partial pressure) first decreased rapidly after the addition of the catalyst to approximately 15 kPa and then increased to reach 19 kPa after 3.5 h; this value was then kept constant during the rest of the reaction. In the presence of antioxidant the oxygen pressure changes in the reaction medium were much smaller.

The $A_{234\ nm}$ curves obtained after addition of the catalyst represent the net production of diene compounds since hematin compounds catalyze both the production of linoleic acid hydroperoxides and their breakdown (Tappel 1962).

These measurements were performed in all experiments in order to check that the reaction conditions, particularly the oxygen tension changes, were reproducible.

In each experiment, headspace samples were analyzed before the addition of the catalyst and then at regular intervals during the reac-

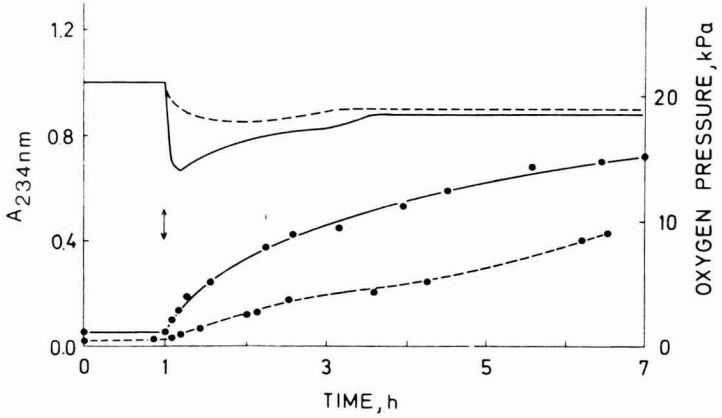

FIG. 4. CHANGES IN OXYGEN TENSION (UPPER CURVES) AND CONJUGATED DIENE COMPOUNDS (LOWER CURVES) DURING OXIDATION AT 20°C OF LINOLEIC ACID CATALYZED BY DENATURED HORSERADISH PEROXIDASE (FULL LINES) AND OF THE SAME SYSTEM CONTAINING 1.6 MMOL n-PROPYL GALLATE PER MOL LINOLEIC ACID (BROKEN LINES).

Addition of the catalyst is indicated by the arrow.

tion. A typical gas chromatogram of a headspace sample is shown in Fig. 5. The identity of some of the major volatile compounds was earlier established by mass spectrometry (Leu 1974) and now rechecked by retention analysis. While waiting for results from a detailed instrumental and sensory analysis of the volatiles formed in lipid oxidation, presently going on at this Institute, n-hexanal, peak No. 18, has been chosen as the major indicator in order to study the versatility of the oxidizing and analyzing systems.

In the headspace chromatograms which were obtained during the experiments the integrator computed area under the n-hexanal peak was used to calculate the n-hexanal concentration in the reaction medium from standard lines. The n-hexanal concentration increment during the reaction in the presence of varying amounts of n-propyl gallate (0, 1.6, 8.0, and 40.0 mmol per mol linoleic acid) is shown in Fig. 6, from which it is apparent that lipid oxidiation can be followed and antioxidative action evaluated by measuring the n-hexanal in the gas phase. n-hexanal is one of the largest peaks in the chromatograms; similar plots of the smaller peaks, n-hept-*trans*-2-enal and n-oct-*trans*-2-enal, (peaks No. 31 and 43) gave essentially the same result (Fig. 7). In this figure one can see, however, that the kinetics of the formation,

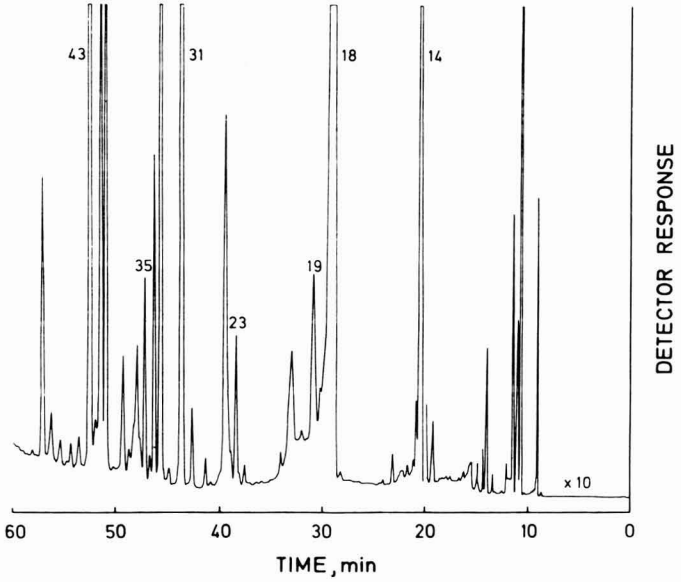

FIG. 5. GAS CHROMATOGRAM OF A 600 ML HEADSPACE SAMPLE OF LINOLEIC ACID, CATALYTICALLY OXIDIZED AT 20°C, 24 H, BY DENATURED HORSERADISH PEROXIDASE.

Peak No.	Compound	Integrator Value · 10^{-4}
14	n-pentanal	4
18	n-hexanal	119
19	n-pentan-1-ol	2
23	n-heptanal	0.7
31	n-hept-*trans*-2-enal	7
35	2-n-pentyl-furan	0.7
43	n-oct-*trans*-2-enal	2

of n-hept-*trans*-2-enal differed from the kinetics of n-oct-*trans*-2-enal production as well as from the kinetics of n-hexanal production as shown in Fig. 6. This is an interesting observation since such information is believed to be valuable for the further understanding of qualitative flavor changes during lipid oxidation.

Since the methods described are intended to be used for studying the antioxidative effect of naturally occurring and process induced antioxidative components in foods, preliminary experiments were carried out with sugar-amino acid reaction compounds using this technique as well. The effectiveness of these additions to inhibit n-hexanal formation is shown in Fig. 8. The results were found to be similar to those in Fig. 6. The additional volatile compounds introduced with this type of additive did not interfere with the gas chromatographic analysis.

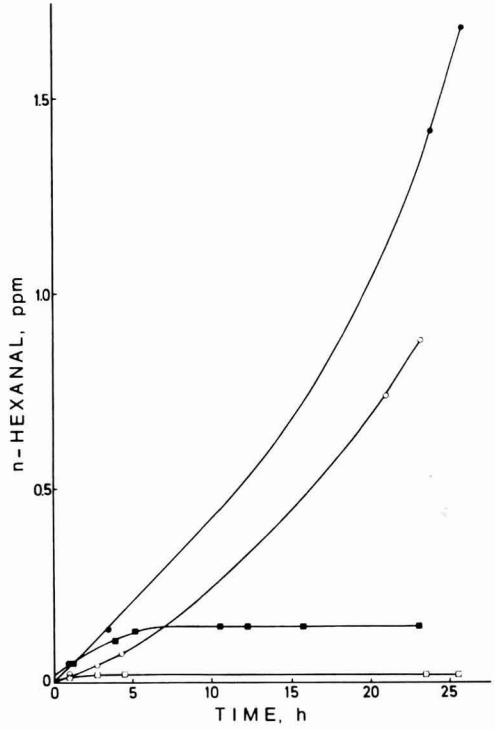

FIG. 6. FORMATION OF n-HEXANAL DURING OXIDATION AT 20°C OF LINOLEIC ACID CATALYZED BY DENATURED HORSERADISH PEROXIDASE AND IN THE PRESENCE OF VARYING AMOUNTS OF n-PROPYL GALLATE

Symbol	mmol n-Propyl Gallate / mol Linoleic Acid
●——●	0
○——○	1.6
■——■	8.0
□——□	40.0

DISCUSSION

Methods for measuring antioxidative effect by polarographic technique have been described previously (Hamilton and Tappel 1963; Yagi 1970; Berner *et al.* 1974; Cort 1974). Various substrates such as emulsified animal or vegetable fats, linoleic acid or methyl linoleate,

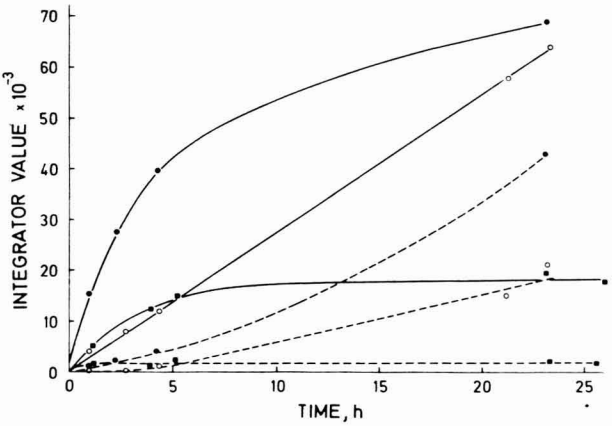

FIG. 7. FORMATION OF n-HEPT-*TRANS*-2-ENAL AND n-OCT-*TRANS*-2-ENAL DURING OXIDATION AT 20°C OF LINOLEIC ACID CATALYZED BY DENATURED HORSERADISH PEROXIDASE AND IN THE PRESENCE OF VARYING AMOUNTS OF n-PROPYL GALLATE.

Symbol	Compound	mmol n-Propyl Gallate / mol Linoleic Acid
●——●	n-hept-*trans*-2-enal	0
○——○	"	1.6
■——■	"	8.0
●- - - - - ●	n-oct-*trans*-2-enal	0
○- - - - - ○	"	1.6
■- - - - - ■	"	8.0

and various catalysts such as hemoglobin, hemin or ferrous sulfate, have been used. The main difference between these methods and the procedure we describe is that we use a closed thermostated reaction cell, ensuring no dissolution of atmospheric oxygen into the system during the oxidation and eliminating the influence of temperature variations, since oxygen electrodes are rather temperature sensitive. The method is very rapid and easy to perform. The reproducibility may, however, be unsatisfactory owing to the instability of the linoleic acid emulsion against autoxidation. This was discussed by Yagi (1970) and Hamilton and Tappel (1963) and the latter concluded that fresh emulsion should be prepared at 30-min intervals. The oxidative changes of the linoleic acid emulsion is a problem mainly when comparisons between the effect of several antioxidants are to be made. As can be seen in Fig. 3, the

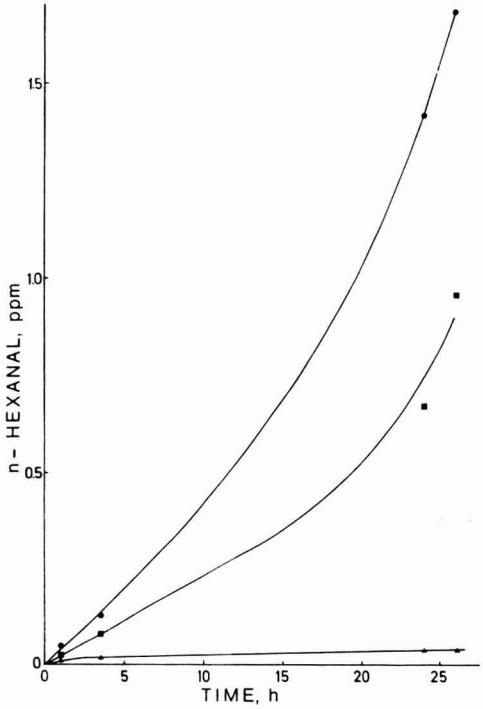

FIG. 8. FORMATION OF n-HEXANAL DURING OXIDATION AT 20°C OF LINOLEIC ACID CATALYZED BY DENATURED HORSERADISH PEROXIDASE AND IN THE PRESENCE OF VARYING AMOUNTS OF BROWNING REACTION PRODUCTS OBTAINED BY REFLUXING 5 MMOL L-ARGININE MONOHYDROCHLORIDE AND 10 MMOL D-XYLOSE IN 5 ML POTASSIUM PHOSPHATE BUFFER (pH 7.0) FOR 5 H.

Symbol	Addition per 400 ml linoleic acid emulsion
●——●	No addition
■——■	100/ul arginine-xylose reaction mixture
▲——▲	250 /ul arginine-xylose reaction mixture

linoleic acid emulsion should preferably have an absorption at 234 nm of about 0.1 or more in this case to minimize the changes in the results during the experimental period. The improved reproducibility is, how-

ever, obtained at the expense of the sensitivity of the method. In order to further reduce the autoxidation of the linoleic acid during the experimental period, the antioxidants to be tested should be mixed with portions of the linoleic acid emulsion at the start of the experiment. A control should be run immediately and the samples containing antioxidant should be kept in the refrigerator until analyzed. When the purpose is to screen substances for antioxidative activity the polarographic method is very suitable. In this case the hydroperoxide content of the linoleic acid emulsion ought to be as low as possible in order to achieve as sensitive measurements as possible.

When using the other two methods, autoxidation of the linoleic acid is no problem since every batch of emulsion is used only once, whereby it is possible to check that the emulsion is of desired quality before starting the test. The spectrophotometric method is very suitable when a large number of samples are to be tested for antioxidative effect. The method was developed mainly for measuring the antioxidative effect of chromatographic fractions when separating food components with antioxidative properties. It also has the advantage of requiring much less antioxidative material than, for example, the polarographic method. From Fig. 1 it can be seen that about 0.25 mmol BHA per mol linoleic acid is required to detect antioxidative effect by the polarographic method, while Table 2 shows that antioxidative effect is measurable at the concentration 0.02 mmol BHA per mol linoleic acid by the spectrophotometric method. Bearing in mind that 2 ml linoleic acid emulsion are used in the spectrophotometric method, while about 6.5 ml are needed in the polarographic method, about 40 times more antioxidative compound is required for the latter method.

In the spectrophotometric method, parameters such as temperature, incubation time, and concentration of linoleic acid in the emulsion can be varied and optimized for a specific purpose. For the sake of convenience we have used 15–20 h (overnight) incubation at 37° C.

The gas chromatographic method is the most time and work consuming method of the three described. The major advantage of the procedure is, however, that the analytical principle basically allows a straightforward comparison of the results with the sensory phenomena caused by lipid oxidation. Moreover, much information is obtained from each analysis.

Owing to the large volume of linoleic acid emulsion used with this technique, considerably more antioxidative material is required for this method as compared with the polarographic and the spectrophotometric method. The method is intended to be used mainly for confirming (by measuring at these late stages of lipoid oxidation as well) anti-

oxidative effect of compounds shown to be antioxidative by one of the previously discussed methods. Furthermore, as the method has shown good reproducibility, it is perhaps the one that is best suited for comparisons between different antioxidants, especially since it has the advantage of allowing the antioxidants to be tested at different occasions.

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THE ISOLATION OF PROTEIN CONCENTRATES FROM PASTURE HERBAGE AND THEIR FRACTIONATION INTO FEED- AND FOOD-GRADE PRODUCTS

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ABSTRACT

The principles of protein extraction from pasture and techniques applied for isolation of unconventional protein- leaf protein concentrates (LPC) from pasture herbage have been described. Comparing various methods used for fractionation of proteins recovered from herbage into food, and/or feed-grade product, advantages of using the ultrafiltration procedure for preparation of protein concentrates (isolates) from pasture were demonstrated. The potential and possible ways of application of the protein extraction system in agricultural practice were also outlined in relation to modern dairy farming operation.

INTRODUCTION

Although green vegetation represents an ample source of good quality protein only a few species, notably leafy vegetables, are at present utilized by man. The main reason for the limited direct consumption of plant material by monogastric organisms is the low protein/fiber ratio and indigestibility of the cellulosic cell walls in green plant leaves. However, since the green vegetation derived from photosynthesis is the primary nondepletable source of food in the world (Phillipson 1973), numerous technologies have been developed over the last 50 years which have made it possible to separate the protein from the accompanying fibrous plant material making the extracted plant proteins available and more usable for direct consumption by man and monogastric animals (Pirie 1971; Olatunbosun *et al.* 1972; Kamalanathan and Devades 1975; Voslosh 1976; Bray 1977; Wilkins *et al.* 1977).

Grasslands, of all crops, have been found to be most outstanding, being high yielding performers in terms of dry matter (DM) and protein production, particularly in temperate and subtropical agricultural

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ecosystems (Ostrowski 1976a, b, 1978b; Vincente-Chandler 1973). Therefore an attempt has been made to use grasslands as a source of protein which could be isolated from green herbage in the form of highly concentrated protein fractions for use in monogastric farm animals' nutrition and utilizing partially deproteinized herbage as a still fully productive fodder for ruminants (Ostrowski 1978a, b).

The principles of protein isolation-extraction from pasture herbage is demonstrated in Fig. 1, indicating alternative stages of mechanical processing of herbage and herbage juice as well as multi-stage conversion of herbage not suitable for direct human and monogastric animal consumption into proteins of food origin representing foods of high nutritional quality.

In practice the processing of pasture is accomplished when the pasture herbage, after harvesting, is mechanically disintegrated in the press (belt or screw type) into green liquor (herbage juice) and fiber residues. Partially deproteinized herbage is then used for feeding cows (Ostrowski 1978b) and herbage juice is further processed in order to recover proteins in protein concentrate form which, being chemically and

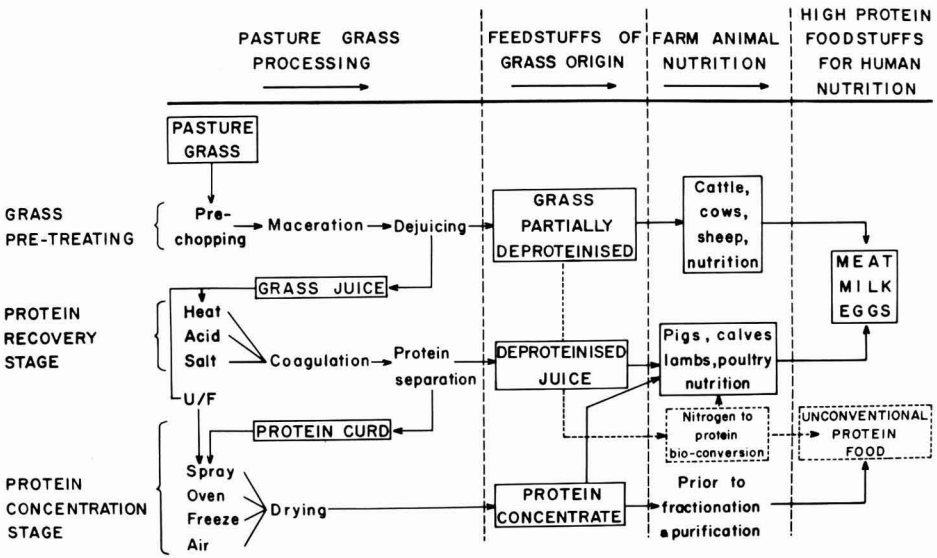

FIG. 1. DIAGRAM OF PROTEIN EXTRACTION FROM GREEN PLANTS USING PASTURE HERBAGE AS AN EXAMPLE

Processing stages and conversion of pasture through feedstuffs into both conventional and/or unconventional edible protein products of food grade, with farm animals and alternatively single cell organisms being pasture nitrogen converters.

biologically equivalent to soybean protein (Ostrowski 1976c), may be used in monogastric farm animal nutrition (Pirie 1971; Ostrowski 1976a).

In general practice, acid and/or heat coagulation is used for the recovery of protein from herbage extract (juice) mainly for use as a feed for poultry. Such a product however, due to its dark green color, bitter taste and strong grassy smell is not acceptable for direct human consumption. Therefore, in order to prepare protein concentrates from herbage which would be more acceptable as a potential novel high protein food supplement, a system of protein extraction from pasture herbage has been developed with alternative separation of protein, the fraction suitable for direct human consumption (Ostrowski 1976a). Within such a system, the chloroplastic material responsible for the green color and much of the grassy flavor is separated by differential heating at 55° C followed by sedimentation, centrifugation or filtration. The remaining soluble proteins (cytoplasmic protein fraction) can be precipitated by heat at 85° C or concentrated without heat precipitation by the use of membrane filtration (Ostrowski 1975). As a result of the protein extraction and fractionation operation, two distinctive protein fractions are separable: the dark green chloroplastic "animal fraction" and the cream-white cytoplasmic "human fraction."

Since protein isolation from pasture herbage followed by various fractionation and purification procedures, as well as biochemical and nutritional evaluations of protein fractions recovered from herbage juice has not yet been reported, a detailed characteristic of the whole protein extraction process from pasture herbage has been described in this paper. Together with the nutritional characteristic of the product isolated from pasture, a potential application of the extraction system to agricultural practice is discussed with particular reference to intensive dairy farming.

MATERIALS AND METHODS

Pasture

A stand of permanent irrigated pasture on a mixture of sandy loam and clay soil types was used as a source of herbage for extraction purposes. Until commencement of the present experiment, the pasture was intensively grazed by a herd of dairy cows. Productive characteristics of the pasture from which herbage has been sampled is shown in Fig. 2, and climatic conditions at which this production was achieved,

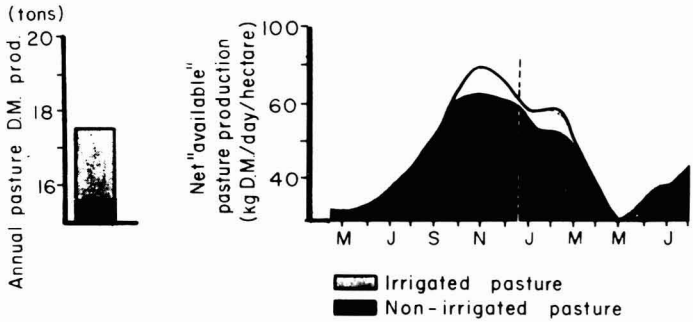

FIG. 2. ANNUAL PRODUCTIVE CHARACTERISTICS OF PERMANENT TOP-DRESSED PASTURE IN AUSTRALIA (VICTORIA) WITH AND WITHOUT IRRIGATION APPLIED DURING THE SUMMER AND AUTUMN SEASONS

as characterized by meteorological observations are summarized in Fig. 3.

Herbage Sampling

A pilot scale operation was performed on two occasions by cutting summer-growing irrigated pasture herbage in its fifth week of regrowth with a forage harvester.

Herbage Processing

Herbage was processed in the Pirie type macerator (Pirie 1971) within 1 h of harvesting.

The juice expressed from herbage in the belt press was filtered by passage through a set of sieves (0.1 mm) and samples for analysis, and/or used for further processing.

Protein Separation From Herbage Juice

Two different scales of juice processing were used for protein separation from the juice: laboratory and pilot-scale extraction procedures.

For evaluation of protein extraction efficiency in the laboratory-scale extraction procedure, juice samples separated from bulk-filtered juices were precipitated at 85°C by steam injection in a glass laboratory precipitation chamber. After cooling by passing the juice through a glass coil immersed in cold water, precipitated coagulum was separated from the supernatant by filtration through a Buchner funnel under vacuum using Whatman No. 54 filter paper. Leaf protein concentrates

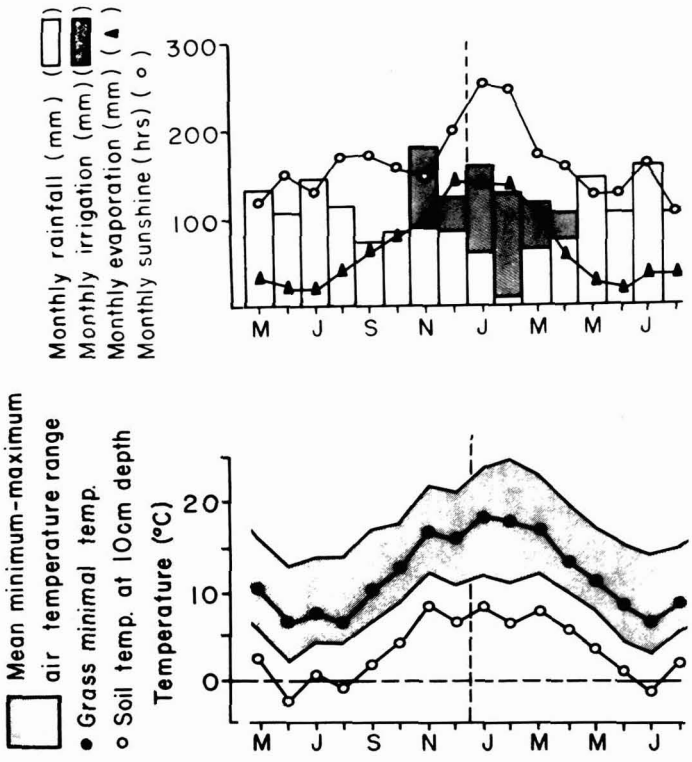

FIG. 3. CLIMATIC CONDITIONS IN WHICH PROTEIN RECOVERY FROM PASTURE HERBAGE HAS BEEN INVESTIGATED

Shadowed area closed within two solid lines indicates the difference between monthly mean highest and lowest recorded air temperature. Black columns built on rainfall figures indicate irrigation (mm).

were obtained by freeze-drying separated protein coagulums, and the yields of dry matter (DM) recovered from herbage were determined. The freeze-dried LPC's were used for further chemical analyses.

In the pilot-scale protein extraction procedure, 2 volumes each of 100 liter juice batches were subdivided into 2 samples; one being processed in a processing line using steam coagulation at 85°C, while the second one was ultrafiltered.

Protein Isolation From Juice Without Fractionation

In the steam coagulation process juice was passed through a precipitation chamber using a steam generator. Juice after precipitation at

85°C was immediately cooled to approximately 18°C in a heat exchanger, followed by separation of the precipitated coagulum by filtration in calico socks.

Using the ultrafiltration procedure for unfractionated protein isolation, juice samples were processed in a laboratory ultrafiltration unit with a nominal 2×10^4 molecular weight cut off DIAFLO membrane. Filtration was carried out at a temperature of 22°C until a 5-fold concentration was obtained in a single UF run. No preservative was used in ultrafiltered juice. Both steam coagulated protein curds and UF concentrates were freeze-dried, and used for further chemical analysis.

Protein Fractionation

To separate the chloroplastic protein fraction (feed-grade) juice was passed through a precipitation chamber using steam with the effective temperature of heated juice 55°C. Filtrate remaining after filtration of coagulum at 55°C was then used for recovery of the cytoplastic protein fraction (food-grade). This was accomplished when 55°C filtrate was passed again through the precipitation chamber with steam heating up to 85°C, followed by the filtration in calico socks.

For isolation of food-grade cytoplasmic fraction by means of ultrafiltration, a Romicon PN-10 hollow fiber cartridge has been used. Filtrate from 55°C heat coagulation procedure was ultrafiltered in a batch system at 40°C with the effective pressure on the membrane 170 KPA and recirculation rate 601 min^{-1}.

All protein concentrates without fractionation and protein fractions isolated from herbage juice in the pilot-scale operation using heat and/or ultrafiltration systems were then used for chemical and nutritional evaluation.

Analytical

Total dry matter (DM) content in herbage and juice was determined after heating in a forced-draught over at 110° for 24 h. Total nitrogen (TN) was determined using the Kjeldahl method as described by the Association of the Official Agricultural Chemists (AOAC 1965). Protein nitrogen was determined by mixing freshly extracted juice with an equal volume of a solution of 20% (w/v) trichloroacetic acid (TCA) and 2% (w/v) silicotungstic acid (STA). The precipitant curd layer was separated by centrifugation for 15 min at 3,000 rpm at 5°C. The TCA/STA precipitants after centrifugation were used for precipitable dry matter determinations and the supernatants for TCA/STA-soluble nitrogen analysis according to the Kjeldahl procedure used for TN

determination. Precipitable nitrogen was calculated from total juice nitrogen subtracting TCA/STA soluble nitrogen.

Ash was determined as the residue remaining after heating in a muffle oven for 18 h at 600°C. Total soluble sugars were analyzed using a procedure with an anthrone indicator. Methionine was determined by the Lorenzo-Andreu (1961) procedure and methionine availability by the method described by Pieniazek *et al.* (1975), and total and available lysine as described by Ostrowski *et al.* (1970). For comparison, lysine availability was measured also using procedures described by Carpenter (1960), Hurrel and Carpenter (1974) and Kakade and Liener (1969). Tryptophan was measured by using the procedure described by Matheson (1974) and tryptophan availability was determined using *Tetrahymena pyryformis* according to Boyne *et al.* (1975). *In vitro* digestibility was determined by a two-stage pepsin-trypsin digestion procedure according to Saunders *et al* (1973). Amino acids were determined using the analytical and sample preparation procedures recommended by Byers (1971a, b) for protein concentrates analysis. Nutritional value of protein in LPC's was determined using rats (AOAC 1965).

RESULTS AND DISCUSSION

Techniques for Separation and Fractionation of Proteins from Herbage Juice

Figure 4 shows average efficiency of pasture herbage processing and balance of protein, ash and sugars as achieved in typical protein extraction operation with heat coagulation at 85°C used for protein extraction without fractionation. In the whole system of protein extraction from pasture herbage one of the factors restricting the efficiency of protein recovery during the extraction process as measured by the yield of protein recovered from herbage juice is the method of protein separation from the juice (Ostrowski 1976a, 1978b). Despite the fact that the most commonly used method to isolate protein from plant extracts is that based on heat and/or acid precipitation (Pirie 1971), such treatments cause the denaturation of proteins which become water insoluble. In this form they are not favored in modern food and feed technology and also may have restricted application to farm practice (e.g. as a milk replacer). Thus, looking at the different methods and techniques for protein recovery from herbage juice, a membrane filtration technique (ultrafiltration) has been chosen as a possible alternative to the precipitation procedure.

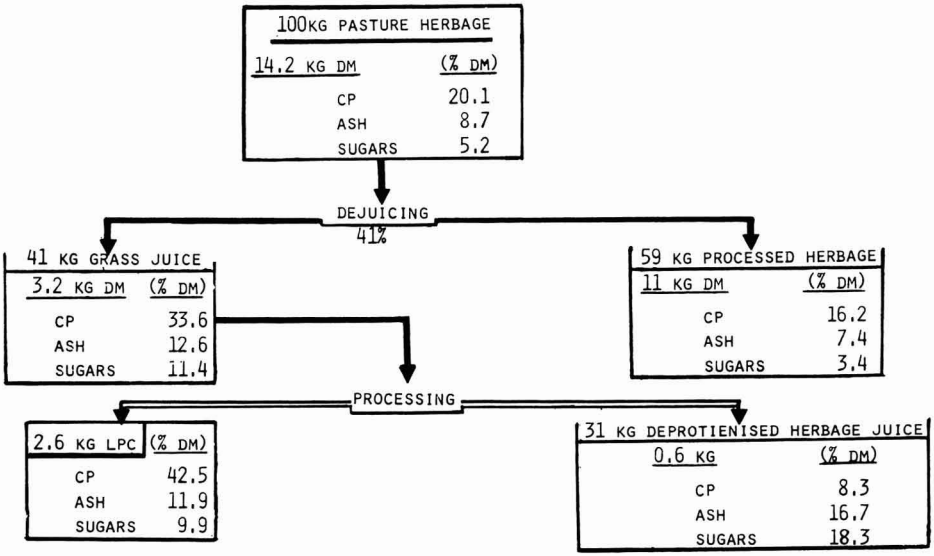

FIG. 4. EFFICIENCY OF PASTURE HERBAGE PROCESSING AS ACHIEVED IN TYPICAL PROTEIN EXTRACTION OPERATION WITH HEAT COAGULATION AT 85°C, AND THE DISTRIBUTION OF DRY MATTER, CRUDE PROTEINS, ASH AND SOLUBLE CARBOHYDRATES BETWEEN THE FRACTIONS OF THE ORIGINAL HERBAGE SUBJECT TO MECHANICAL AND CHEMICAL PROCESSING

In recent years when suitable commercial ultrafiltration membranes appeared on the market, ultrafiltration became one of the cheapest protein isolation and concentration methods (Payne *et al.* 1973). Thus, the ultrafiltration technique has been applied for protein isolation, fractionation and concentration as an alternative method to heat at 55 and 85°C and/or acid protein coagulation in herbage juices processed within the system of protein extraction from pasture (Ostrowski 1975, 1976a).

The ultrafiltration has already been reported by Singh *et al.* (1974), Tragardh (1974), Knuckles *et al.* (1975) and Ostrowski (1975, 1978a), as useful in concentrating proteins in herbage juices by removing 80 to 90% of the water and non-protein components. The final product obtained after drying, protein concentrate, still remains water-soluble.

There are several alternatives which can be applied to herbage juice concentration and fractionation. After ultrafiltration of herbage juice without fractionation the protein concentrate of feed grade, containing a mixture of chloroplastic and cytoplasmic proteins, has a similar chemical composition as heat precipitated (85°C) concentrates (Table 1). When the chloroplastic fraction was removed by heat precipitation

Table 1. Production efficiency and chemical characteristics of LPC obtained from pasture herbage processed alternatively without or with fractionation into concentrates of feed and food grade products using different processing and fractionation techniques

Measurement[1]	Protein Concentrate from Pasture Herbage				
	Heat Precipitation			Membrane Filtration	
		Fraction			
	Without Fractionation	Chloroplastic (55°C)	Cytoplasmic (55/85°C)	Without Fractionation	Cytoplasmic Fraction
Protein concentrate (grade)	Feed	Feed	Food	Feed	Food
LPC's Production Efficiency					
Yield of LPC (kg DM from 100 kg of fresh herbage)	2.6	2.2	0.4	3.3	0.4
Protein nitrogen recovery (recovered protein as percent of the total N × 6.25 in herbage)	38.7	32.1	6.2	44.1	6.6
Protein yield (g per 1 kg herbage DM)	77.8	64.6	12.5	89.7	13.2
Chemical characteristic of LPC (% DM)					
Crude Protein (N × 6.25)	42.5	41.2	57.4	46.8	52.0
Ash	11.9	13.1	5.5	9.6	3.6
Soluble carbohydrates	9.9	11.7	9.1	6.7	6.4
Total essential amino acids (EAA)[2] (g per 100 g recovered)	44.8	44.3	47.4	48.7	49.0

[1] Each value represents mean from eight processed herbage
[2] Cystine excluded

at 55°C, proteins, cytoplasmic fraction, not precipitated at this temperature were recovered by precipitation at 85°C as a water insoluble product or by ultrafiltration, without protein denaturation, as a water soluble protein concentrate (isolates). Different membranes used in the ultrafiltration process result in slightly different efficiency levels (Ostrowski 1976c). Since ultrafiltration of the whole herbage juice without fractionation is a long and high energy consuming process, the most advantageous method of protein separation appeared to be steam coagulation of chloroplastic (feed grade) fraction at 55°C followed by membrane filtration for recovery of cytoplasmic (food grade) proteins (Ostrowski 1976a). Figure 5 presents a schematic diagram of the average separation of protein fractions from juice extracted from one tonne of fresh weight pasture herbage showing approximate recoveries of feed — and food — grade protein fractions as achieved in this study. The diagram also indicates the possible ways of utilizing the deproteinized liquor in farm animal nutrition by its use as a substrate for microbial growth allowing for the production of approximately 14

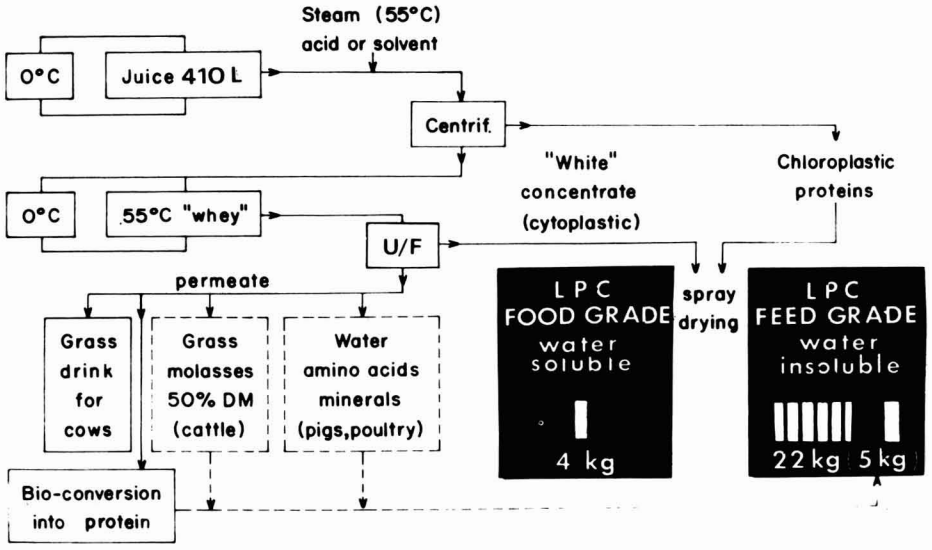

FIG. 5. PROCESSING OF JUICE EXTRACTED FROM PASTURE HERBAGE AND AVERAGE BALANCE OF DRY MATTER AND PROTEIN AS ACHIEVED DURING FRACTIONATION OF HERBAGE PROTEINS INTO CHLOROPLASTIC — FEED GRADE AND CYTOPLASMIC — FOOD GRADE PROTEIN FRACTIONS

Based on processing of juice extracted from one tonne of fresh weight herbage as in Fig. 4.

to 20 g of microbial protein from one liter of deproteinized herbage juice. When not being used the liquor containing minerals and nitrogen compounds is returned to pasture as topdressing.

Protein Fractions as Recovered From Pasture Herbage

In practice there are three major fractions which can be easily separated from herbage juice when the permeate (filtrate) obtained from the whole juice filtration process using the membrane with a nominal 6.8×10^4 MW cutoff level. The largest fraction represents proteins of a molecular weight above 6.8×10^4 (approximately 57–80% of total recoverable proteins) and the smallest fraction being approximately 5% of the total represents proteins of below 6×10^3 MW (Ostrowski unpublished results). In addition, to precipitable proteins the fraction below 6×10^3 MW contains non-precipitable amino-nitrogen compounds in the form of free amino acids, peptides and polypeptides as well as macromolecules formed through condensations of amino-nitrogen. The protein fraction of molecular weight below 6.8×10^4, being approximately 20–25% of the total recoverable protein, is cream-white in color and tasteless after concentration. It is comparable with the cytoplastic fraction which is separated from juice using fractionated steam coagulation.

Chemical and Nutritional Characteristics of Proteins Separated from Herbage Juice

As a result of different methods of protein separation and/or fractionating of proteins extracted from pasture herbage ultrafiltration (UF) appeared to be a superior procedure to heat precipitation (Table 1) from a production efficiency point of view. Protein concentrates obtained by the latter procedure were also inferior to UF from a protein concentration point of view.

Both chloroplastic fraction and unfractionated LPC's in general have similar amino acid characteristics. This was opposed to the cytoplasmic fraction, food grade being superior to LPC feed grade. It has been noted that cytoplasmic protein fraction contains more lysine, histidine and tryptophan and less leucine as compared to chloroplastic protein fractions (Table 2). Methionine and other amino acids were in both fractions at similar levels. However, in total, ultrafiltered protein concentrates contained more essential amino acids than heat precipitated ones.

The biological value of chloroplastic feed-grade protein fraction, included in the standard diet for rats, as measured by the Protein

Table 2. The amino acid composition of LPC's recovered from pasture herbage by either unfractionated heat coagulation (85°C) and membrane filtration or by fractionating with the use of heat (55°C) followed by membrane filtration and comparison with the range of amino acid concentrations in several leaf proteins (Byers 1971) and with FAO (1965) provisional recommendations (g amino acid per 100 g recovered amino acids)

	Protein Concentrate from Pasture Herbage[1]					Reference		
	Heat Precipitation			Membrane Filtration				
		Fraction						
Amino acid	Without Fractionation (85°C)	Chloroplastic (55°C)	Cytoplasmic (55/85°C)	Without Fractionation	Cytoplasmic Fraction	Byers (1971 b) (range)		FAO
Histidine	2.2	2.0	2.6	2.0	2.9	1.8	2.8	-
Isoleucine	4.6	4.5	4.7	4.6	4.6	4.5	5.5	4.2
Leucine	9.5	9.9	9.3	9.9	8.7	8.8	10.2	4.8
Lysine	4.8	4.6	5.7	6.2	6.6	5.6	7.3	4.2
Methionine	1.9	1.8	2.0	2.0	1.9	1.6	2.6	2.2
Phenyloalanine	6.1	6.2	6.0	5.9	5.9	5.5	6.8	2.8
Threonine	4.3	4.0	4.8	5.0	6.3	4.7	5.8	2.8
Tryptophan	1.3	1.3	1.8	2.0	2.1	1.2	2.3	1.4
Tyrosine	3.9	3.8	4.0	4.3	4.1	3.7	4.9	2.8
Valine	6.2	6.2	6.5	6.7	6.3	5.9	6.9	4.2

[1] Each value represents the mean of four determinations

Efficiency Ratio (PER), was substantially lower than that recorded with cytoplasmic food-grade fraction. Supplementation of the diets with lysine, methionine and tryptophan indicated methionine as the first limiting amino acid in cytoplasmic fraction but in the chloroplastic fraction lysine and tryptophan were limiting too (Table 3).

Despite the total lysine "sufficiency" in both LPC's — feed grade, the response of test animals to lysine added, may be an indication of loss in lysine availability during processing which was greater in the fractionated chloroplastic LPC as opposed to the unfractionated LPC. This can be explained by heat processing (Lund 1973) since in the presence of reducing sugars, proteins are degraded via the Maillard reaction, basic amino acids being especially reactive. Even though the heat coagulated LPC's were supplemented with methionine and lysine they had a lower PER value than that observed with casein (2.8). An increase in PER due to tryptophan supplementation may indicate that, due to processing, tryptophan became limiting also in feed grade LPC's. This may be due to the lowering in tryptophan availability. Of all the essential amino acids, lysine and threonine were pointed out by Lund (1973) as the most heat labile, but it was shown by Meredith *et al.* (1974) that during processing histidine, threonine and valine are also subject to an even higher degree of degradation than are methionine and lysine. Wallace (1973) however, indicated that processed protein products tend to be limited by the sulphur amino acids rather than by lysine and so damage to the sulfur amino acids and supplementation with these is generally more significant than damage to lysine and supplementation with lysine. The results presented in Table 4 would indicate that this is so. Fetuga *et al.* (1973) on the other hand, showed that apart from methionine, both lysine and tryptophan are the amino acids in shortest supply in most proteins of plant origin. However, both these amino acids in the analyzed LPC's were above the FAO (1965) amino acid standard.

In general, concentrations of the essential amino acids detected in LPC's, despite the technique used for their production, were in the range of concentrations as reported by Byers (1971b). Byers (1971a) reported, however, that the method of protein separation from juice may influence the amount of lysine in the final product. This influence was also distinctive in LPC's recovered by different methods in reference to lysine.

The total essential amino acids and microbiologically determined availability correspond with the nutritional value of LPC's measured in biological tests. A similar relationship between PER values and amino acid composition of LPC's was shown by Hansen and Eggum (1973),

Table 3. Nutritional characteristics of LPC obtained from pasture herbage processed alternatively without or with fractionation into concentrates of feed and food grade products using different processing and fractionation techniques

Measurement[1]	Protein Concentrate from Pasture Herbage				
	Heat Precipitation			Membrane Filtration	
	Without Fractionation (85°C)	Fraction		Without Fractionation	Cytoplasmic Fraction
		Chloroplastic (55°C)	Cytoplasmic (55/85°C)		
Protein Concentrate (grade)	Feed	Feed	Food	Feed	Food
Protein digestibility (%)					
In vivo	73	71	80	78	82
In vitro	85	78	84	80	93
Availability (%)					
- Lysine	77	73	82	81	86
- Methionine	82	80	81	85	91
- Tryptophan	78	75	80	72	94
Protein Efficiency Ratio (PER)					
LPC supplemented with DL methionine (0.2%)	1.4	1.1	2.0	2.2	2.6
LPC supplemented with DL methionine (0.2%) and L-Lysine (0.5%)	2.2	1.9	2.4	2.7	2.6
LPC supplemented with DL methionine (0.2%), L-Lysine (0.5%) and L-tryptophan (0.3%)	2.7	2.6	2.8	2.6	2.8

[1] Each value represents mean from eight processed herbage
[2] Casein control group PER: 2.8; All groups of rats fed LPC without amino acid supplementation gave negative weight gains

Sikka *et al.* (1975) and between PER and lysine availability by Ostrowski *et al.* (1972). However, because various methods used for lysine availability determination are showing different sensitivities to a particular type of lysine damage during protein concentrate processing (Ostrowski 1978c), it was essential to compare the degree of lysine destruction in protein fractions recovered by different techniques with the use of various analytical methods. Table 4 shows that chemically determined lysine availability showed large variation due to the method being used for availability determination. Variation in availability, however, was greater due to the method of determination within the analyzed protein concentrate rather than due to the technique used for protein recovery.

Both *in vivo* and *in vitro* digestibilities of both LPC's feed grade obtained by different production procedures are in the range of values reported by Byers (1971b), Sabba Rau *et al.* (1969, 1972) and Hartmann *et al.* (1967) to be satisfactory for such a type of product, but lower than those reported by Akeson and Stahmann (1965) and Saunders *et al.* (1973). Digestibilities *in vitro*, however, when determined in various experimental conditions were substantially higher with longer duration of the digestion and/or with higher enzyme concentrations (Table 5).

Irrespective of the type of enzyme and its concentration, 1 or 2 step digestion procedure and the duration of the digestion, the protein digestibilities in ultrafiltered LPC — food grades were similar to those recorded with LPC precipitated at 85°C. Protein digestibilities in ultrafiltered concentrate determined at both lower enzyme concentrations and shorter digestion durations were slightly higher than in heat coagulated LPC.

The results as presented in this paper indicate an enormous potential in protein of both feed- and food-grade production particularly from the grasslands growing in temperate and subtropical climatic conditions (Vincente-Chandler 1973) Whether quantities and quality of the recovered herbage protein can be achieved in real practice, in routine farm operations, is a matter for agricultural engineering involvement in the whole protein extraction process and optimization of the efficiency of the entire extraction system (Brown *et al.* 1975; Spedding 1977) and which has been shown earlier (Ostrowski 1975) depends on many factors limiting the final protein recovery from pasture. Further research is needed in order to develop the whole-flexible system of protein extraction feasible enough to be introduced to dairy farming practice in various agricultural conditions specific to a particular country, state and/or climatic region.

Table 4. Availability of lysine in various protein concentrates recovered from pasture herbage as determined by different chemical procedures

Measurement	Protein Concentrates (grade)					
		Extracted from Herbage				
		Heat Precipitation			U/F[1]	
	Soyabean Meal (feed)	85°C (feed)	55°C (feed)	55/85°C (food)	(A) (feed)	(B) (feed)
Protein (N × 6.25) (% DM)	44.6	42.5	41.2	57.4	46.8	52.0
Total lysine (mg/g)[2]	19.8	20.4	18.9	32.7	29.0	34.3
Lysine availability (%) as measured by method:						
FDNB - reactive lysine: "direct" method (Carpenter 1960)	92	83	78	82	75	88
FDNB - reactive lysine: "difference" method (Ostrowski *et al.* 1970)	93	72	72	77	74	80
TNBS - reactive lysine: "direct" method (Kakade and Liener 1969)	94	79	75	81	79	86
Dye - binding method (Acid Orange 12) (Hurrel and Carpenter 1974)	105	117	122	110	108	103

[1] Ultrafiltration procedure using DIAFLO membrane: (A) without fractionation, (B) Cytoplasmic fraction from juice after 55°C precipitation

[2] As determined by short column chromatography

[3] FDNB, fluorodinitrobenzene; TNBS, Trinitrobenzene sulphonic acid

Table 5. The effect of the type and quantity of enzyme used in the *in vitro* digestion procedures and two digestion length intervals on protein digestibility of heat coagulated and ultrafiltered LPCs - food grade

	One Step Digestion						Two Step Digestion					
	Papain			Pepsin			Pepsin/trypsin			Pepsin/pancreatin		
	% digestibility			% digestibility			% digestibility			% digestibility		
Duration of Digestion	mg	55/85°C	UF	mg	55/85°C	UF	mg	55/85°C	UF	mg	55/85°C	UF
12 h	1	77	80	1	94	95	5/5	95	96	1/4	96	96
	10	93	93	10	95	95	10/5	96	96	2.5/4	97	97
24 h	1	80	85	1	95	95	5/5	96	96	1/4	97	97
	10	97	97	10	95	96	10/5	96	97	2.5/4	97	99

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THE NATIONAL FOOD IRRADIATION PROGRAM CONDUCTED BY THE DEPARTMENT OF THE ARMY

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ABSTRACT

This article reviews the history of the food irradiation program in the USA and gives the status and future plans for research and development in food irradiation as of August 1976.

INTRODUCTION

During the last 200 years we in the United States have learned to cultivate our land so effectively that we reap a surplus of food — enough to feed 40 persons for each one working on production. We have learned to dig for minerals and use them to build everything that imagination and inventiveness can create. We have learned to harness the diversified energy resources to do the work for us. Before the beginning of this century, our technology had already surpassed Europe's technology, and by the end of World War II, our technology was a wonder to the rest of the world. Since then each country exposed to this technological wonder has sought to import and adopt it as fast as technical training and education of their people permitted. As a consequence, the world is changing at a faster rate than ever before.

This fascinating 200-year evolution, spearheaded by man's ingenuity in taming the forces of nature, led us into the nuclear age. This new age changed our outlook. We had been obsessed in conquering nature. We had gained knowledge, but, at the same time, greater destructive capability than ever before. Our chief challenges now were to retain the beauties of nature and to maintain peace throughout the world. We had harnessed the forces of nature. Now we had to preserve our environment and to harness the forces within man himself.

At the beginning man witnessed and feared the destructive power of

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his new knowledge about the forces in the nucleus of the atom. It was the destiny of this nation to be the first to understand nuclear power and to spearhead the course of mankind towards using this power for peaceful purposes.

One of the most important milestones on this route was President Eisenhower's "Atoms for Peace Program," which he proposed in his address to the General Assembly of the United Nations on December 8, 1953.

The dormant energy in the nucleus of the atom could be released to power our tools and heat our houses. Radioisotopes could be used not only in medicine to cure cancer, but also in many scientific investigations where the radioisotopes could show us the position and route of atomic elements so that we could trace them in physical, chemical, and biological processes. Atomic energy could be used to increase production of fertilizers. Radioisotopes used as tracer elements could aid in determining the most effective application of the fertilizers. Nuclear radiation could be used for sterilization of medical supplies. Radiation could be used for genetic transmutations in plant breeding aimed at increasing the quantity and the quality of food. Radiation could also be used to preserve the produced food by cutting down the large food wastage due to insects, parasites, and bacteria. Food could be made available to the hungry people all over the world regardless of the absence of costly refrigeration facilities and mechanized distribution systems. Not only would we reduce hunger, but we would also improve health by reducing or eliminating food-borne diseases. Consumers all over the world would benefit.

This "Atoms for Peace Program" has set an example of a course for mankind to follow in the next 200 years. To maintain peace and to tame the forces within man himself, we have to eliminate hunger and malnutrition for all.

Exploratory investigations during the first half of the century had shown how very effective radiation was in eliminating microbial growth, but the high costs of radiation sources precluded its use for preserving food. The technology evolution had now made preservation by irradiation economically feasible. The early ideas were sometimes molded by dreams of radiation as a panacea for preservation of food. This early optimism was an important driving force through most of the 1950's, and it resulted in tremendous efforts in developing this food preservation technology and in proving its safety. In the United States much of this work was spearheaded by U.S. Army food research laboratories and the Office of the Surgeon General of the Army. In the following discussion we will review the Army's efforts in this great

undertaking through our early short- and long-term studies, problems and achievements, cooperative work with other agencies, technological advances, current studies, and scheduled plans for the ultimate attainment of our goals.

Short-Term Toxicological Studies

By 1942 the U.S. Army was supporting some exploratory work in food irradiation at the Massachusetts Institute of Technology. In 1948, the Army Medical Nutrition Laboratory, Denver, Colorado, initiated toxicity studies of irradiated foods (Kraybill 1955). By 1955, the Army systematically had carried out short-term (usually 8 to 12 weeks) toxicity studies of 45 foods. In no case were any toxic effects found (Progress Report on Atomic Energy Research 1956). These short-term animal feeding studies were followed by short-term feeding studies using human test subjects. Subsequently, many more food items were tested in short-term animal feeding studies.

The 54 food items listed in Table 1 were tested in the years 1955 and 1959 in 7 short-term animal feeding studies using human volunteers (Progress Report on Atomic Energy Research 1956; Plough *et al.* 1960; Kraybill 1958). In these tests the caloric intake of irradiated foods was 32 to 100% for a period of 15 days. A thorough medical examination of the individuals was made, and clinical procedure was followed to check many of the measurable physiological and biochemical indicators of these individuals; in no case were there any indications whatever of toxic effects due to consumption of irradiated foods.

Long-Term Toxicological Studies

In 1956, following the completion of short-term studies on many of the food items, The Surgeon General initiated a systematic, long-term, animal feeding study program to check the toxicity and the nutritional quality of 22 representative irradiated foods (Read 1960; Reber *et al.* 1966). The protocols were made by consulting extensively with a great many specialists from the National Research Council and Food and Drug Administration. These protocols conformed to the best standards of that time. The 22 foods fed to rats, dogs, mice, and monkeys for 2 years to determine their possible chronic toxicological effects, carcinogenicity, and nutritional adequacy are listed in Table 2.

The many long-term animal feeding studies were of unparalleled scope and magnitude. Never before had any food processing method been tested so thoroughly.

Table 1. Fifty-four foods tested on humans in short-term toxicological studies

Meat Items	Fish Items	Vegetable Items	Fruit Items	Cereal Product Items	Miscellaneous Items
Bacon	Cod	Asparagus	Dried apricots	Bread	Dessert powder
Corned beef	Haddock	Green beans	Cherries	Crackers	Powdered whole milk
Ground beef	Salmon	Lima beans	Dried fruit compote	Cereal bar	Peanut butter
Beef steak	Shrimp	Beets	Melon balls	Flour	Pineapple jam
Chicken	Tuna	Brussel sprouts	Oranges	Macaroni	Strawberry jam
Chicken stew		Cabbage	Orange juice	Nut roll	Sugar
Frankfurters		Carrots	Peaches	Pound cake	
Ground ham		Cauliflower	Dried pears	Rice	
Ham steak		Celery	Strawberries		
Ground pork		Cole slaw			
Sausage		Corn			
		Mushrooms			
		Peas			
		Sweet potatoes			
		White potatoes			

Table 2. Twenty-two foods tested in the long-term toxicological studies during 1956-1959 period

Ground beef	Chicken	Corn
Pork loin	Chicken stew	Flour
Bacon	Cabbage	Fruit compote
Beef stew	Carrots	Pineapple jam
Tuna	Green beans	Peaches
Shrimp	White potatoes	Oranges
Codfish	Sweet potatoes	Evaporated milk
		Dried eggs

Contraction of the Army Program in 1959

Concurrently with the long-term animal feeding studies, the Army made plans to erect a food-irradiation pilot plant. Inevitably, problems unrelated to toxicity of irradiated foods were encountered in the animal feeding studies, and these problems often could not be explained without further testing. Therefore, on October 22, 1959, the Director of Research and Development, Department of the Army, suspended the erection of the planned food-irradiation pilot plant in Stockton, California (National Food Irradiation Research Program 1959). Most people assumed this decision indicated that irradiated foods were not safe. This misunderstanding sent a shock wave around the world, impeding or halting food irradiation research everywhere (Nucleonics 1959). Although Kraybill made it clear (Kraybill 1960) that there was no evidence of any toxicity in irradiated foods, the impact of the fiscal decisions already made could not be reversed.

The Completion of the Long-Term Toxicological Studies in 1965. The Food Irradiation Process is Found to be Safe

The Office of The Surgeon General of the Army, who had responsibility for the long-term toxicity testing, continued the animal feeding testing to clarify and resolve the problems. These tests, which continued through 1964, confirmed that the problems encountered were due to our deficient knowledge about nutrition in general and were unrelated to the irradiation processing of the food. In 1965, after completing the evaluation of the many long-term toxicological studies of the 22 representative food items, The Surgeon General's scientists concluded that:

"food irradiated up to absorbed doses of 5.6 megarads with Co-60 source of gamma radiation or with electrons with energies up to 10

million electron volts have been found to be wholesome; i.e., safe, and nutritionally adequate," (Radiation Processing of Foods 1965).

Clearly the Office of the Surgeon General of the Army concluded that the 22 food items tested were representative of all foods, that the great many tests conducted proved the safety of foods preserved by ionizing radiation, and that the food irradiation process consequently, should get a broad or general clearance. That such broad clearance was a consequence of negative finding of toxicity in all the 22 tested foods was in accordance with views of the many scientists who had planned the entire approach to resolving the question of wholesomeness of irradiated foods, including the scientists of the Food and Drug Administration (FDA) (Commissioner of Food and Drugs 1960).

Participation by the Atomic Energy Commission and Clearances by the Food and Drug Administration of Individual Items 1960-1968

The Army had spearheaded the National Food Irradiation Research Program through the 1950's. In January, 1960, after the contraction of the Army Program in the fall of 1959, the Atomic Energy Commission (AEC) initiated a food irradiation research program with emphasis on low dose application. The Army program then became more concerned with high dose applications, especially radappertization (sterilization) of meats, poultry, and fish.

While the toxicological questions were considered resolved, the Army and the AEC continued to support research and development aimed at improving the irradiation engineering and the quality of irradiated foods with regard to taste, color, stability, microbiological safety, and packaging. The Army also petitioned the FDA for clearance of bacon, which packed under vacuum was fairly stable in storage and acceptable. The clearance was granted on February 8, 1963. FDA further granted clearance for irradiated wheat and wheat products on August 21, 1963, irradiated for insect disinfestation; for irradiated white potatoes on June 30, 1964, irradiated for sprout inhibition; and for irradiated packaging materials in the years 1964 to 1967 to be used in contact with foods.

The Petition to FDA for Clearance of Ham

The Army petitioned FDA on August 15, 1966 for clearance of ham packaged under vacuum and radappertized at room temperature. This ham was stable under storage at room temperature and highly accep-

table. In January, 1966, FDA raised questions about the validity of clearing ham based on the data obtained in the studies with bacon and pork, and FDA was also concerned about the loss of thiamine in irradiated pork. In March, 1967, the National Academy of Sciences-National Research Council Committee on Radiation Preservation of Food and The Army Surgeon General's Advisory Committee on Nutrition reviewed the questions posed by FDA. The Committees endorsed as valid the interpolation of pork and bacon data to ham. The Committees maintained that man could get sufficient thiamine from other dietary sources to meet the daily requirements. In April, 1968, the FDA nevertheless turned the petition down on the grounds that the data submitted were insufficient to prove safety. This denial of approval of irradiated ham resulted in great reduction of research and development of irradiated foods throughout the world. Many proponents of food irradiation became frustrated and stopped promoting the R and D efforts. In discussion with FDA officials, it became clear that new wholesomeness studies were mandatory to satisfy current FDA requirements (Status of the Food Irradiation Program 1968).

Renewed Long-Term Toxicological Studies on Beef

A new protocol for animal feeding studies on ham was then designed by the scientists at Natick Research and Development Command and at the Office of The Surgeon General in close cooperation with FDA's scientists. Meanwhile, the food technology research and development at Natick had resulted in good quality radappertized beef which was highly stable in storage when blanched at 343°K (70°C), vacuum packed, and irradiated at 243°K (-30°C). Beef, which is the most important meat item in the American diet, was then selected (rather than ham) to be the first meat for testing in the renewed wholesomeness studies. Beef also had the advantage over ham that it did not contain any nitrites or nitrates, which by then had become suspect. The new animal feeding studies were very extensive. It was considered reasonable to be cautious and to start with only one meat item. The contract for the animal feeding studies of beef was awarded in March, 1971, to a private contractor, who started the tests with the first generation of animals in the fall of 1971.

The animal feeding studies were designed to compare any measureable biological indicator of experimental animals fed irradiated beef against the same indicators of control animals fed nonirradiated beef. The animals fed irradiated beef were divided into 2 groups, 1 group fed gamma ray irradiated beef and another group fed electron irradiated

beef. The control animals were likewise divided into 2 groups, 1 fed heat-sterilized beef, the other group fed beef stored frozen. The two groups fed irradiated beef can be considered equivalent because the gamma rays generate the fast electrons, which in turn are responsible for practically all the ionizations and excitations. In both cases, therefore, we are dealing with electron irradiated beef. In one case the electrons are generated by gamma rays, and in the other case the electrons are generated by accelerators. Also, the two control groups can be considered equivalent, because both are assumed to be inert from a toxicological point of view. Some scientists do prefer, however, to consider the frozen beef as the proper control, because heat destroys some of the nutrients, while others would actually prefer the heat-sterilized beef as the control, because heat may inactivate some toxic compounds possibly present in the beef, and also because radappertization is intended to replace heat-sterilized beef rather than frozen, stored beef. To satisfy each and every scientist, the studies were designed with the four separate groups. These animal feeding studies were completed on December 31, 1976. Details of these studies have been previously described (Raica and Baker 1972, Johnson *et al.* 1974; Baker 1976).

Technological Improvements

Concurrent with the animal feeding studies, the food technology research and development at Natick improved the texture, color acceptance, and storage stability of many other food items. These improvements have been obtained by inactivating proteolytic and autolytic enzymes in the meats at 343 to 348°K (70 to 75°C) prior to irradiation. This preirradiation treatment resulted in less off-flavors and provided increased storage stability. The packaging under vacuum resulted in reduction of oxidation, rancidity, and peroxides. Irradiation of the meat in the frozen state reduced the radiation chemical processes occurring in the water in the meat (approximately 2/3 by weight). This factor is especially important for some of the water soluble micronutrients like vitamin B_1 which acts as a scavenger for the radicals produced in the water and is thereby destroyed. When the meat is irradiated at room temperature, the B_1 vitamin is destroyed to the same extent as in the heat sterilization process. On the other hand, when irradiated in the frozen state, the vitamin destruction is small. Since the withdrawal of the ham petition in 1968, the research in the radiation chemistry of the proteins, the lipids, the carbohydrates, and the vitamins has continued and has been well elucidated by use of pulse radiolysis techniques, modern electrophoretic techniques, fractionation of

volatiles, gas chromatography, and liquid chromatography followed by mass spectrometric analysis. The conventional electron spin resonance (ESR) techniques have been used to study the fate of radicals. These studies will soon be supplemented by fast ESR techniques. The principal value of these chemical studies is for extrapolating and interpolating data obtained in the animal feeding studies and for investigating irradiation effects as a function of temperature and dose.

The radappertizing (sterilizing) irradiation dose for meats irradiated at 243°K (-30°C) is found to be around 40 kJ/kg (= 4 million rad) for non-cured meats and around 30 kJ/kg (= 3 million rad) for cured meats. A radappertizing dose is the irradiation dose that will reduce the initial number N of *C. botulinum* spores to a number $N \cdot 10^{-12}$.

The radiation source technology and the irradiation technology have also improved considerably since the 1960's, due to the greatly increased irradiation processing in other branches of industry: in the medical industry for sterilizing of medical products; in the cable industry for producing shrinkable films and for polymerizing the plastic in monomer impregnated wood for greater hardness and durability; in the auto industry for curing coatings and paints; and in the garment industry for grafting monomers to fibers for crosslinking and for polymerizing monomers.

Renewed Long-Term Toxicological Studies on Chicken, Pork, and Ham

In 1975, after a thorough review of the progress made, the Assistant Secretary of the Army for Research and Development directed an accelerated program to assess wholesomeness concurrently for three additional radappertized meat items: chicken, pork, and ham. The research protocols for the animal feeding study portion of the wholesomeness studies of these three items have been made by the scientists within the Army in consultation with a great many experts in the United States and abroad, including those with the National Research Council Committees and with the Food and Drug Administration. The contracts for these studies were awarded June 1, 1976 and were monitored by The Army Surgeon General's Office. The meats for the first year consumption by the animals have been processed by industry and irradiated at Natick. As in the case of the beef studies, research on food technology, microbiology, radiation chemistry, induced activity, and irradiation processing as well as the overall direction of the food irradiation program are the responsibilities of U.S. Army Natick Research and Development Command.

The flow diagram showing the major operations and milestones as a function of time are shown in Fig. 1A to 1D.

While such flow diagrams give a good overview and serve well as a management tool, perturbation of plans involving animal studies of this magnitude are rather common. Having learned from the difficulties encountered in the beef studies, we are hopeful, however, that we will be able to progress on schedule.

Future Petitions to FDA

The petition to FDA for approval of irradiated beef was submitted in the fall of 1977. We hope to submit petitions to FDA for clearance of chicken, pork and ham in 1981. The petitions will be in six volumes; each volume will be written by specialists in the subjects and reviewed by a great many experts. The table of contents for the beef petition is shown in Table 3, "Animal Feeding Studies." Volume V in the Table will be the main part and contain most of the supporting data for the petition to FDA.

Based on our present data, we believe it likely that we will succeed in proving to FDA that enzyme-inactivated radappertized beef, chicken, pork, and ham are wholesome. We are hopeful that we will also succeed in expanding FDA approvals of the above items to cover a broad spectrum of foods. The petitions for expansions of the approvals will be supported by numerous other feeding studies sponsored by the U.S. Army in the 1950's and the 1960's, by AEC in the 1960's, as well as studies done in many other countries (for instance, Japan, England, the Netherlands, Canada, and India) and by the International Project in the Field of Food Irradiation in Karlsruhe, Germany. We are also hopeful that gradually the world will come to recognize that the food irradiation program, when properly used, will significantly help reduce suffering from hunger, malnutrition, and food-borne diseases, and that the process in no way impairs nutrition of the food nor compromises safety in its consumption. As a consequence, throughout the world there will be fewer people hungry and fewer people suffering from food-borne diseases over the next 200 years.

Table 3. Table of contents for the beef petition

Vol. I. Irradiation Technology
- A. Nature of Radiation to be Used
- B. Irradiation Facilities
- C. Irradiation Dosimetry
- D. Induced Radioactivity
- E. Irradiation Control

Vol. II. Processing and Packaging Technology
- A. Processing and Handling of Beef
- B. Other Beef Formulations
- C. Packaging
- D. Proposed Regulations

Vol. III. Microbiology
- A. Microflora in Beef
- B. Radiation Effect on Vegetative Bacteria
- C. Radiation Effect on Spore Forming Bacteria
- D. Radiation Effect on *C. botulinum*
- E. Radiation Effect on Virus and Rickettsia
- F. Effect on Enzyme Inactivation
- G. Effect of Irradiation Temperature
- H. Sanitary Microbiological Standards

Vol. IV. Radiation Chemistry of Food
- A. Basic Concepts
- B. Proteins
- C. Fats and Lipids
- D. Carbohydrates
- E. Vitamins
- F. Minerals
- G. Free Radicals; "Stored Energy"
- H. Review of Radiation Chemistry

Vol. V. Animal Feeding Studies
- A. Toxicity and Carcinogenicity of the Total Diet
- B. Mutagenicity
- C. Teratogenicity
- D. Antinutritional
- E. Protein Efficiency
- F. Toxicity and Carcinogenicity of the Radiolytic Products
- G. Review of all Related Animal Feeding Studies

Vol. VI. Proposed Regulations and Controls for Irradiation of Beef and Distribution of Beef
- A. Licensing Control and Operation Control of Food Irradiation Facility
- B. Food Processing Controls and Labeling Requirements
- C. Sanitary and Microbiological Standards and Handling of the Food During Processing

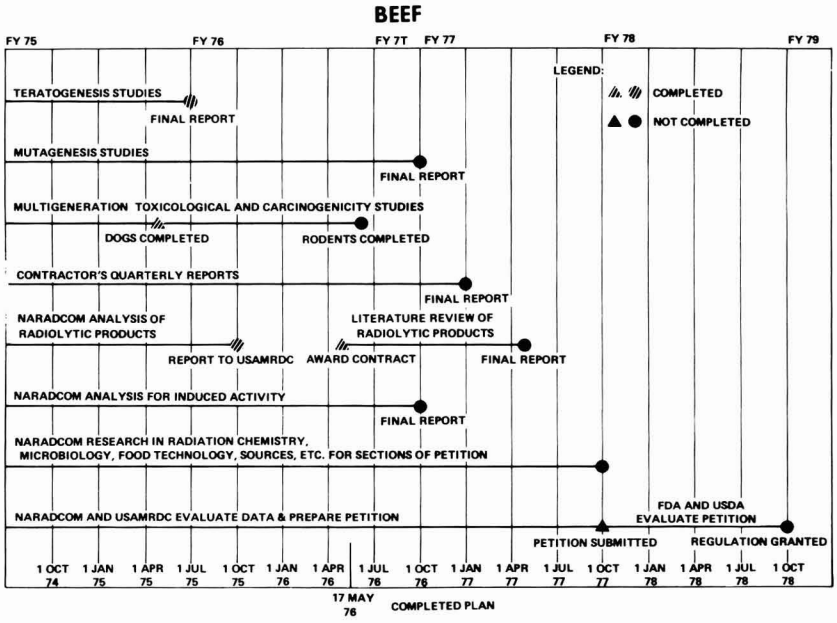

FIG. 1A. FLOW-DIAGRAM FOR THE WHOLESOMENESS STUDIES ON RADAPPERTIZED (47 TO 71 kJ/kg) BEEF AND FOR PETITIONING FDA FOR APPROVAL

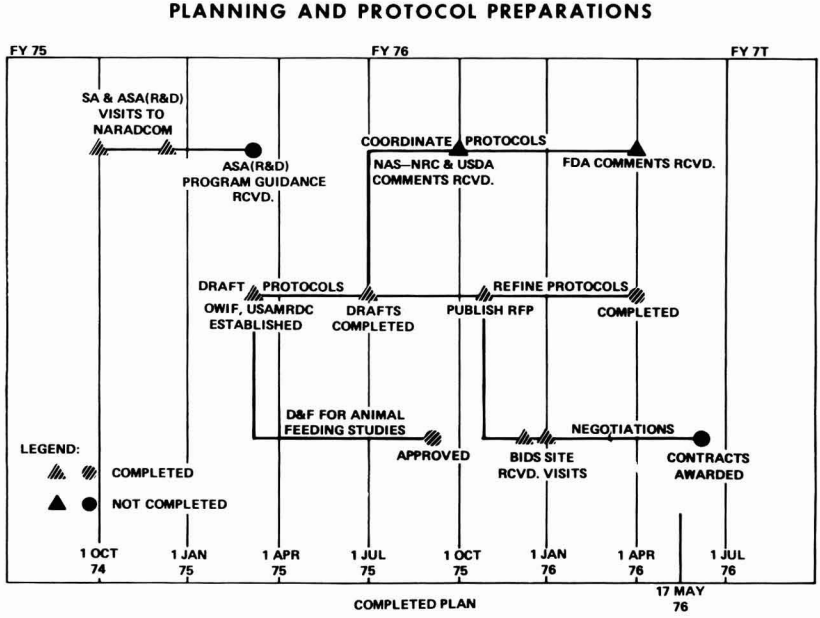

FIG. 1B FLOW-DIAGRAM FOR PREPARATION OF THE PROTOCOLS FOR THE ANIMAL FEEDING STUDIES OF RADAPPERTIZED HAM, PORK, AND CHICKEN AND CONTRACTING THE RESEARCH TO A PRIVATE CONTRACTOR

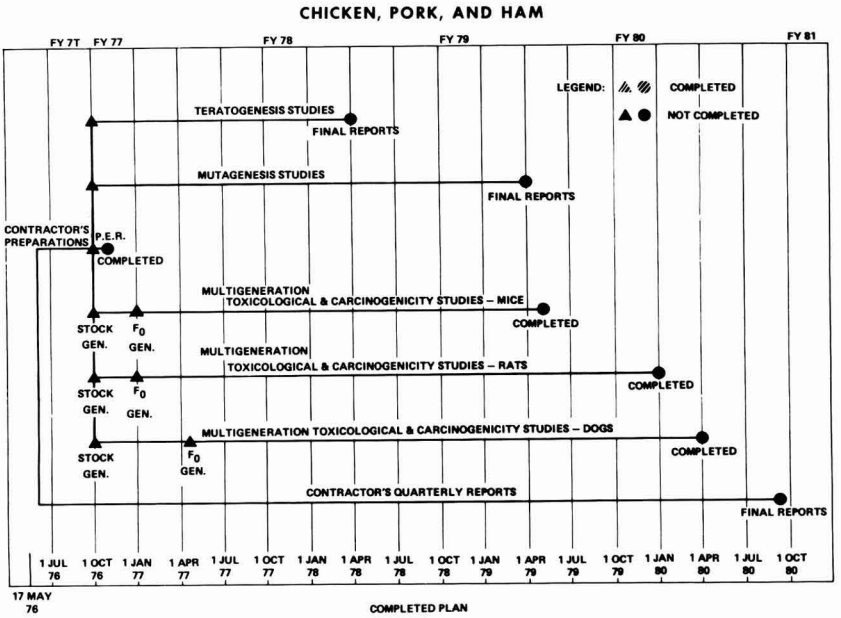

FIG. 1C FLOW-DIAGRAM FOR THE ANIMAL FEEDING STUDIES ON HAM, PORK, AND CHICKEN DONE BY CONTRACTORS

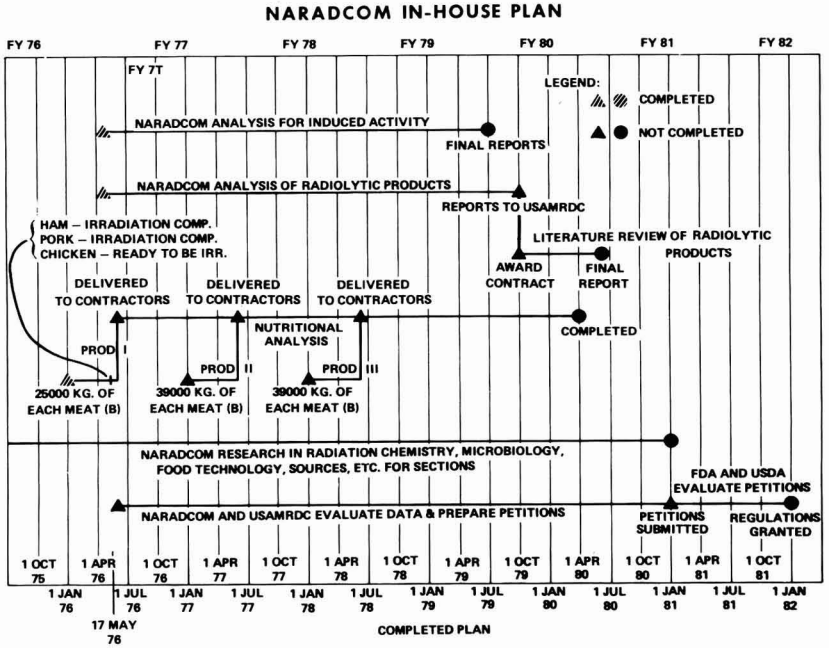

FIG. 1D FLOW-DIAGRAM FOR THE RESEARCH AT NATICK RESEARCH AND DEVELOPMENT COMMAND (NARADCOM) RELEVANT TO THE WHOLESOMENESS STUDIES OF RADAPPERTIZED HAM, PORK AND CHICKEN

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THE ACRIDITY FACTOR IN TARO PROCESSING[1] [2]

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ABSTRACT

Taro (Colocasia esculenta *L. Schott*), *a tropical, edible aroid, contains an irritant preventing its corm from being eaten raw or incompletely cooked. The irritation is thought to be associated with needles of calcium oxalate called raphides which are released from special cells called idioblasts. The irritation is removed by prolonged baking or boiling. To find a suitable processing method to eliminate the acridity and to make the product stable toward microbial and chemical degradation, some of the properties of the acridity factor were investigated. Results suggest that the acridity of taro is not caused solely by the calcium oxalte crystals, but is also associated with a boiling water and ethanol labile factor. Separation of the idioblasts reduces the acridity. Extraction with ethanol either removes the factor or renders the cell's ability to deliver the factor useless. The rat appears to be a useful animal in differentiating the acridity in taro roots.*

INTRODUCTION

Taro, (*Colocasia esculenta* L. Schott) is an edible aroid which is grown widely as a subsistence crop. It thrives in warm humid conditions where other crops are unsuited. Historically, various forms of taro flour have been marketed, but have never been able to compete with grains. The absence of a simple method to render the products stable to spoilage is a main reason for the lack of development of the roots as an item of commerce.

Taro contains an irritant which prevents eating of the raw corms. The irritant is thought to be associated with special cells called idioblasts

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which contain needles of calcium oxalate called raphides (Sakai *et al.* 1974). These raphides are released from the cells when disturbed, and are thought by some to be the cause of irritation (Black 1918). Others (Osisiogu *et al.* 1974; Suzuki 1975) have recently claimed that the irritant resulted from the presence of a factor which Suzuki has reported as a diglycoside of 3,4 dihydroxybenzaldehyde.

Cooking for a long time either by baking or boiling removes the irritant. Such action, however, gelatinizes the starch granules and renders the food subject to microbiological deterioration. The initial transformation of the gelatinized product via a lactic acid type fermentation results in the traditional poi, a taro paste, of the Hawaiians.

The work reported here is an attempt to find methods of processing which will quickly eliminate the acridity of taro and render it stable. In order to accomplish this goal, some of the properties of the acrid factor were investigated.

MATERIALS AND METHODS

Taro corms were obtained from the Honolulu Poi Company and are of the variety commonly called *Lehua maoli.* This variety contains anthocyanins (Chan *et al.* 1975) which gives the cooked corm a pinkish-grey color, hence it is also known as red taro. Another variety, Bun-long commonly called Chinese taro (Whitney *et al.* 1939) was obtained from the Kauai Station of the Hawaii Agricultural Experiment Station. The latter is considered to be less acrid and is referred to as a table taro. Species of *Alocasia cucullata* were also used and obtained from the Lyon Arboretum of the University of Hawaii.

Light microscopy using polarized and unpolarized white light was employed to study the morphology of idioblasts and raphide crystals before and after heat and 95% ethanol treatment. Thin sections of stems from *Alocasia cucullata* were used for heat treatment studies. Sections were studied and then heated for 1.5 h in a boiling water bath. Morphological changes in idioblast and crystal structure were noted.

Taro was fractionated for feeding studies by peeling and grinding in a meat grinder. This ground red taro was then blended in a Waring blender at high speed for 2 to 3 min. Approximately 5 kg of blended taro was mixed with 20 liters of taro water in a cylindrical jar and the top layer of starch suspension was removed every 15 min for 3 h. Starch was collected after centrifuging the pooled collection at 12,000 $\times$ *g* for 5 min and tested for acridity. The sediment also was collected by centrifugation and 300-400 g of it immersed for 2 h in 95% ethanol at

room temperature. The ethanol treated fractions were then centrifuged, collected and treated with hot air to evaporate the ethanol. All fractions were freeze dried to dryness. The acridity was qualitatively assessed by 3 individuals by tasting and indicated by an intensity scale from 0 to 3.

The acceptability and nutritive properties were assessed by a short-term rat feeding study. Male, weanling, Sprague-Dawley rats were fed a semi-purified complete diet containing 50% of either raw dried taro, taro starch, a water washed dried precipitate of taro after starch removal, or a water washed, ethanol extracted, dried precipitate. A 50% corn starch dietary group was used as a comparative control. Eight animals per dietary treatment were fed the various diets ad libitum for 21 days. The diet composition is shown in Table 1. All diets were mixed using a Hobart mixer for 10 min.

RESULTS AND DISCUSSION

After boiling, idioblasts from stems of *Alocasia cucullata* showed no morphological alterations. The raphide crystal appeared unchanged except for the disappearance of a slightly enlarged, bubble-like structure located in the middle of the cell.

Investigations of the raphide crystal and idioblast structures from red taro corms before and after boiling and ethanol treatment indicated that the only morphological change associated with each treatment was the loss of the bubble-like structure in the middle of the cells. However, both treatments destroyed the acridity of red taro. In fact, the starch-free fraction of ground taro (fraction 2) was found to lose its acridity in less than 5 min when exposed to boiling water. (No time studies were done for ethanol treatment). The acridity appears to be independent of

Table 1. Experimental diets

Ingredients	Percent
Casein (vitamin-free)	22.0
Corn Oil	5.0
Minerals (P&H)	4.0
Vitamins (NBC)	2.0
Sucrose	12.0
Fiber (Non-nutritive)	5.0
Taro or taro fractions	50.0

the calcium content as indicated in Table 2. These findings suggest that the acridity of taro is not caused by the calcium oxalate crystals alone, but there is also a boiling water and ethanol labile factor associated with acridity.

As seen in Table 3, rats fed taro starch had similar mean weight gains as the control, cornstarch fed group. Feeding raw taro, or taro cooked in a microwave oven for 1-1/2 min similarly depressed both weight gains and food intake. A further, significant depression was observed in the rats fed the water-washed precipitate fraction of taro. This depression was reversed when the taro precipitate was extracted with ethanol. There is a distinct aversion, but no grossly observable toxic

Table 2. Acridity and calcium content in various taro fractions or extracts

Fraction	Degree of Acridity[1]	Calcium content[2]
Blended taro with starch	++	.06%
Taro with starch extracted but untreated	+++	.16%
Starch extract	0	.03%
Sediment after EtOH treatment	0	.20%
Sediment after boiling (1.5 h)	0	—

[1] 0 = no acridity
+ = some acridity
++ = definitely acridic
+++ = extremely acridic

[2] Comparison of Chinese (Bun-long) taro to red taro indicated that the former contains less calcium (.05%). The percentage of calcium in red taro is .06%. Also the number of raphides in Chinese taro was seen to be much less than in red taro. A quantitative assessment is presently in progress

Table 3. Weight gains and food intake of weanling, male rats fed diets containing 50% taro or specific fractions of taro for 21 days.

Diets	Number of Animals	Weight Gain[2]	Food Intake[2]
Corn starch	8	144 ± 8^{a}	372 ± 18
Taro starch	8	138 ± 12^{a}	341 ± 26
Taro (raw, dried)	8	121 ± 10^{b}	331 ± 27
Taro (microwave cooked 1-1/2 min)	8	122 ± 12^{b}	334 ± 13
Taro precipitate[1]	8	103 ± 20^{c}	303 ± 19
Taro precipitate—extracted with EtOH	8	135 ± 17^{ab}	343 ± 19

[1] Residue after starch is harvested from water mixture.
[2] Mean ± S.D.; common superscript letters indicate non-significance ($P \geq 0.05$).

response, to consuming raw taro and especially to the taro precipitate fraction. This dietary repugnance is reversed when ethanol is used to extract or neutralize the causative factor(s). Upon gross autopsy, no abnormalities were observed, including no inflammatory edema of the tongue and mouth. Edema and inflammatory exudates have been seen in tongues of rats dosed with the juice of the highly irritating, oxalic acid containing ornamental plant *Dieffenbachia* (Fochtman *et al.* 1969).

It is well-known that Bun-long taro is not as acridic as red taro. During the course of these investigations it became apparent that the former also does not contain as many idioblasts as red taro. An investigation to quantitate the number of idioblasts per unit area in various taros is presently being done. A further observation is that when a rat had a choice between raw, red taro and less acrid Bun-long (Chinese) taro, he would choose to eat the Chinese taro. Thus, the rat appears to be a discriminating animal model in taro feeding studies. The comparison with the Chinese variety is instructive in that it suggests that naturally occurring variations in acridity may be found. This further suggests that perhaps completely non-acrid types may be developed. From a processing point of view, removal of these cells will accomplish the same thing. Present work is directed toward finding simple methods of removing the cells or else destroying the factor *in situ*.

CONCLUSIONS

Studies indicate that the acridity factor in taro is associated with the cells containing the raphides. However, it is not clear whether the needles themselves are the acridity factor. Nonetheless, it is apparent that acridity can be reduced by separation of the cells. Cooking will usually remove the acridity, but the fact that cooking for several hours does not destroy the raphides tends to suggest another factor. The action of alcohol may be envisaged as either extracting the factor or rendering the cell's ability to deliver the factor useless.

The rat appears to be a useful animal in differentiating the acridity of taro products.

ACKNOWLEDGMENT

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DOSIMETRY IN SUPPORT OF WHOLESOMENESS STUDIES

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ABSTRACT

Interest in dosimetry procedures in the context of a large-scale processing situation exceeds the purely documentary aspects of this report. The numerous combinations afforded by the various types, strengths and configurations of irradiation sources and the possibilities for various conveyors and other facility design factors impacting on irradiation logistics render a completely general treatment of dosimetry procedures in such instances almost impossible. While the exact combination of these various factors represented by the irradiation facilities at NARADCOM may be duplicated nowhere else, the dosimetry procedures documented in this report offer both experience and solutions that might be more generally useful. Therefore, this report complements and supplements more general discussions found in the literature and cited in the text.

INTRODUCTION

The animal feeding studies represent a crucial part of the effort to prove the safety and wholesomeness of meats preserved by exposure to a sterilizing dose of ionizing radiation (radappertization). Two groups of radappertized test product are required for these studies. One group consists of product irradiated using Co-60 gamma rays, and the other, of product radappertized with electrons from an electron linear accelerator (LINAC). The logistics of producing, storing and feeding require that relatively large quantities of product be irradiated within a short period of time and within prescribed limits of dose and temperature. The task of producing the necessary radappertized product under these conditions provided the opportunity and the necessity to formalize various aspects of the irradiation procedures. This codification

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was especially desirable in order to be able to systematically document the treatment given to the product. The records maintained and the data gathered both during the actual processing and in the preparation phase are an invaluable aid in demonstrating that the product was irradiated within the imposed limits. The contributions that dosimetry made to this effort is the subject of this paper.

The two radiation sources employed in these studies present quite disparate requirements regarding the type of dosimetry data necessary to define the irradiation procedures and to calibrate the irradiation sources. Therefore, the dosimetry procedures employed in obtaining the necessary data to support all phases of the irradiation processing for both the LINAC and the megacurie Co-60 source are most logically discussed separately. To facilitate these discussions, a separate section describing the methodology used in measuring dose has been included.

General Description of Dosimeters

For the work to be discussed in the subsequent section five types of dosimeters were used. Of these, three are classified as chemical dosimeters, one is a film dosimeter, and one is a physical dosimeter.

The chemical dosimeters are all aqueous systems and based on the radiolytic conversion of iron from the ferrous (+2) to ferric (+3) state. The extent of this conversion, i.e., the amount of Fe^{3+} produced, is related to the dose by Equation (1):

$$\text{Dose} = \frac{\Delta[Fe^{+3}]}{G\rho} \times 9.649 \times 10^{6} \text{ Grays} \qquad (1)$$

where:

$\Delta\ Fe^{+3}$ = molar concentration of ferric ions produced

G = number of ferric ions produced per 100 eV of energy absorbed

ρ = solution density

and

9.649×10^{6} = a grouping of various conversion factors.

Spectrophotometric analysis is an accurate and facile method for evaluating the concentration of Fe^{+3} produced. Using the standard

expression relating optical absorbance (A), extinction coefficient (ϵ), path length (L), and concentration (C):

$$A = \epsilon LC \tag{2}$$

the expression for $\Delta[Fe^{+3}]$ becomes:

$$\Delta[Fe^{+3}] = \frac{\Delta A\lambda F_d}{\epsilon_\lambda^{Fe^{+3}} L} \tag{3}$$

where:

ΔA_λ = absorbance at wavelength λ due to irradiation of sample
= A_λ (after irradiation) — A_λ (Before irradiation)

L = optical path length in cm (1 cm unless otherwise noted)

$\epsilon_\lambda^{Fe^{+3}}$ = molar extinction coefficient for Fe^{3+} at wavelength λ in ℓ cm^{-1} $mole^{-1}$ and for the solution composition in which Fe^{3+} is actually measured

F_d = dilution factor
= volume of diluted solution/volume of aliquot of irradiated solution.

The dose equation then becomes:

$$\text{Dose} = \Delta A_\lambda \underbrace{\frac{F_d}{G \cdot \rho \cdot \epsilon_\lambda^{Fe^{+3}}} \times 9.649 \times 10^6}_{K} \text{ Grays}$$

Since the factors in the bracket are experimentally determinable and are constant for a given dosimetry system, they are grouped together with the numerical constant as a conversion factor K.

The equation used in practice then becomes:

$$\text{Dose} = K\Delta A \tag{5}$$

Instead of independently measuring $\epsilon_\lambda^{Fe^{+3}}$ and G for each system, a simplification is made to determine conversion factors for each system. One system is chosen as a standard and used to very accurately

and precisely define the dose in a standard geometry. A second system is then calibrated against the first by direct replacement in the calibrated geometry and irradiated to known doses. The conversion factor is then obtained by using Equation (5). A third system may then be calibrated against the first by direct replacement or the now calibrated second system may be used to calibrate another irradiation geometry whose dose range is more appropriate to the third system. This particular cross calibration method for obtaining the conversion factors is, of course, predicated upon the availability of irradiation sources that make such a procedure feasible. These facilities are available at NARADCOM.

The conversion factor determinations proceeded in the following sequence:

1. Calibration of a 3 ampule irradiation geometry in the NUMEC 340 irradiator using the Fricke dosimeter.
2. Calibration of the regular ferrous-cupric (Reg. Fe/Cu) dosimeter by direct substitution in that geometry.
3. Calibration of an irradiation geometry on the NLAB Irradiator using the Reg. Fe/Cu.
4. Calibration of the extended range ferrous-cupric dosimeter by direct substitution in that geometry.

For the Fricke dosimeter, the G value is taken as 15.6 ± 0.2 (Allen 1961) and $\epsilon_{305}^{Fe^{+3}}$ as 2201 ± 0.4%. This gives a conversion factor of K = 2.74×10^2 Grays/ΔA. The other conversion factors measured relative to this are:

Reg. Fe/Cu K = 6.43×10^3 Grays/ΔA
6x Fe/Cu K = 7.74×10^4 Grays/ΔA

It should be emphasized that these calibration factors are valid only for the dosimeters as prepared and evaluated according to the procedures to be discussed.

Preparation of Dosimetry Solutions and Evaluation After Irradiation

Fricke. The Fricke dosimeter solution is prepared by dissolving 0.278 g $FeSO_4 \cdot 7H_2O$ and 0.057 g NaCl in one liter of 0.8 N H_2SO_4 (22.5 ml of concentrated acid per liter). The irradiated solution is read spectrophotometrically at a wavelength of 305 nm and a tempera-

ture of 298K using quartz cuvettes of 1 cm pathlength. All absorbances are taken relative to a distilled water reference.

Regular Ferrous—Cupric (Reg. Fe/Cu). The Reg. Fe/Cu dosimeter solution is prepared by dissolving 0.278 g $FeSO_4 \cdot 7H_2O$ and 2.50 g $CuSO_4 \cdot 5H_2O$ in one liter of 0.01 N H_2SO_4 which may be prepared by diluting 12.5 ml of 0.8 H_2SO_4 to one liter with distilled water. In practice 12.5 ml of 0.8 N H_2SO_4 is added to a one-liter volumetric flask approximately 2/3 full of distilled water, the inorganic salts added and this solution made up to the one liter mark. The irradiated solution is read spectrophotometrically at 302.5 nm in a 1 cm quartz cuvette and at 298K. All absorbances are taken relative to a distilled water reference.

Extended Range Ferrous—Cupric (6x Fe/Cu). The 6x Fe/Cu dosimeter solution is prepared by dissolving 1.67 g $Fe\,SO_4 \cdot 7H_2O$ and 15.00 g $CuSO_4 \cdot 5H_2O$ in one liter of 0.01 N H_2SO_4. The 0.01 N H_2SO_4 may be prepared as described for the Reg. Fe/Cu dosimeter. The irradiated solution is prepared for reading by taking a 1 ml aliquot and diluting it to 10 ml with 0.8 N H_2SO_4. This solution is read spectrophotometrically at 302.5 nm and at a temperature of 298K using a 1 cm quartz cuvette. Distilled water is used as a reference.

Preparation of Dosimeter Ampules. After preparation, the dosimeter solutions are pipetted into 5 ml glass ampules (NEUTRAGLAS, COLOR BREAK, Kimble #120124). The dosimeter ampules are sealed using an oxygen-gas flame. When the dosimeters are ready for spectrophotometric evaluation, the tops are broken off at the "color break" band and the solution extracted for either dilution or direct transfer to the quartz cuvette.

Water Calorimeter. The water calorimeter, used exclusively for LINAC dosimetry, involves a simple physical measurement — the temperature rise in a mass of water due to the absorption of energy. As seen in Fig. 1 the calorimeter is physically a short cylindrical polystyrene container 14.5 cm in diameter and 2.5 cm high filled with water into which a copper-constantan thermocouple probe is inserted. A watertight seal is provided by a small rubber stopper through which the thermocouple wire is threaded. Thermal insulation is provided by two 30.5 × 30.5 × 5 cm styrofoam blocks in which recesses of appropriate size have been milled to provide a snug fit for the calorimeter dish. The thermocouple junction potential is read using a potentiometer and a thermoelectric ice point reference in conjunction with an electronic galvanometer as the null indicator in a wheatstone bridge arrangement. The thermo-

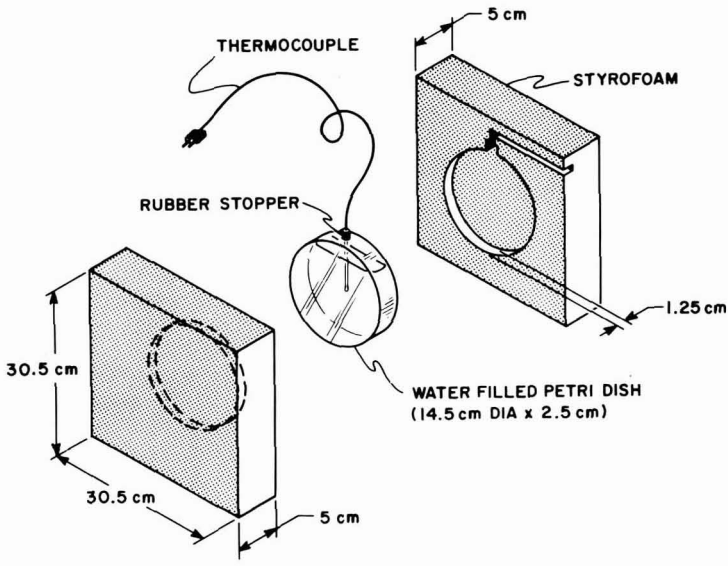

FIG. 1. THE WATER CALORIMETER

Drawing shows the various components of a calorimeter, their dimensions and spatial relationships in the final assembly.

couple reading is proportional to temperature and is directly convertible to it by reference to a table of standard values (Anon. 1973).

The basic dose equation is:

$$\text{Dose(Gray)} = \underbrace{\frac{\sum_{i=1}^{N} m_i c_i}{\sum_{i=1}^{N} m_i}}_{K} \times 4.186 \times 10^3 \, \Delta T \qquad (6)$$

where

m_i = Mass of the i-th calorimeter component, e.g., plastic dish, water, rubber stopper, etc.

c_i = Heat capacity of that component

4.186×10^3 = Conversion of units and physical constants

ΔT = Change in temperature (Kelvin).

Equation (6) reduces to:

$$\text{Dose} = K\Delta T \tag{7}$$

where K is the calorimeter constant and contains all the information pertaining to the calorimeter itself.

The steps involved in running a calorimeter are illustrated in the time-temperature profile of Fig. 2. The calorimeter is preheated to some dose and allowed to cool without disturbance. At some point after a regular cooling curve has been established, a time sequence is started and thermocouple readings are taken every 2 min to a total of 10 readings. The calorimeter is then sent to be irradiated. The time when the front end of the calorimeter is being irradiated is recorded and is later used to calculate the time t_c. After irradiation, a regular agitate-rest-read cycle is initiated and the thermocouple reading taken at 1-min. intervals until at least ten points on the downslope side have been obtained. These data are entered into a computer program which calculates the cooling curves as a linear least squares fit and extrapolates both curves to the common time, t_c, which is the time when the calorimeter had progressed half way along the irradiation path.

The table of standard thermocouple values is then searched and the program, using an interpolation procedure, calculates the extrapolated initial (T_i) and final (T_f) temperatures. From these two values the change in temperature, $\Delta T = T_f - T_i$, is obtained. Using data about the calorimeter already entered, the calorimeter constant, K, is calculated and the dose obtained using Equation (7).

A few comments are appropriate about the calculation of the calorimeter constant. The composition of a typical calorimeter is listed in Table 1.

The weights of the water and the dish are slightly variable. These weights are, of course, measured when the calorimeter is first used and are checked periodically thereafter. The mass and cross sectional area of the thermocouple probe is kept as small as practical. This is important from the standpoint of responsiveness of the probe and heat conduction out of the calorimeter. The rubber stopper is also kept as small as is practical to give a watertight seal. As can be seen from Table 2, if the thermocouple and stopper were neglected in the calculation of K, errors of about 0.03% and 0.06% respectively would be incurred.

This calorimeter is of the type commonly referred to as quasi-adiabatic. (Holm *et al.* 1970). Equation (6) is strictly valid only if the energy absorbed by the i items is transferred to the water as thermal energy and thus evidenced as a temperature rise. What this means in

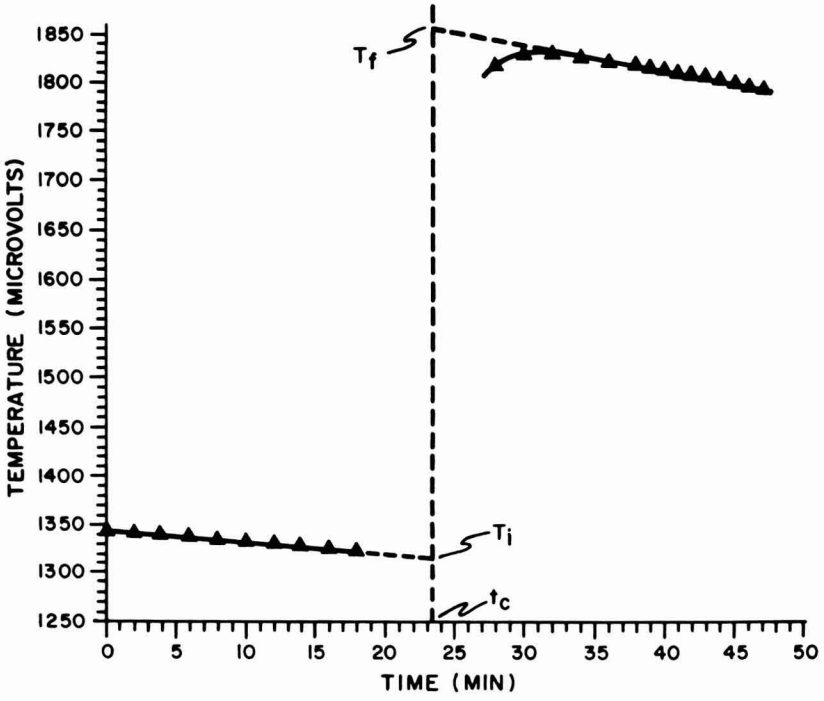

FIG. 2. CALORIMETER TIME-TEMPERATURE PROFILE

Graph demonstrates temperature data collected for a typical calorimeter run. The solid triangles indicate the actual thermocouple readings. The time midway through the irradiation to which the pre- and post-irradiation cooling curves are extrapolated is designated by t_c. The two temperatures obtained by extrapolation are T_i and T_f.

practice is that some portion of the styrofoam insulation must be considered as part of the calorimeter body. As shown in Table 1, this has been estimated at 30 g.

PVC Dosimeter. The PVC dosimeter is a film of heat press polished polyvinylchloride (Bakelite VSA3310) especially procured for use as a dosimeter. Colorless prior to irradiation it turns various hues of green/brown upon irradiation. Irradiated PVC films are evaluated photometrically and the resulting absorbance change correlated with radiation dose. Although irradiated PVC contains several strong optical absorption bands, the one at 395 nm is monitored. A postirradiation treatment consisting of heating the film to 338K for 15 min has been shown to be optimum for developing and stabilizing the irradiation induced color (Jarrett *et al.* 1968).

Table 1. Components of a typical calorimeter

Component	Weight (g)	Specific Heat (cal K^{-1} g^{-1})
(1) Plastic Petri Dish	50	0.32
(2) Water	400	1.0
(3) Thermocouple:		
Copper Wire	0.034	0.093
Constantan Wire	0.036	0.10
Glass Fibre Insulation	0.10	0.2
(4) Rubber Stopper	0.5	0.4
(5) Styrafoam Insulation	30	0.32

Table 2. Contributions of various calorimeter components

N[a]	$K = \sum_{i=1}^{N} m_i c_i \sum_{i=1}^{N} m_i$ [b]	K(N)/(K(N-1)[c]
5	0.8859	1.042
4	0.92357	1.0006
3	0.92415	1.0003
2	0.92444	

[a]N is the number of the component listed in Table 1
[b]The value of K is calculated with N as the maximum value of the index i
[c]The error that would be incurred if successive components were neglected in calculating K

Since PVC films inherently record very fine gradations of dose over areas limited only by the size of the film itself, several types of instrumentation have been devised to extract this detailed information. Both a spectrophotometer and a densitometer have been adapted to read 1.25 cm wide strips of film. A narrow band pass filter is used in the densitometer to monitor the 395 nm peak. From these strips of film and using either of these readout techniques, one dimensional dose variations are obtained. Two dimensional information may be presented in the form of iso-dose curves by utilizing the encoding/isodensitracer feature of the recording densitometer. A more detailed account of these techniques is contained elsewhere (Jarrett *et al.* 1968).

PVC films are used as dosimeters primarily on the LINAC. Consequently, the films are calibrated on the LINAC in conjunction with the water calorimeter. A typical irradiation geometry for such a calibra-

tion consists of placing strips of the film on the face of a calorimeter. Other convenient methods include placing films on the face of a block of Lucite or between two pieces of Lucite.

Production Go/No Go Dosimeters. The handling of large volumes of product through multiple stages and by many people makes it highly probable that some confusion might arise as to whether or not a particular package had been irradiated. To circumvent this problem, dye impregnated PVC dots in the form of self-adhering labels (Avery DETEX) were used. Normally yellow/orange these dots turn red upon irradiation to about 5K Grays and thereby indicate whether or not the package to which they are attached has been irradiated.

Cobalt Disometry Procedures

The cobalt radappertization process, discussed in more detail by MacDonald is of the batch type (MacDonald 1976). A description of the process as far as it impacts on the dosimetry task may be reduced, however, to three identifiable steps:

1. Cans of food packed in boxes are arranged in the predetermined irradiation configuration.
2. The cobalt-60 source is brought into the irradiation position.
3. The product is exposed to the irradiation source for a predetermined time at the conclusion of which, the source is returned to its storage position.

The dose determining factors are:

1. The fixed geometric relationship between sample and source,
2. The exposure time to the irradiation source,
3. The dose the samples receive while the source is in motion between the storage position and the irradiation position,

The dosimetry considerations are thus reduced to two tasks: First, to define an irradiation configuration such that the maximum/minimum total dose ratio is not greater than 1.50 for any of the product within its boundaries (and for any total dose to be delivered); Secondly, having related the dose at any point in such a configuration to the dose at any other point, to relate the dose at several especially chosen points to the dose determining parameter, exposure time.

The selection of an irradiation configuration is primarily dictated by the source homogeneity. The large, 2 plaque, 3 megacurie, Co-60

source used in these irradiations is described by MacDonald. Basic information about the dose homogeneity for any plane between the two source plaques was obtained from an involved computation program using the geometry of the source elements and their curie strengths as the input information. These calculations were made using NARADCOM's UNIVAC 1106 computer. Typical output formulated as a 3-dimensional topographical plot is shown in Fig. 3. Experimental confirmation of these calculations was provided by studies using regular ferrous-cupric (Fe/Cu) and extended range ferrous-cupric (6xFe/Cu) dosimeter solutions and polyvinylchloride (PVC) film strips affixed to a 1.8 m × 2.4 m plywood board suspended midway between the 2 source plaques and parallel to them. A matrix was drawn on the board to aid in sample position identification and doses were measured at various positions. The resulting data from these experiments was reduced to an isodose presentation (Fig. 4) similar to the format of the computer output. The agreement between the two lends credence to the computer program's ability to predict planar dose variation.

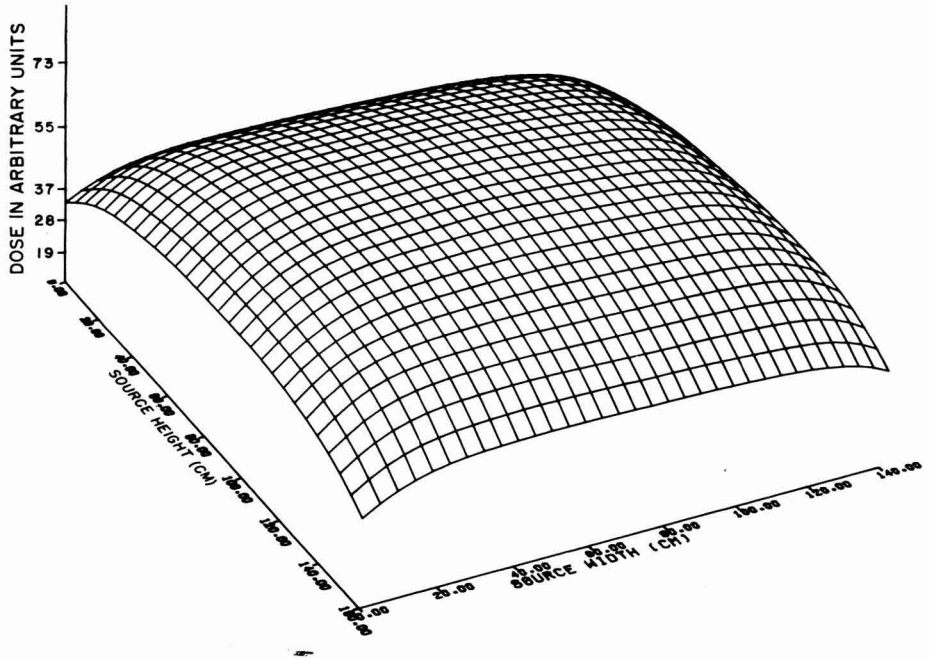

FIG. 3. TOPOGRAPHICAL REPRESENTATION OF PREDICTED PLANAR DOSE HOMOGENEITY

Computer generated plot shows dose variation for a plane midway between the two source plaques.

The total dose variation for any finitely thick sample configuration is obtained by adding to the planar variation the dose variation thru the thickness of the product, the depth dose. Experience has shown that an average depth dose for a 18 cm thick product is about 16%. Therefore, a planar variation of approximately 30% can be allowed and still maintain the required 1.50 value for the maximum/minimum dose ratio. An overlay of product box outlines on an isodose curve representation of the planar dose variation (Fig. 4) illustrates the product configuration decided upon. In practice this configuration consists of 8 cartons of cans with either twelve 404 × 700 cans or twenty-four 404 × 309 cans per carton centered in a carrier and the carrier positioned against the physical stops in the irradiation cell.

In practice the total dose delivered to any point in the configuration

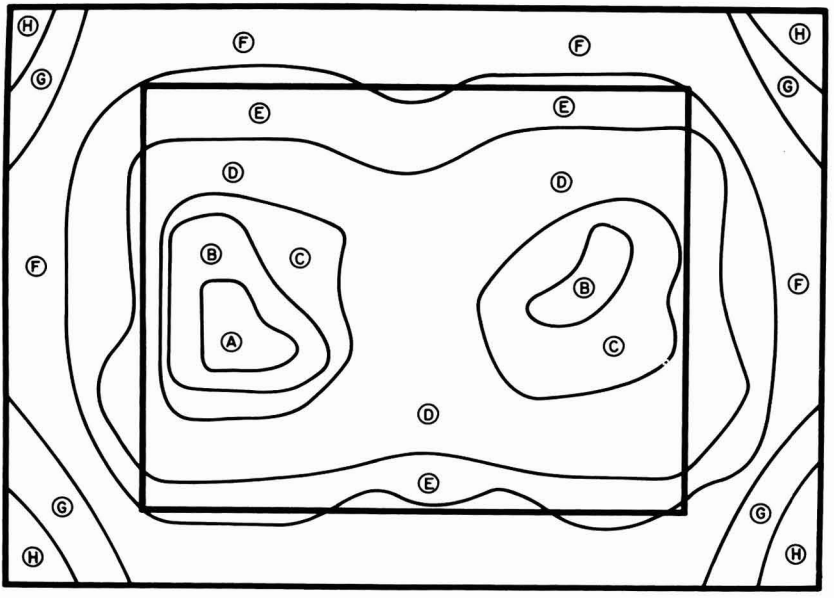

FIG. 4. ISODOSE REPRESENTATION OF EXPERIMENTALLY DETERMINED DOSE HOMOGENEITY

Experimentally determined dose rate values drawn as isodose curves. The large outer rectangle represents the dimensions of the Co-60 source. The inner rectangle represents the area covered by the product. The various areas of equal dose range are labeled A thru G. Normalizing relative to area D, the ratios represented by the other areas are: A-107%; B-106%; C-104%; D-100%; E-94%; F-92%; G-73%.

is the sum of two contributions — that from the dose rate and that from the transient dose.

$$\text{Total Dose} = \text{Dose Rate} \times \text{Irradiation Time} + \text{Transient Dose} \quad (8)$$

Recalling that the transient dose is that dose delivered as a result of the source being raised from the storage position to the irradiation position and then returned to the storage position, the exposure or irradiation time is measured when the source is in the fully raised position, i.e. in the irradiation configuration. Depending on specific conditions, the transient dose can constitute a significant portion of the total dose. An illustration of this point for various realistic combinations of total and transient dose levels is given in Table 3. The distribution of the transient dose is determined primarily by the vertical travel of the source. Hence, the transient dose has a strong positional dependence in the vertical direction (shown later in Fig. 6) and is relatively independent of position in the horizontal direction.

Experimentally, the dosimetry task reduces to determining dose rate and transient dose values. When talking about the total dose, dose rate or transient dose, we refer to one point in the target. The variation in these values is another consideration. One experiment that can be performed is to make the exposure time very short so that it is, for all intent, zero. In this case Equation (8) becomes:

$$\text{Total Dose} = \text{Transient Dose} \quad (9)$$

The dose rate now may be determined by using Equation (8) rearranged:

$$\text{Dose Rate} = \frac{\text{Total Dose} - \text{Transient Dose}}{\text{Irradiation Time}} \quad (10)$$

If the transient dose is known to be small — or the error its neglect would contribute is acceptable (Table 3) — then the expression for dose rate simplifies to:

$$\text{Dose Rate} = \frac{\text{Total Dose}}{\text{Irrad. Time}} \quad (11)$$

Because of its ever present contribution to the total dose, the transient dose will also have an effect on the maximum/minimum dose

Table 3. Contribution[1] of transient dose to total dose transient doses[2]

Total Dose[2]	.40	.45	.50	.55	.60	.65	.70	.75	.80
2.00	25.00	29.03	33.33	37.93	42.86	48.15	53.85	60.00	66.67
4.00	11.11	12.68	14.29	15.94	17.65	19.40	21.21	23.08	25.00
6.00	7.14	8.11	9.09	10.09	11.11	12.15	13.21	14.29	15.38
8.00	5.26	5.96	6.67	7.38	8.11	8.84	9.59	10.34	11.11
10.00	4.17	4.71	5.26	5.82	6.38	6.95	7.53	8.11	8.70
15.00	2.74	3.09	3.45	3.81	4.17	4.53	4.90	5.26	5.63
20.00	2.04	2.30	2.56	2.83	3.09	3.36	3.63	3.90	4.17
25.00	1.63	1.83	2.04	2.25	2.46	2.67	2.88	3.09	3.31
30.00	1.35	1.52	1.69	1.87	2.04	2.21	2.39	2.56	2.74
35.00	1.16	1.30	1.45	1.60	1.74	1.89	2.04	2.19	2.34
40.00	1.01	1.14	1.27	1.39	1.52	1.65	1.78	1.91	2.04
45.00	.90	1.01	1.12	1.24	1.35	1.47	1.58	1.69	1.81
50.00	.81	.91	1.01	1.11	1.21	1.32	1.42	1.52	1.63
55.00	.73	.82	.92	1.01	1.10	1.20	1.29	1.38	1.48
60.00	.67	.76	.84	.93	1.01	1.18	1.18	1.27	1.35
65.00	.62	.70	.78	.85	.93	1.01	1.09	1.17	1.25
70.00	.57	.65	.72	.79	.86	.94	1.01	1.08	1.16
75.00	.54	.60	.67	.74	.81	.87	.94	1.01	1.08
80.00	.50	.57	.63	.69	.76	.82	.88	.95	1.01
85.00	.47	.53	.59	.65	.71	.77	.83	.89	.95

[1] Calculated by: $\left(\frac{\text{Total Dose}}{\text{Total Dose - Transient Dose}}\right) - 1 \times 100$, entries in the table illustrate the percent effect of neglecting the transient dose entirely

[2] Units are k Grays

ratio. The actual maximum/minimum total dose ratio approaches the dose rate ratio asymptotically as the total dose level increases. It is very convenient and very common to use the dose rate ratio as an index of the dose variation between points in an irradiation configuration. In most cases encountered in practice, the dose rate ratio is a valid index.

Having selected the configuration in which the product is to be irradiated and understanding in general terms the dosimetry data required and how it may be obtained, the next step is to perform the actual pre-irradiation calibration. A complete calibration is normally performed in four steps. All of these steps require the use of either product or product simulating material (phantoms) arranged in the exact irradiation configuration. Several steps in the calibration procedure require placing dosimeter ampules at various positions in the phantom. We have found long grain rice to accurately represent the product and to be much more amenable to reproducible dosimeter

placement. The initial step before any calibration experiments are attempted, then, is to fill cans with long grain rice and to close them.

In the first experiment ampules of 6xFe/Cu are affixed to the front surface of each of the 96 cans and irradiated for an accurately measured period of time to obtain a total dose in the 40-50 kGy range. The results of this experiment give a planar total dose variation and permit the pinpointing of the areas of maximum/minimum dose (Table 4a). The next step is to determine the transient dose for this planar configuration. This is done by placing Reg. Fe/Cu dosimeters where the 6xFe/Cu dosimeters were in the previous experiment and accumulating an adequate total dose (e.g. 5kGy maximum) by raising and lowering the source 4 or 5 times with zero irradiation ("up") time. Using Equation (9), the transient dose for each position is the total dose thus measured divided by the number of raise-lower cycles. The dose rates for the planar configuration can now be calculated using equation (10). From the results so far (Table 4b,c) the areas of maximum/minimum surface dose can be ascertained using dose rates as the index of variation. The maximum dose rate position for the whole configuration will be a surface position, either front or back, while the minimum dose rate position will lie on some midplane. A common position for the minimum dose is, for example, in the center of a can.

The next step is to do some depth dose measurements for selected can positions. Such information will, for the cans with the lower surface dose rates, determine the minimum dose for the entire configuration and will, for the cans with the higher front surface dose rates, determine whether it is the front or back surface which is higher. Depth dose experiments are done by taping 6xFe/Cu dosimeters together to form a stack and locating this stack on the axis of the can. Again, using rice as the phantom material facilitates the location of these dosimeters as well as serving as an accurate representation of the actual product.

A total of ten dosimeter readings are thus available at each position to define the depth dose. The positions chosen for this treatment were E1, F1, K3, K4 and K5. As an added check on the surface dose 6xFe/Cu dosimeters are taped on the front surface of selected cans in each of these areas (e.g. D1, E1, F1, D4, E1, F1). As previously, these dosimeters are irradiated to a total dose in the 40-50 kGy region, the irradiation time being accurately measured. This experiment is repeated using Reg. Fe/Cu dosimeters and five raise-lower cycles to obtain transient dose values for these positions. Again, using Equation (10) dose rates are calculated and the depth dose is plotted as dose rate against ampule position (Fig. 5). The maximum dose rate in a can is not at any actual dosimeter position but at the can's inner surface.

Table 4. Dose and dose rate dependence on position in the front plane of the irradiation configuration

	A	B	C	D	E	F	G	H	I	J	K
(1)	2.779	2.678	2.647	2.562	2.500	2.492	2.562	2.655	0.000	0.000	0.000
(2)	2.810	2.825	2.678	2.687	2.585	2.562	2.601	2.701	2.817	2.887	2.949
(3)	2.949	2.941	2.887	2.856	2.817	2.732	2.740	2.872	3.003	3.026	3.081
(4)	2.949	2.949	2.872	2.872	2.833	2.779	2.717	2.833	2.933	2.949	3.034
(5)	2.895	2.833	2.848	2.910	2.856	2.740	2.794	2.903	2.949	3.011	3.065
(6)	2.879	2.895	2.918	2.887	0.000	2.771	2.794	2.872	2.972	3.026	3.026
(7)	2.817	2.833	2.825	2.794	2.771	2.655	2.732	2.856	2.895	2.949	3.026
(8)	2.794	2.794	2.732	2.663	2.639	2.601	2.655	2.694	2.755	2.833	2.895
(9)	2.794	2.717	2.717	2.639	2.616	2.585	2.601	2.655	2.743	2.817	2.879

(a) Total dose distribution for a 25 min irradiation. Dose is in 10^4 Grays
Maximum Dose = 3.081×10^4 Grays in row 3, column K.
Minimum Dose = 2.492×10^4 Grays in row 1, column F. Maximum/minimum ratio = 1.236

	A	B	C	D	E	F	G	H	I	J	K
(1)	1.099	1.059	1.047	1.013	.988	.985	1.013	1.050	0.000	0.000	0.000
(2)	1.109	1.115	1.056	1.059	1.019	1.010	1.025	1.065	1.112	1.140	1.164
(3)	1.161	1.158	1.136	1.124	1.109	1.074	1.078	1.130	1.183	1.192	1.214

(4)	1.158	1.158	1.127	1.127	1.112	1.090	1.065	1.112	1.152	1.158	1.192
(5)	1.133	1.108	1.115	1.139	1.118	1.071	1.093	1.136	1.155	1.180	1.201
(6)	1.124	1.130	1.140	1.127	0.000	1.081	1.090	1.121	1.161	1.133	1.183
(7)	1.096	1.102	1.099	1.087	1.078	1.031	1.062	1.112	1.127	1.149	1.180
(8)	1.084	1.084	1.059	1.031	1.022	1.006	1.028	1.043	1.068	1.099	1.124
(9)	1.081	1.050	1.050	1.019	1.009	.997	1.003	1.025	1.056	1.090	1.115

(b) Dose rate distribution. Dose rates in units of 10^3 Grays/min

	A	B	C	D	E	F	G	H	I	J	K
(1)	11.63	7.55	6.29	2.83	.31	0.00	2.83	6.60	0.00	0.00	0.00
(2)	12.59	13.22	7.24	7.56	3.47	2.53	4.10	8.18	12.90	15.73	18.25
(3)	17.92	17.61	15.41	14.15	12.58	9.12	9.43	14.78	20.12	21.07	23.27
(4)	17.62	17.62	14.47	14.47	12.90	10.70	8.18	12.90	16.99	17.62	21.08
(5)	15.09	12.58	13.20	15.72	13.52	8.80	11.00	15.40	17.29	19.81	22.01
(6)	14.16	14.79	15.73	14.47	0.00	9.76	10.70	13.84	17.93	20.13	20.13
(7)	11.32	11.95	11.63	10.37	9.43	4.71	7.86	12.89	14.46	16.66	19.81
(8)	10.05	10.05	7.53	4.70	3.76	2.19	4.39	5.96	8.48	11.62	14.14
(9)	9.74	6.60	6.60	3.46	2.51	1.25	1.88	4.08	7.23	10.69	13.20

(c) Dose rate variation distribution. Expressed as percent relative to minimum dose rate in position F1

Interpolation is made between the dose measured in the ampules on the surface and that measured in the first ampule of the depth dose stack. Fig. 5 illustrates this procedure.

The actual maximum dose rate in the entire configuration will be just such an interpolated value obtained as illustrated. Dose variations may now be calculated by taking appropriate ratios of maximum/minimum dose rates. Taken within a can this ratio is the depth dose; taken for the entire configuration it is the dose variation.

All the necessary dosimetry information has now been collected. The results in summary are as follows:

1. From the planar total dose and transient dose experiments the dose rate and transient dose values for the front plane of the cans are obtained. From the dose rates in Table 4b and the variations tabulated in 4c, the positions of the maximum and minimum front planar dose rates are K3 and F1, respectively. The vertical transient dose dependence is given by Fig. 6 for the front plane.

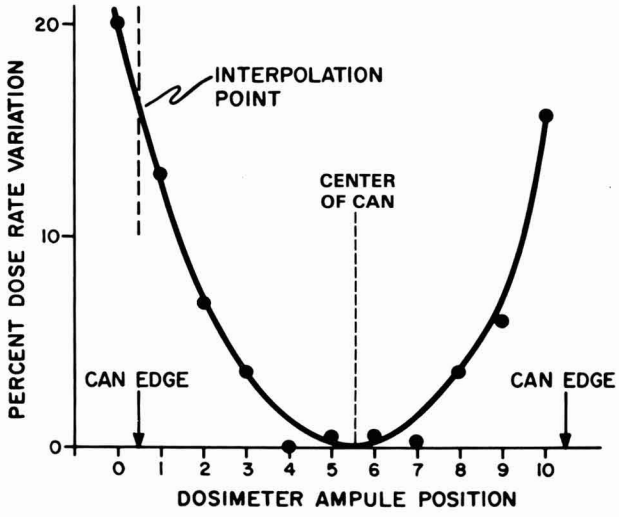

FIG. 5. DEPTH DOSE CURVE FOR Co-60 IRRADIATED PRODUCT

Dosimeter ampule position is plotted against the dose rate at each position normalized against the dose rate at the center of the product. Arrows (↓) indicate the positions of the can boundaries. The interpolation procedure is illustrated by the intersection of the vertical dotted line with the depth dose curve.

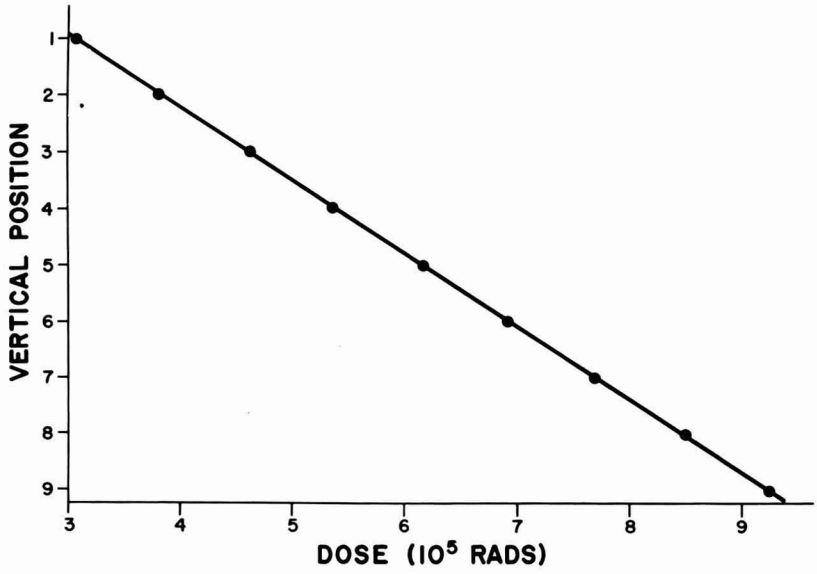

FIG. 6. VERTICAL DEPENDENCE OF THE TRANSIENT DOSE

Transient dose is plotted against vertical position. Dosimeters were placed on the faces of the product cans and the total accumulated dose divided by the number of raise-lower cycles.

2. From the depth dose series of experiments (Fig. 5) the variation along the axis of the can was found to be 16%, a ratio of 1.16 between the dose at the can edge and at its center. The dose measured on the outside of the can was 3.5% (a ratio of 1.035) higher than the interpolated value at the edge of the can.
3. Maximum Dose Rate at the edge of the can in position (K3) is:

$$= \frac{1.214 \times 10^3}{1.035} = 1.172 \times 10^3 \text{ Gy/min}$$

Minimum Dose Rate at the center of F1 is:

$$\frac{\text{Interpolated dose rate at F1}}{\text{Depth dose variation}}$$

$$= \frac{0.985 \times 10^3}{1.035 \times 1.16} = 0.820 \times 10^3 \text{ Gy/min}$$

Dose Rate Variation = 1.429 = 42.9% variation

The main purpose of the procedure followed to this point is to identify the locations, even the general areas, where the maximum and minimum dose rates occur. Shorter confirmatory experiments may now be conducted to either more precisely define the maximum/minimum location or more accurately determine their values. These confirmatory experiments usually consist of further depth dose determinations including the dose value on the outside of the can. Confirmatory calibrations are conducted before and after each irradiation session.

Most of the dosimetry effort in support of the cobalt irradiation is placed in the pre- and postirradiation calibrations. Actual production controls are provided solely by fixing and monitoring the irradiation parameters. The product is boxed and the boxes fitted into the carrier such that geometric variations are minimal. The carrier and source have physical stops in the irradiation cell to guarantee the reproducible positioning of the carrier relative to the source. The remaining irradiation parameter, the irradiation time, is measured automatically and recorded. Details of the cobalt facility operation and the collection and recording of pertinent parameters are covered by MacDonald (1976). The PVC color dot go/no-go dosimeter is affixed to each can as well as to each carton of cans to prevent possible mixing of irradiated and nonirradiated product.

LINAC Dosimetry Procedures

Despite obvious dissimilarities both conceptual and operational between the LINAC and cobalt radappertization processes, the basic systematic used in performing the cobalt dosimetry are applicable to the LINAC dosimetry. The overall task can be divided into three separate phases:

1. A precalibration phase that includes the gathering of support information to aid in the selection and definition of an irradiation geometry,
2. The actual calibration of the irradiation geometry including confirmation of the expected dose variation and the necessary relation of dose to LINAC operating parameters,
3. Production controls.

The LINAC radappertization process is described in detail by Rees and Caspersen (1976). For dosimetry purposes the following two steps suffice to describe the process:

1. The pouches of food arranged in an irradiation box are loaded onto a conveyor car.

2. The conveyor car is moved past the scanning electron beam at some constant velocity.

The irradiation dose from the LINAC is delivered via a scanned, pulsed beam of electrons impinging on a sample moving perpendicularly to the plane of the scanning beam. The net result is the inscription of a pattern of contiguous V's consisting of overlapping pulses of electrons. The parameters controlling the irradiation dose are the average current (I) in the beam, the sample velocity (V) and the scan height (H).

$$\text{Dose} \propto \frac{I}{VH} \tag{12}$$

The scan rate is held constant at 0.5 Hz.

As with the cobalt, the precalibration consists chiefly of gathering certain basic information on which to base the selection of an irradiation configuration and to estimate the dose variation. This information can be divided into the following three categories:

1. Planar dose homogeneity
2. Depth dose
3. Edge effects

The uniformity of the dose delivered to a given plane is measured with PVC. PVC is, for example, attached to the front of a carrier and irradiated. The results for a single 1.25 cm strip hung vertically are shown in Fig. 7.

The scan height is defined as the distance between the points of half-maximum dose (the distance AA′). A series of such vertical strips placed at various horizontal positions or a sheet of PVC film covering the front of the carrier shows there is no horizontal variation, i.e. the area defined by BB′ × the length of the carrier has no observable dose variation.

The consideration of depth dose is probably the most important single factor influencing the selection of an irradiation configuration. It is beyond the scope of this paper to discuss in much detail a subject rooted so deeply in the basic physics of the interaction of radiation with matter. Both theoretical and measured depth dose curves are available for many materials. (Humphreys *et al.* 1973; Brynjolfsson *et al.* 1963). The shape of such curves depends on both the energy of the incident radiation and the material with which the radiation is interacting. Since the electron energy for the food irradiation is specified as 10 MeV, our depth dose considerations are confined to this energy.

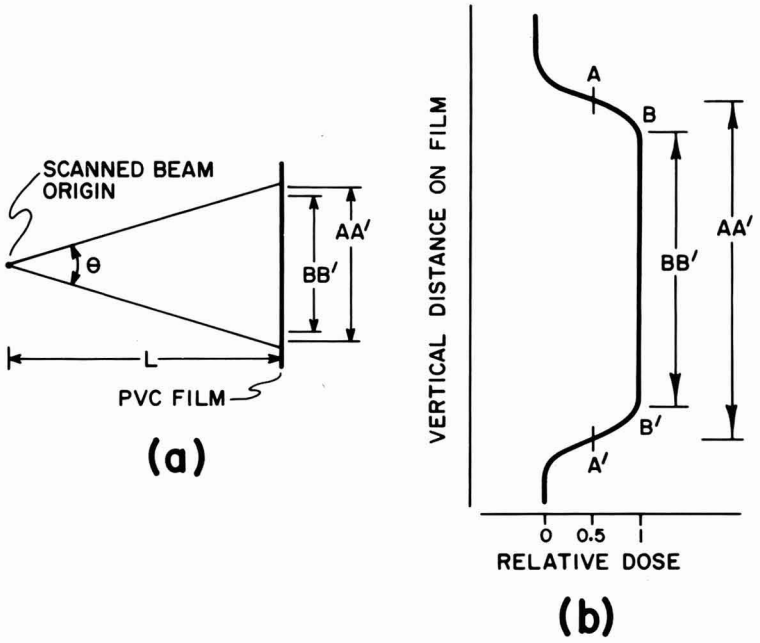

FIG. 7. SCAN HEIGHT DETERMINATION USING PVC FILM STRIPS

(a) Schematic diagram shows relation of scan height to scan angle, θ, and distance, L, from the beam origin. L $\sim$ 1m and θ = 0.4 radians.

(b) Diagrammatic plot of relative dose against position on the PVC strip. The scan height, AA, is the vertical distance between the half dose points. The vertical limits of the planar dose homogeneity are designated by BB′.

Our depth dose distribution studies have emphasized the use of films, PVC in particular, to obtain the necessary information. Conceptually, there are two approaches to doing such film studies. The films may be pressed intimately together to form a stack which is then irradiated such that the beam axis is perpendicular to the face of the stack. A plot of the dose on each film versus its position in the stack gives a curve related to the depth dose, to which corrections for film thickness and density can be made. Such experiments give, of course, depth dose in the film material, which may not be of particular interest. Using the stack technique, depth dose curves may be obtained for other materials by interspersing the dose monitoring films among layers of the subject material. While the films cause only a slight perturbation, the number of data points is also reduced.

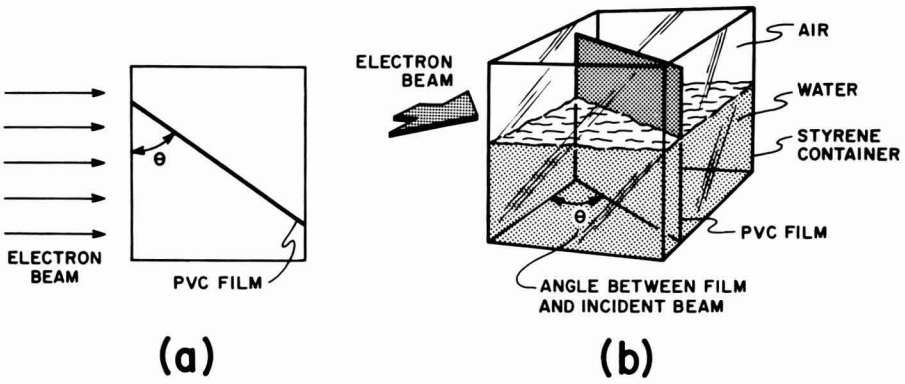

FIG. 8. DETERMINATION OF LINAC DEPTH DOSE CURVES USING THE WEDGE TECHNIQUE

(a) Diagram shows relation of electron beam and PVC film on which dose is recorded.

(b) Experimental arrangement used in determining depth dose in water.

The other approach is referred to as the wedge technique. The dose monitoring film is placed on a diagonal through a block of the material of interest (Fig. 8). Using the recording densitometer mode of evaluating PVC films and making corrections for the incident angle between the beam axis and the film, many dose points are obtained through the depth of the material.

Experimentally we measured the depth dose in water using the wedge technique. As shown in Fig. 8b, a PVC film was supported in a water filled polystyrene container. Two cases were studied. For the case of an infinitely thick sample, the water thickness was 10 cm. To simulate actual product thicknesses, a depth of 2.5 cm was used. The results are shown in Fig. 9 for 7, 9 and 10 MeV, and a water depth of 10 cm.

An independent effort was made by one of the authors (RDJ) in collaboration with Humphreys, McLaughlin and Chappel of the U.S. National Bureau of Standards. This study used radiochromic dye dosimeters to study depth dose in aluminum, styrene, carbon, and beef. Besides the obvious relevance of the subject matter, the applicability of these results to the subject under discussion is made even more acute by the use of NARADCOM's LINAC and some of the instrumentation used for the dosimetry work already discussed. Both the stack and wedge techniques were used in these studies.

Edge effects refer in general to the dose perturbation at the interface between two dissimilar materials caused by non-equilibrium scattering phenomena. For food irradiation the edge effect is observed primarily

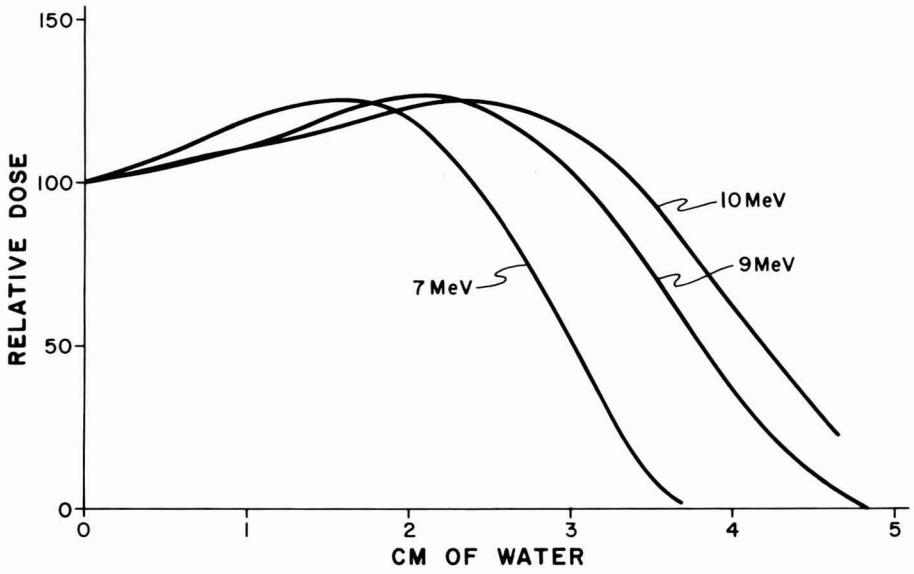

FIG. 9. LINAC DEPTH DOSE CURVES

Doses were measured in water using the wedge technique.

at the periphery of the product but is also evidenced at voids. The magnitude of this effect was investigated in two ways. First, the PVC depth dose experiment already described yielded some of this information. The peripheral edge effect is, after all, the change in the shape of the depth dose as the air/product interface is approached. Again, using the experimental arrangement shown in Fig. 8 with the PVC film protruding about 5 cm above the air/water interface the radiation scattered out of the water and into the air is recorded quite dramatically by the PVC (Fig. 10) using the isodensitracer mode of representing the optical absorbance.

The isodensitracer representation results from the encoding in a specific symbolic format of incremental changes in the absorbance. Thus, a one line print out of an ordered series of dots, dashes and spaces contains the information found in a more conventional graph of absorbance versus distance. The two dimensional picture derived from a series of such one dimensional encoded graphs (Fig. 10) gives what is essentially an iso-dose display of the dose variation recorded on the entire PVC film.

Two cases are shown. One in which the film is at an angle sufficient to monitor the entire 10 cm depth of the sample (A) and the other (B) in which only the first 3 cm is monitored. In the first case (A) the iso-

dose distribution of the radiation scattered out of the interface region and into the air is evidenced as a "flaring" effect. This effect is not as pronounced in B where the iso-dose presentation of the depth dose in water is seen in more detail as in the dose variations in the interface region. While isodose representative of the data are useful in giving an overview, other presentations are more effective in gaining a more quantitative understanding.

Using the normal densitometric presentation, the PVC film is read in two directions, E → W and S → N. Reading in the E → W direction yields traces which are related to the conventional depth dose curves. A series of such traces starting at the interface and proceeding into the water at 2 mm increments is shown in Fig. 11a as a three dimensional plot. For clarity, this 3-dimensional representation is viewed from 2 perspectives. Reading in the S → N direction yields a series of traces which proceed from the water phase, through the interface region and into the air phase. Two such traces are shown in Fig. 11b. At the entrance dose position, there is no perturbation as the interface is traversed, while at the exit dose position (depth = 2.5 g/cm^2) a dose variation of approximately 25% is observed. These traces illustrate the edge effect at the two extremes of the irradiation configuration. The variation at

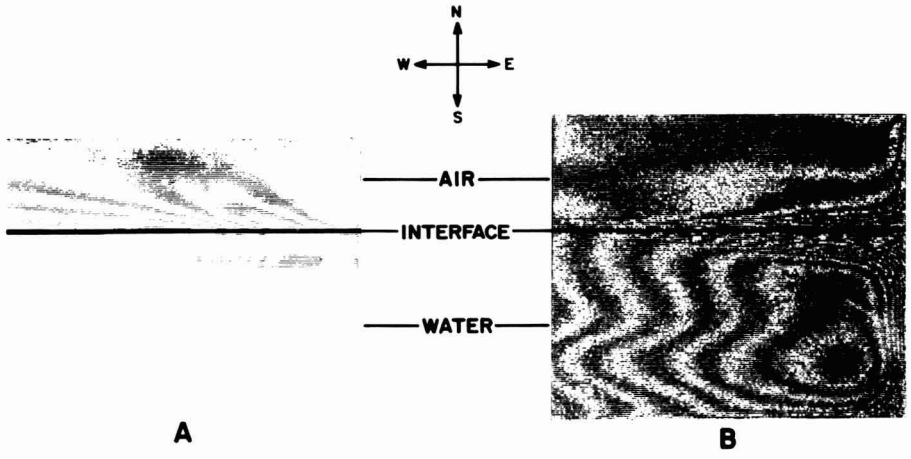

FIG. 10. ISODOSE REPRESENTATION OF THE DEPTH DOSE AND THE DOSE VARIATION AT THE AIR/WATER INTERFACE

Experimental data obtained by the isodensitometric readout of films irradiated using the wedge techniques. The interface line denotes the air/water interface. The electron beam enters from the right side (E). For A, $\theta = 62^\circ$ (Fig. 8); for B, $\theta = 20^\circ$.

intermediate depth is best visualized by reference to Fig. 11a. In addition to determining the magnitude of the edge effect, it is also seen that its influence in the water (product) phase extends to about 1.5 cm back from the interface.

a. Data for these three dimensional plots of the depth dose data illustrating the interface effect was obtained by the wedge technique using a PVC film along the diagonal of a block of agar gel. Depth dose curves are at 2 mm increments starting at the interface and proceeding into the water phase.
b. Two dimensional illustration of the interface effect is shown for 2 positions along the depth dose profile — at the entrance and exit positions.

The restrictions that the results of these pre-calibration experiments imposed led to the following conclusions:

1. The depth of sample should be about 2.5 cm of unit density material (2.5 g/cm^2).
2. The product should be confined to the area of no planar dose variation, i.e., limited in the vertical direction only and then to within the distance as defined by the scan height determination (BB, Fig. 7).
3. The magnitude of the edge effect is, as has been shown, variable and depends on where it is evaluated. A 25% effect is a very conservative estimate of its magnitude and covers the worst situations in which edge effects could be encountered. The edge effect contribution is, of course, applied to the lowest dose point found as a result of all other considerations.

Fig. 12 summarizes in graphic form the dose variation information upon which the choice of irradiation configuration was based. The depth dose curve presented was measured for a 2.5 cm thick sample using the wedge technique. Contrasting this curve for a sample of finite thickness with those for 10 cm thick samples (Fig. 9) two effects are observed. The position of the peak dose is changed. The general shape of the curve is altered resulting in a dose at the 2.5 cm depth (exit dose) that is only 12% above the entrance dose. Using numerical integration methods, the average dose was found to be 10% above the entrance dose. Applying the 25% edge effect correction to the exit dose, the edge dose is found to be 89.5% of the entrance dose. Taking 5% margins on either side of the edge and peak doses, the minimum

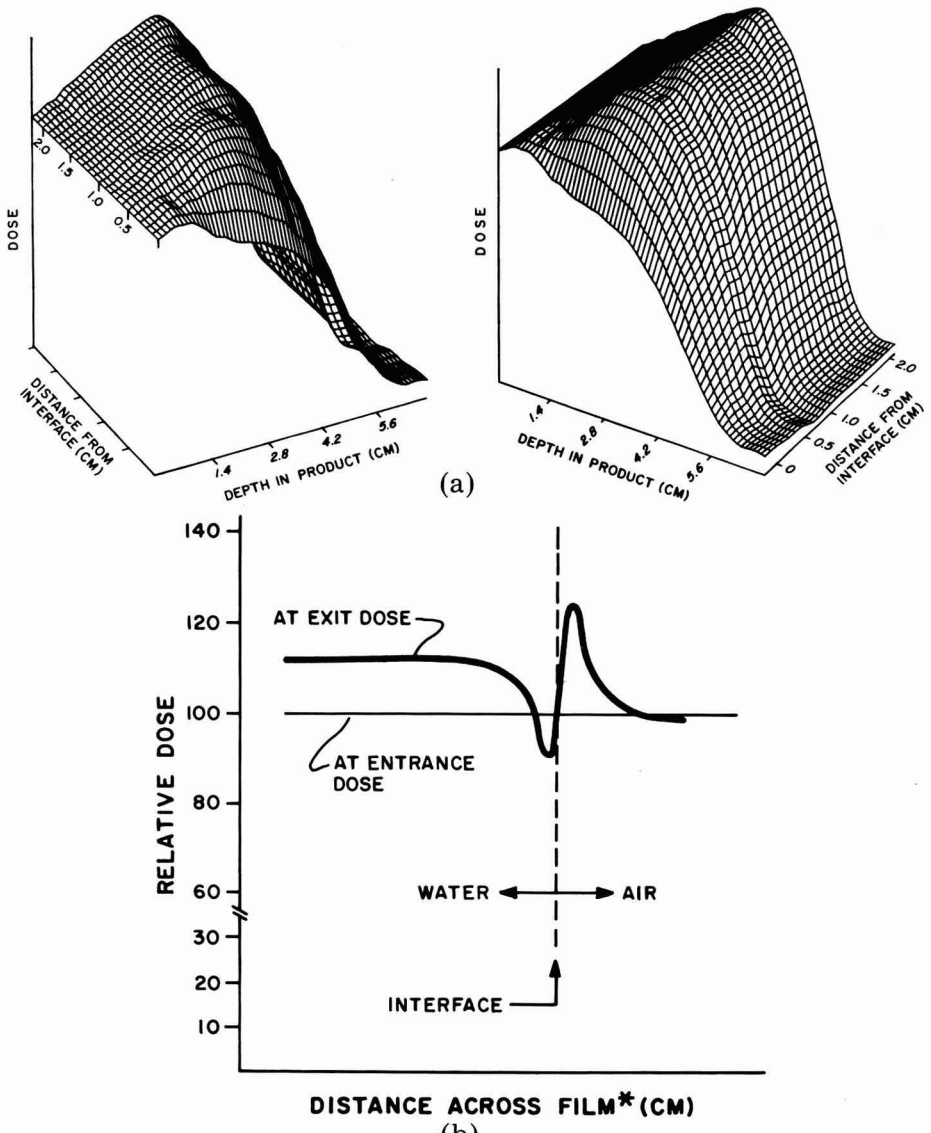

FIG. 11. TWO AND THREE DIMENSIONAL REPRESENTATIONS OF THE INTERFACE EFFECT

(a) Date for these three dimensional plots of the depth dose data illustrating the interface effect was obtained by the wedge technique using a PVC film along the diagonal of a block of agar gel. Depth dose curves are at 2 mm increments starting at the interface and proceeding into the water phase.

(b) Two dimensional illustration of the interface effect is shown for two positions along the depth dose profile — at the entrance and exit positions.

*Distance across film is the transverse direction in the N ↔ S direction of Figure 10.

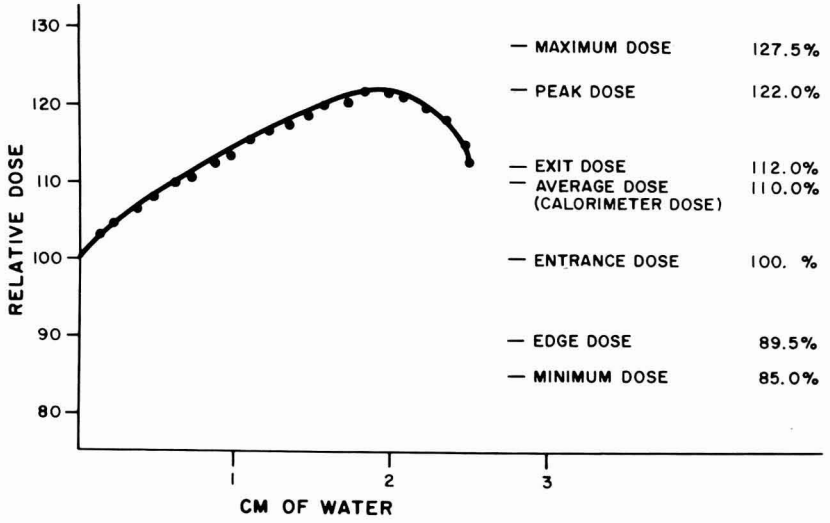

FIG. 12. LINAC DEPTH DOSE CURVE AT 10 MeV FOR A 2.5 cm SAMPLE

The right hand side of the figure summarizes the dose variation for LINAC irradiations using the 2.5 g/cm^2 configuration. The maximum and minimum doses allow a 5% margin beyond experimentally measured doses.

and maximum doses are calculated to be respectively 85% below and 27.5% above the entrance dose.

As in the cobalt case the total irradiation configuration implies certain geometric constraints. For the LINAC this chiefly involves the position of the irradiation package relative to the scanned beam. In the vertical direction, the packages must be located totally within the area of planar dose homogeneity, as previously discussed relative to Fig. 7. On the horizontal beam axis the package must be a fixed distance from the scan origin. Again referring to Fig. 7(a), it can be seen that variation in the distance L will be reflected as a scan height variation and thus as a dose variation via Equation (12). It is appropriate to mention in conjunction with this discussion on scan height variations as a function of distance from the beam origin that various measurements taken in this direction, e.g., depth dose curves, have not been corrected for scan height variations. There is some question concerning how data such as depth dose data should be presented. To reflect only the depth dose phenomena, the values at each depth position should be normalized using the value of the entrance dose at that depth. For our work, we normalize using the entrance dose at the front of the target. Thus

our depth dose curves reflect actual values which, for a report of this nature, is the only valid way of presenting the data. Variation in scan height in our particular case contributes a dose error of approximately 1% for a 1 cm variation in L. Thus, for example, in Fig. 9 the peak dose occurring at about 2.2 cm and 122% above the entrance dose would have a corrected value of 124.7% if correction is made for the scan height at 2.2 cm. This same argument is valid where doses at different depths are compared. For product irradiations these geometric variations are reflected in how the irradiation package is positioned on the conveyor car and how the conveyor car is positioned in front of the scanning beam. As with the cobalt, the sum of all these considerations, the irradiation package and its presentation to the irradiation sources, constitutes the total irradiation configuration.

The calibration of the irradiation geometry primarily involves establishing the relationship between some dose in the irradiation geometry and the LINAC operating parameters. This correlation is made via the water calorimeter. The dose variation aspects of the calibration have already been completed during the precalibration phase. Confirmatory experiments are, of course, performed at this point.

It can now be seen that the dimensions of the water calorimeter (Section 1) are not fortuitous. In fact, the calorimeter was designed to measure the dose in a 2.5 g/cm^2 sample — an irradiation configuration at 10 MeV whose convenience is not unique to the irradiation of food. The dose measured by the calorimeter is, by definition, the average dose for that configuration. Therefore, for "average dose" in Fig. 12 may be substituted "calorimeter dose". We may now recall Equation (12) and write it as:

$$\text{Dose} = \frac{K'}{H} \cdot \frac{I}{V} = K \cdot \frac{I}{V} \tag{13}$$

where the proportionality constant K is understood to apply only to a single scan height, H — constant scan height being part of the irradiation configuration definition. Verification of the scan height is done by electronic means (6) and by the PVC vertical strip method already described.

The pre-irradiation calibration (or irradiation configuration calibration) consists primarily of verification of the K value. This is done very straightforwardly by determining the dose with the calorimeter and recording the average beam current (I) and conveyor velocity (V) used. Plotting values of the ratio I/V as the abscissa and calorimeter dose

values on the ordinate, the slope of the resulting straight line is the K value and has units kGy Volts per milliampere.

As indicated earlier, there is a considerable emphasis on production dosimetry for the LINAC processing. Not least among the justifications for this emphasis is the desire to acquire a large body of data and the accompanying experience about the various fluctuations and deviations that occur when large quantities of actual product are processed. The daily routine followed during the LINAC processing includes the following steps involving dosimetry.

1. Before any food is irradiated a calorimeter and a special dosimetry box are run.
2. During the food processing a 6× Fe/Cu dosimeter is run on the leading edge of the product box for approximately every fifth run.
3. A PVC strip is run on the back of every styrofoam box.

A calorimeter provides not only a daily confirmation of the K values but serves as a general systems check. The special dosimetry box consists of a styrofoam box (used to hold the food) in which are placed 2 water filled, 2.5 cm thick polystyrene dishes, a wooden tube holder containing three 6× Fe/Cu dosimeters, and a strip of PVC film on the back side of the box placed horizontally so that it is directly behind the water filled dishes and the wooden holder and extends the entire length of the box. The water filled dishes represent the product. The 6× Fe/Cu dosimeters in the wooden block represent an irradiation configuration such that the average dose in the ampule is the same as the calorimeter average dose. The irradiation of this special dosimetry box is, then, a daily, minicalibration of the PVC and 6× Fe/Cu dosimeters under processing conditions. While the 6× Fe/Cu dosimeter run on every fifth run is not in an ideal irradiation geometry, it does provide an index of the processing reproducibility and reliability. The PVC strips, on the other hand, are useful in studying the fluctuations that occur within a run as well as providing a run to run comparison.

The various LINAC operating parameters such as beam current, conveyor velocity, scan height, scan position, and beam centering are dynamically monitored electronically by the LINAC operators during irradiation. The production dosimetry, particularly the PVC dosimetry, complements these efforts in detecting error trends or malfunctions and in determining which of the product was affected. The large body of data resulting from the daily production dosimetry, while difficult to reduce to a convenient or meaningful form for presentation, does

demonstrate the compliance of the irradiation processing with the restrictions imposed on it.

A PVC color dot go/no-go dosimeter was affixed to the smallest unit irradiated (in this case the individual pouchs) as well as to every larger packaging unit actually irradiated.

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EFFECTS OF OZONE TREATMENT ON MICROFLORA OF POULTRY MEAT[1]

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ABSTRACT

Ozone was produced using a Welsbach ozonator and measured by an iodometric titration method. Microbiological characteristics of ozone treated and air treated control samples were studied. Ozone treated broiler parts had consistently lower microbial counts than the control parts during the entire refrigerated observation period. Using log total microbial counts of 7.0 cm^2 *as a spoilage criterion, broiler parts treated with ozone had extended shelf-lives for* 2.4 *days. Ozone treated samples contained about* 52.7% *gram-positive cocci, while the air treated control samples had* 39.6% *gram-positive cocci. Air treated control samples had* 22.4% *gram-negative rods while the ozone treated samples had only* 12.7% *gram-negative rods. Studies using microflora from spoiled poultry meat have also demonstrated that ozone treatment preferentially destroyed gram-negative rod-type organisms.*

INTRODUCTION

Ozone is a powerful oxidizing agent, attacking almost all organic compounds and destorying the most elementary forms of life, such as fungi and bacteria. Ozone has also been used as a postharvest chemical for controlling postharvest decay of strawberries, peaches, and lettuce (Thompson 1971). Kolodyanaya and Suponina (1975) reported that ozone destroyed pathogenic microflora on the surface of potatoes stored in an ozone-containing environment.

Kaess and Weidemann (1968) exposed fresh beef to 0.15 to 5 mg/m^3 of ozone mixed with air and examined the beef for microbial counts. Some meat samples were inoculated with meat spoilage non-pigmented and pigmented *Pseudomonas* spp., *Candida scotti* and *Thamnidium*

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and *Penicillium* spp. They reported that the population densities of non-pigmented *Pseudomonas* spp. and of *Candida scotti* showed a significant decrease at concentrations over 2 mg/m^3 of ozone. The lag phase of pigmented *Pseudomonas* spp. was retarded with lower concentrations, but there was less effect on non-pigmented *Pseudomonas* spp. The lag phase of *Thamnidium* and *Penicillium* was greatly increased under concentrations of 0.16 to 5.0 mg/m^3 ozone. Fournaud and Lauret (1972) studied the influence of ozone on the surface microbial flora of beef during refrigeration and thawing. They found that atmospheres containing controlled additions of ozone had little effect on the surface microflora of meat, even with an ozone concentration of 500 ppm; this is attributed to a possible protective effect of meat due to reaction between the ozone, fat, and proteins. Haraguchi *et al.* (1969) studied the preserving effect of ozone on fresh jack mackerel (*Trachurus trachurus*) and shimaaji (*Caranx mertensi*). They reported that viable bacterial counts of the skin surface of the gutted fish, soaked in 3% NaCl solution containing 0.6 ppm of ozone for 30 to 60 min, decreased 1/100 to 1/1000 times more than those of the control fish. The storage life of the fish was lengthened 1.2 to 1.6 times by ozone treatment once every 2 days.

The possible formation of carcinogenic chlorinated compounds in water (Brungs 1973) has raised questions on the use of chlorine in the poultry processing operation. The present work was conducted to study the possible extension of poultry meat shelf life by the ozonation of chilling water and to study the effects of ozone on poultry meat microflora.

MATERIALS AND METHODS

Sample Preparation

Broiler Parts. Commercial type frozen broiler carcasses were obtained from a poultry processing plant. Carcasses were cut-up into two halves, then further separated into breast, thigh, drumstick and wing portions. Only thigh and breast parts were used in this study.

Poultry Meat Microbial Suspensions. Chicken neck samples were obtained from a poultry processing plant and stored in a -18°C freezer. Before the study, 5 pieces of frozen chicken necks were defrosted and washed with 200 ml of distilled water. Sixty milliters of the chicken neck microbial suspension was diluted with ice cold distilled water to 600 ml.

Chicken necks of the same source were stored at 4°C for 28 days to obtain spoiled chicken neck samples. Three pieces of rotten chicken necks were washed with 200 ml distilled water. Twenty milliliters of this rotten chicken neck microbial suspension were diluted with ice cold distilled water to a final volume of 600 ml. The distilled water used in this study was sterile.

Ozone Treatment. Ozone was produced from purified, extra dry oxygen using a Welsbach Model T-816 ozonator (Welsbach Ozone System Corp., Philadelphia, PA). For broiler parts, eight pieces of the cut-up broilers were weighed and soaked in the same quantity of ice cold water. Using a washing bottle disperser, ozone at 3.88 mg/liter under a flow rate of 2050 ml/min was dispersed through the broiler parts for 20 min. Control treatments were made using compressed air under the same flow rate condition as the ozone treatment. The control and ozone treated broiler parts were drained to remove the excess water and stored at 4 to 5°C in polyethylene poultry bags. For poultry microbial suspension, the ozone outlet was connected to a 500-ml washing bottle, which contained 300 ml of the microbial suspensions. To a second and third washing bottle, 2% Kl solution was used to absorb the excess ozone. The washing bottles containing the microbial suspension were maintained in ice slush. The poultry microbial suspensions were treated with ozone at 2.48 mg/liter for a predetermined time under a flow rate of 3175 ml/min. Again, control treatments were made using compressed air under the same flow rate. Each study was repeated three times.

Microbiological Method

Total Count. Microbial samples of the broiler parts were obtained by swabbing the skin for 30 s in different directions with sterile cotton swabs. An area of 1 $in.^2$ or 6.45 cm^2, described by a sterile aluminum foil template was assayed. Serial dilutions of the swabs were made and plated with standard method agar (BBL). Plates were incubated for 72 h at 20°C to determine the psychrotrophic counts, 48 h at 37°C to determine the mesophilic counts, and 10 days at 7°C to determine the psychrophilic counts.

For poultry microbial suspensions, total microbial counts were made immediately following treatment. One ml of the sample was pipetted into serial dilution tubes containing 9 ml of diluent. Mesophilic, psychrotrophic and psychrophilic counts were made as described previously.

Coliform Most Probable Number (MPN). Methods for the coliform test as described by Thatcher and Clark (1968) were followed.

Isolation of Colonies. Psychrotrophic count plates were used. Fifty to 60 colonies were randomly removed from the plates containing 10 to 30 colonies; cultures were purified by the streak-plate technique.

Identification of Microorganisms. The purified cultures were transferred to standard method agar slants and incubated for 24 h at 25°C. Fresh cultures were used for the identification. The gram stain, motility, oxidative and fermentative metabolism, cytochrome oxidase, and catalase tests were made according to the methods described by Collins and Lyne (1970). The scheme of Freeman *et al.* (1976) was used to identify the isolates.

RESULTS AND DISCUSSION

Effect of Ozone on the Microbial Counts of Refrigerated Broiler Parts

The ozone treated broiler parts had consistently lower microbial counts than the control parts during the entire refrigeration period (Fig. 1). Immediately after ozone treatment, the total microbial counts at

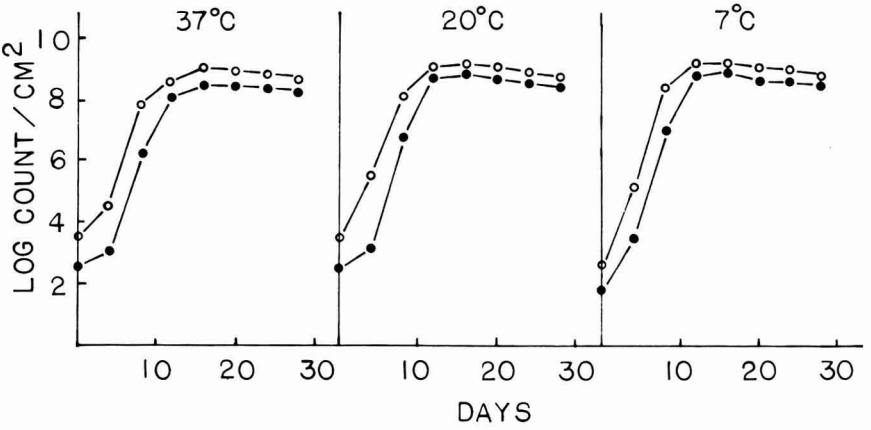

FIG. 1. EFFECTS OF OZONE TREATMENT ON TOTAL MICROBIAL COUNTS AT 37, 20, AND 7°C

Cut-up broiler parts in equal amounts of ice water were treated with ozone at 3.88 mg/liter for 20 min under a flow rate of 2050 ml/min. The treated parts were drained and stored at 2 to 4°C in polyethylene bags. Control treatments were made by using compressed air. ○ control treated sample; ● ozone treated sample.

37, 20 and 7°C were reduced 90.5, 90.5 and 86.0%, accordingly, as compared to the air treated controls. Results indicated that ozone has a slightly stronger destruction power for the mesophiles and the psychrotrophs than for the psychrophiles. The initial 7°C counts were lower than the 37°C and the 20°C counts for both the ozone treated and control parts.

According to Essary *et al.* (1958), broilers were considered spoiled when the number of psychrotrophs reached a log number of 7.0/sq cm. This log microbial count of 7.0/sq cm has been accepted as a shelf-life determinating criterion for broiler carcasses (Cox *et al.* 1974). With this in mind, an extension of 2.4 days in shelf-life was found when broiler parts in ice water were treated with ozone at 3.88 mg/liter for 20 min under a flow rate of 2050 ml/min as compared to the air treated control.

Ozone Treatment and Fresh Poultry Meat Microflora

After a fresh microbial suspension was treated with an ozone concentration of 2.48 mg/liter for 5 min under a flow rate of 3175 ml/min, its log total count at 20°C was reduced from 2.15 per ml to 1.53 per ml. Also, the log MPN of coliforms was reduced from 1.38 to 0.36.

A total of 113 cultures were isolated and purified from the 20°C total count plates with 55 cultures from the ozone treated sample and 58 cultures from the control sample. Gram-positive cocci dominated the microbial population on the ozone treated sample (Table 1). Ozone treated samples contained about 52.7% gram-positive cocci, while the control samples had 39.6% gram-positive cocci. The percentages of gram-positive rods for the ozone treated samples and the control samples were about the same. The control had 22.4% gram-negative rods, while the ozone treated samples had only 12.7%. It could be postulated that ozone was more effective against gram-negative rod-type organisms than any of the other types.

Ozone Treatment and Spoilage Poultry Meat Microflora

Ozone treatment drastically reduced the total count at 20°C for spoilage microorganisms. After a spoilage microbial suspension was treated with an ozone concentration of 2.48% mg/liter for 9 min at a flow rate of 3175 ml/min, its log total count of 8.30 per ml was reduced to 4.95 per ml and the log MPN of coliforms was reduced from 4.97 per ml to 2.63 per ml.

Table 1. Incidence of microflora from air and ozone treated fresh poultry meat microbial suspensions[1]

Gram-stain and Morphology	Microbial Groups[2]	Air Treated		Ozone Treated	
		No. of Isolates	% of Isolates	No. of Isolates	% of Isolates
G+ cocci		23	39.6	29	52.7
G+ rod		22	37.9	19	34.5
	A (*Microbacterium*)	9	15.5	6	10.9
	B (*Lactobacilli*)	6	10.3	12	21.8
	Corynebacterium	7	12.1	1	1.8
G⁻ rod		13	22.4	7	12.7
	C (*P. fluorescens* and *P. putida*)	2	3.45	1	1.82
	E (*Psuedomonas sp.*)	3	5.20	2	3.64
	G (*P. putrefaciens*)	2	3.45	—	—
	K (*Acinetobacter*)	2	3.45	1	1.82
	N (*Flavobacterium*)	2	3.45	2	3.64
	—Others—	2	3.45	1	1.82

[1] Fresh poultry meat suspensions were treated with 2.48 mg/liter ozone and air for 5 min under a flow rate of 3175 ml/min
[2] Microbial groups as described by Freeman *et al.* (1976) were followed

Table 2. Incidence of microflora from air and ozone treated spoiled poultry meat microbial suspensions[1]

Gram-stain and Morphology	Microbial Groups[2]	Air Treated		Ozone Treated	
		No. of Isolates	% of Isolates	No. of Isolates	% of Isolates
G+ rod		4	6.8	7	11.7
	A (*Microbacterium*)	4	6.8	6	10.0
	B (*Lactobacilli*)	—	—	1	1.7
G⁻ rod		55	93.2	53	88.3
	E (*Psuedomonas sp.*)	53	89.8	51	85.0
	G (*P. putrefaciens*)	—	—	2	3.3
	—Others—	2	3.4	—	—

[1] Spoiled poultry meat suspensions were treated with 2.48 mg/liter ozone and air for 9 min under a flow rate of 3175 ml/min
[2] Microbial groups as described by Freeman *et al.* (1976) were followed

A total of 119 cultures were obtained with 60 cultures from the ozone treated sample and 59 cultures from the control sample. The gram-negative rod-type organisms dominated and represented 93.2% of the isolates. The results agree with Ayres (1960), and Arafa and Chen (1977). They suggested that the principle microflora responsible for the spoilage of poultry meat were the gram-negative rod-type organisms. After ozone treatment, the number of gram-negative rod-type organisms was reduced and represented 88.3% of the isolates (Table 2). A slightly higher incidence of gram-positive rods was observed for the ozone treated sample than for the control, 11.7% versus 6.8%.

This study has clearly demonstrated that ozone treatment of broiler parts effectively reduced gram-negative microoganisms on the products. This ozone treatment does not affect the multiplication of the surviving microorganisms. With additional studies on the effect of ozone on the chemical, physical, and organoleptic characteristics of broiler meat, using ozone in the chilling water could be an alternative to chlorination by offering a product with a longer shelf-life.

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HASSON, E. P. and LATIES, G. G. 1976. Separation and characterization of potato lipid acylhydrolases. Plant Physiol. *57*, 142–147.

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