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Edited by T. P. Labuza, University of Minnesota

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COMPARISON OF THE EFFICACY OF THREE POTENTIAL POULTRY PRESERVATIVES: GLUTARALDEHYDE, IODACETAMIDE, AND POLY (HEXAMETHYLENEBIGUANIDE HYDROCHLORIDE)

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ABSTRACT

Freshly processed poultry carcasses were dipped in 500 *ppm solutions of glutaraldehyde, iodoacetamide, and poly (hexamethylenebiguanide hydrochloride), or PHMB, for* 2 *h. They were then drained, packed in polyethylene bags, and held at the storage temperature of* 2 *°C. Total aerobic psychrophile counts and sensory evaluation indicated that the PHMB had the best preservative effect followed by that of iodoacetamide and glutaraldehyde. The shelf-lives of the PHMB, iodoacetamide, and glutaraldehyde-treated birds were found to be* 27.4, 21.6, *and* 15.3 *days, respectively, compared to the* 10.7 *days shelf-life of water-treated controls.*

INTRODUCTION

In recent years, particularly after the 1967 FDA ban on chlorotetracycline, there have been many attempts to find an effective poultry preservative. While the main objective of these attempts was to extend the "refrigerated shelf-life" of fresh poultry, there has been considerable variation in the treatment procedure and the storage temperature used. Cox *et al.* (1974) extended poultry shelf-life from 1—5 days by dipping drumsticks for 1—3 min in 1—5% solutions of succinic acid at 24 and 60 °C. The pH's of 1, 3, and 5% solutions were 2.7, 2.4, and 2.3 respectively. Following treatment, the drumsticks were stored at 1.1 °C for the first 24 h and then at 4.4 °C till spoiled. Islam *et al.* (1978) prolonged the freshness of poultry for 2—4 days at 5 °C by dipping chicken carcasses in 0.05—0.5% solutions of chloroacetamide and iodoacetamide. The pH range of these solutions was between 7.0—7.5. Arafa and Chen (1978) extended shelf-life of cut-up broiler parts for 6—7 days at a storage temperature of 2—4 °C by dipping them in a 1% ascorbic

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acid solution of pH 2.75. Thomson *et al.* (1977) extended broiler shelf-life for 6 days at 2 °C by a 10 min prechill in 0.5% glutaraldehyde solution adjusted to a pH of 8.6. Recently Islam (1979) extended the shelf-life of poultry carcasses for 15 days at 2 °C by immersion in 0.04% solution of poly (hexamethylenebiguanide hydrochloride), PHMB, which had a pH of 6.34.

In view of the above variations, particularly in chemical concentration, pH, and the storage temperature, it is rather difficult to compare the effectiveness of these potential poultry preservatives. A valid comparison would provide a helpful guide for further studies involving chemical residue, toxicity, etc. on the most promising preservative. Hence, this study was designed to compare the efficacy of three potential poultry preservatives: glutaraldehyde, iodoacetamide, and poly (hexamethylenebiguanide hydrochloride) under similar experimental conditions. Compounds exhibiting preservative effect under acidic conditions were not included in this study because the low pH's, particularly those below 4.0, have been shown to retard the growth of poultry spoilage bacteria regardless of chemical structure (Mountney and O'Malley 1965; Islam *et al.* 1978).

MATERIALS AND METHODS

Chemicals

Iodoacetamide in crystalline form and glutaraldehyde as a 25% solution were purchased from Pfaltz and Bauer, Inc. of Stamford, Connecticut. Poly (hexamethylenebiguanide hydrochloride) was obtained as a 20% solution ("Vantocil IB") from the ICI-Americas, Wilmington, Delaware.

Preparation of Dipping Solutions

Four double layered 30 gal. size plastic bags were placed in rigid containers for easier handling. Fourteen liters of distilled water were poured into each of these bags and appropriate amounts of the 3 chemicals were added to the 3 bags for obtaining solutions of 500 ppm each. The fourth bag without any chemical served as the control. The bags were then stored in a walk-in cold room (2±1 °C) for at least a 12 h period prior to use. Small portions (about 25 ml) from each of these solutions were withdrawn with sterile pipettes and the pH's determined by a Beckman digital pH meter. No attempt was made to adjust the pH since that would involve an additional step if these chemicals were to be used in the poultry processing plant.

Sample Preparation and the Chemical Dip

Twenty eight freshly processed broiler carcasses were brought to the laboratory in ice-packed styrofoam containers from the Shorgood poultry plant at Milford, Delaware. The carcasses, in groups of seven, were randomly dipped in the solutions for 2 h at $2 \pm 1\,°C$. They were drained for 10 min on alcohol-swabbed metal racks, packaged in gallon-size clear polyethylene bags, and stored in an incubator at $2 \pm 0.2\,°C$. Four birds from each group were designated for bacterial count and three for sensory evaluation during the storage.

Bacterial Count

Since several studies (Ayres *et al.* 1950; Walker and Ayres 1956; Nagle *et al.* 1960; Arafa and Chen 1975) indicated psychrophiles to be the most dominant organisms in spoiled poultry, total aerobic psychrophile counts of the birds were obtained during the storage period at 5-day intervals. The procedures followed for swabbing, incubation and enumeration were the same as described by Islam (1978). Out of the 4 birds designated for bacterial count, 2 were swabbed on the right breast before and after dipping on day 0, and then on the left breast on day 5 and 10. The remaining 2 birds from each group were swabbed on days 15, 20, 25, and 30. Lack of swabbing space on individual birds necessitated this type of split-sampling procedure. Also, this method minimized the chances of contamination since each bird was handled 4 times, instead of 8, over the 30-day storage period.

Sensory Evaluation

Our earlier experience indicated that the water treated (control) chicken carcasses remain fresh for 10—11 days at a storage temperature of 2 °C. Hence, in this study the sensory evaluation began after a week. On the eighth day of storage and every alternate day thereafter, a five-membered semi-trained panel consisting of departmental faculty and graduate students examined the carcasses for the development of off-odor. Individual judges opened each bag, smelled the carcass and then rated the off-odor on a 5-point hedonic scale where 1, 2, 3, 4, and 5 represent none, slight, moderate, strong, and very strong respectively (Thomson *et al.* 1977). Detection of typical spoilage odor was rated as 4, and hence a bird was deemed to be acceptable as long as it maintained a score of 3 or less. When a bird received a rating of 5 by at least 3 of the 5 panel members it was removed from the observation.

Shelf-life Assessment and Statistical Analysis

The "refrigerated shelf-life" for each bird was calculated based on the number of days it maintained the off-odor score of 3 or less during storage at 2±.2°C. The shelf-life data were then analyzed as a 4 × 5 factorial in a completely randomized design with the chemical treatments and the judges as the main effects. Duncan's multiple range test was carried out to delineate any statistical significant difference among the treatments (Larmond 1970). A parallel analysis was also carried out, in a 12 × 5 factorial arrangement with the chicken carcasses and the judges as the main effects, in order to find if there was any significant difference among the birds within the treatment groups.

RESULTS AND DISCUSSION

pH of Dipping Solutions

The pH's of 500 ppm solutions of glutaraldehyde, iodoacetamide, and PHMB were found to be 6.37, 6.95, and 6.37 respectively at about 2°C. Distilled water used as a control had a pH value of 6.46. The pH values in this range were not likely to exert any preservative effect on the poultry (Montney and O'Malley 1965).

Bacterial Count

The bacterial count obtained from the surfaces of treated birds are presented in Fig. 1. Immersion in the 500 ppm solutions of the 3 chemicals resulted in marked decrease in the total aerobic psychrophile counts on the chicken carcasses. The bactericidal effect of PHMB, however, was much greater than that of glutaraldehyde or iodoacetamide. The average initial psychrophile count on the untreated birds was found to be about 10^3 organisms/cm^2 of skin. After 2 h exposure of PHMB solution the birds had so few organisms that no count could be obtained even at the lowest dilution used (10 fold). The psychrophile counts on the glutaraldehyde and iodoacetamide — treated birds were slightly more than 10^2 organisms/cm^2 of skin. As observed by Islam *et al.* (1978), for the control birds immersed in water, there was a slight decrease in the psychrophile count which may be attributed to the mechanical rinsing effect.

When swabbed on the 5th day of storage, the PHMB-treated carcasses again had too few organisms to count (The unobtainable counts have been estimated and linked with a dotted line in Fig. 1). However, at this stage there was a clear trend in the psychrophile counts of the birds treated with the three chemicals. The control birds had the highest

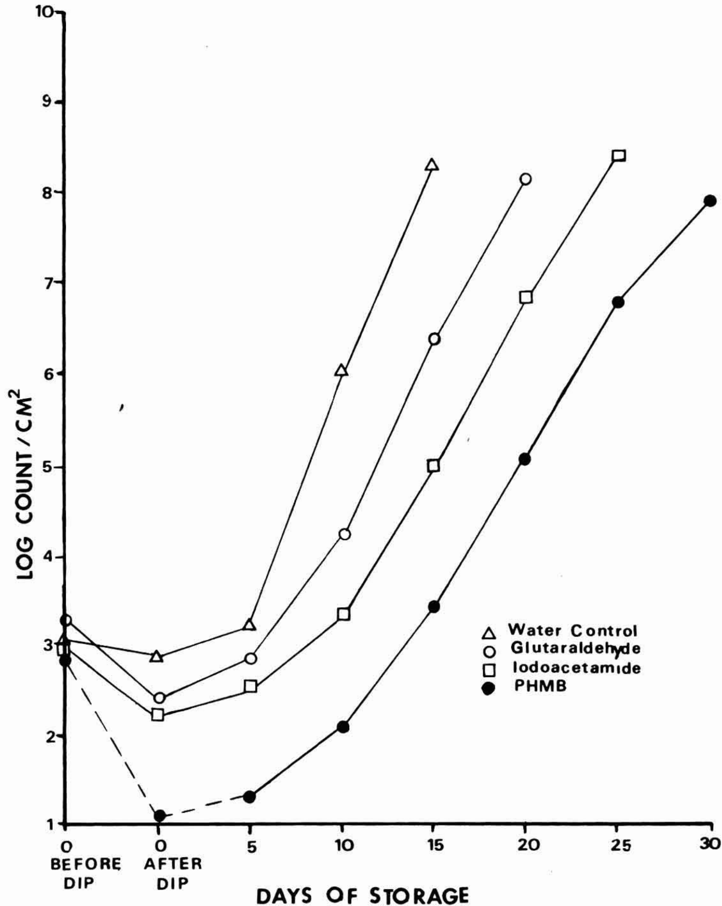

FIG. 1. TOTAL AEROBIC PSYCHROPHILE COUNTS OF BROILER CARCASSES TREATED WITH 500 PPM SOLUTIONS OF GLUTARALDEHYDE, IODOACETAMIDE, AND POLY (HEXAMETHYLENEBIGUANIDE HYDROCHLORIDE), AND STORED AT 2±0.2°C

(Points linked with dotted lines represent estimated counts).

counts which were followed by the counts on birds treated with glutaraldehyde, iodoacetamide, and PHMB respectively. This trend in bacterial counts continued throughout the duration of the experiment.

Sensory Evaluation and the Shelf-Life

The above trend in the total psychrophile counts was reflected by the sensory response of the panel members. Data on the development of off-

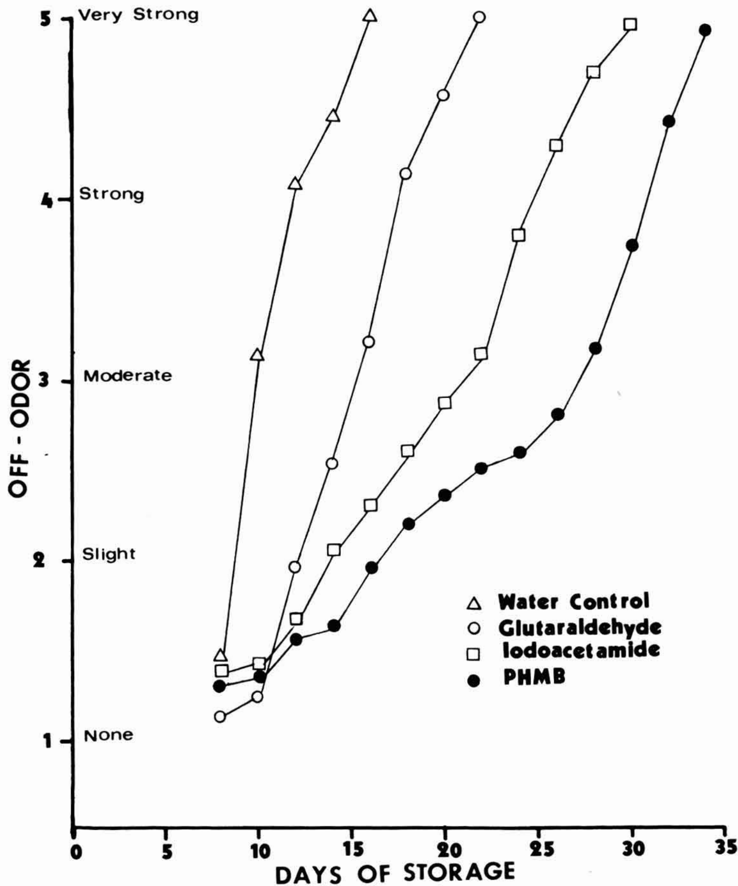

FIG. 2. OFF-ODOR DEVELOPMENT IN BROILER CARCASSES TREATED WITH 500 PPM SOLUTIONS OF GLUTARALDEHYDE, IODOACETAMIDE, AND POLY (HEXAMETHYLENEBIGUANIDE HYDROCHLORIDE), AND STORED AT 2±0.2°C

odor during the storage are presented in Fig. 2. The ratings by the 5 judges on each bird were averaged and then the mean score of the 3 birds per treatment was plotted for each evaluation day. On the first day of the sensory evaluation (that is, on the 8th day of the storage) there was very little difference in the off-odor scores of the birds. However, by the 12th day, the control carcasses had developed a strong off-odor while the chemically treated carcasses exhibited slight or no off-odor. By the 15th day of storage, there was considerable difference in the off-odor scores of the birds treated with the 3 chemicals. The glutaraldehyde treated birds

had the most off-odor and the PHMB-treated birds the least. Subsequently, this difference widened leading to the development of strong off-odor on glutaraldehyde, iodoacetamide, and PHMB-treated birds around the 18th, 26th and 32nd day of storage respectively.

Shelf-life, as mentioned earlier, was calculated based on the number of days the birds maintained the off-odor score of 3 or less. Table 1 presents the shelf-lives of birds treated with the 3 chemicals. Analysis of variance on the 12 × 5 factorial arrangement showed a highly significant difference (P = 0.01) among the shelf-lives of the 12 birds used in sensory evaluation. The Duncan's multiple range test indicated that there was no significant difference (P ∓ 0.01) among the shelf-life values of the 3 control birds. However, within each of the other 3 groups there were significant differences (P = 0.01).

Table 1. Shelf-lives of glutaraldehyde, iodoacetamide, and poly (hexamethylenebiguanide hydrochloride) treated poultry at storage temperature of 2 °C

	Shelf-life (days)[1]	
Treatment (500 ppm)	Individual Birds Mean ± S.D.[2]	Overall Mean for Each Treatment
Water (control)	10.2 ± 0.8a	
	10.6 ± 1.1a	10.7 w
	11.4 ± 0.09a	
Glutaraldehyde	14.0 ± 0.7b	
	15.4 ± 0.9bc	15.3 x
	16.4 ± 1.1c	
Iodoacetamide	19.6 ± 1.1d	
	21.4 ± 1.8e	21.6 y
	23.8 ± 0.8f	
Poly (hexamethylene-biguanide hydrochloride)	25.4 ± 1.7g	
	27.2 ± 1.9h	27.4 z
	29.6 ± 1.7i	

[1]Means not followed by the same letters within each column are significantly different (P = 0.01) according to Duncan's multiple range test.
[2]Standard deviation; five judgements on each bird

The overall mean for the control birds was 10.7 days (Table 1). Glutaraldehyde, iodoacetamide, and PHMB treated birds had average shelf-lives of 15.3, 21.6, and 27.4 days, respectively, at storage temperature of 2 °C. The analysis of variance and Duncan's multiple

range test on the 4 × 5 factorial arrangement indicated that there was a highly significant difference (P = 0.01) between each of these 4 values. In regard to efficacy, the PHMB extended shelf-life by 16.7 days while the iodoacetamide and glutaraldehyde extended by 10.9 and 4.6 days, respectively, beyond the 10.7 days shelf-life of control birds. The result with iodoacetamide, at storage temperature of 2 °C, was rather surprising since Islam *et al.* (1978) using the same concentration found an extension of only 2 days at the storage temperature of 5 °C. Obviously the 3 °C difference in storage temperature was responsible for this large increase in the shelf-life of iodoacetamide treated birds. Direct immersion for 2 h in 500 ppm glutaraldehyde should have given much longer shelf-life since Thomson *et al.* (1977) obtained 6 days extension by only 10 min prechill in 0.5% glutaraldehyde (pH 8.6). Perhaps the unadjusted pH (6.37) was responsible for this relatively short shelf-life. This observation seems to confirm the need for alkaline pH for optimum antimicrobial effect of glutaraldehyde (Borick *et al.* 1964). As observed by Thomson *et al.* (1977), the panel members in this study also noticed the reddening of feather follicles of the glutaraldehyde-treated birds.

Undoubtedly, the preservative effect of poly (hexamethylene-biguanide hydrochloride) is far superior to that of glutaraldehyde and iodoacetamide. PHMB has been marketed for several years, particularly in European countries, as a disinfectant for food processing and brewery fermentation equipment (Boardman 1969). It has also been used as a swimming pool sanitizer (ICI 1973b) and for short-term preservation of cattlehides and sheepskins (Haines 1973; ICI 1973a). A U.S. patent has been issued for preserving beverages by incorporating PHMB up to a level of 50 ppm (Strandskov and Bockleman 1975). In view of these demonstrated applications, and the results of this investigation, PHMB seems to have an excellent potential for use as a poultry preservative. Additional studies are currently being planned to optimize the preservative effect of PHMB and also to monitor its residue on the treated birds as they would move through the various stages of the marketing and the consuming channel.

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RELIABILITY OF THE TINPLATE CAN FOR PACKAGING OF RADAPPERTIZED BEEF UNDER PRODUCTION CONDITIONS

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ABSTRACT

The tinplate can has performed successfully for over a century as a container for thermal processed foods. As a container for irradiation processed foods, its physical, chemical, and protective characteristics had to be evaluated anew. In providing gamma ray irradiated beef in the tinplate can for wholesomeness studies, the reliability of this container for radappertized beef was determined over a significant production period. The production data showed that it is feasible to reliably prepare the cans of beef in a commercial plant, transport the frozen cans of beef packed in fiberboard boxes on commercial equipment, irradiate the frozen cans of beef, transport the radappertized cans of beef at ambient temperature a distance of $3.5 \times 10^6 m$ *(1200 miles), and store the non-refrigerated cans of beef over a 2-year period without any adverse effects on the beef.*

INTRODUCTION

The tinplate can has performed successfully for over a century as a container for thermally processed foods. As a container for irradiation processed foods, its physical, chemical, and protective characteristics had to be evaluated anew. Laboratory evaluation and small-scale production tests showed that the tinplate can, with certain enamels and end-sealing compounds, is acceptable for packaging meat and poultry products irradiated to a dose of 70 kJ/kg (7 Mrad) at temperatures to −60°C (Killoran *et al.* 1974). How this container would perform under strict and definitive criteria during commercial production, irradiation processing, shipment and storage, remained unanswered. In providing frozen control (FC), thermally processed (TP), gamma ray irradiated (GAM), and electron irradiated (ELE) beef for the wholesomeness

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study (Raica and Becker 1973), a concomitant study was performed to determine the reliability of the tinplate can as a container for radappertized beef over a significant production period.

This paper presents the integrity data that were obtained for the tinplate can under the production conditions at Oscar Mayer & Co., Madison, Wisconsin, for the FC, TP, and GAM beef, under the irradiation processing conditions of the GAM beef at the U.S. Army Natick Research and Development Command (NARADCOM), and under the storage conditions of the FC, TP, and GAM at Industrial Bio-Test, Inc., Northbrook, Illinois, where the wholesomeness study was performed.

EXPERIMENTAL

Packaging and Packing of Cans of Beef

The preparation of the beef for seven productions at the Oscar Mayer plant was described by Heiligman *et al.* 1976. The retort process for the TP beef was performed at the Oscar Mayer plant (Cohen 1974). Gamma ray processing was described by MacDonald (1976). The GAM beef and the FC beef were packaged in 404 × 700 tinplate cans with 1.25 kg of beef per can. The TP beef was packaged in 404 × 202 tinplate cans with 369 g of beef in each can. All the cans were coated inside with the epoxy-phenolic enamel and striped at the inside seam. The end-sealing compound was the blend of cured and uncured isobutylene-isoprene copolymer.

The GAM and FC beef were vacuum-sealed on a manually operated, four-station, can closing machine. The TP beef was vacuum-sealed on an automatic production type machine. In both cases, the chamber pressure was set at 7.2 kPa, corrected to standard conditions of temperature and pressure. The cans of beef were packed in V3c fiberboard boxes equipped with top and bottom pads and full height liners and partitions. GAM and FC beef were frozen within 24 h after the can closing operation.

Transportation and Storage of Cans of Beef

The TP and FC beef, the latter maintained at −20°C, were shipped 120 miles from Oscar Mayer & Co., Madison, Wisconsin, to Industrial Bio-Test, Inc. in Northbrook, Illinois, for the wholesomeness study. The GAM beef was shipped 1200 miles while frozen (−25°C) from Oscar Mayer & Co. to NARADCOM for the irradiation processing at 47 kJ/kg to 71 kJ/kg at −35° ± 5°C (MacDonald 1976). The irradiation

processed beef was then shipped at ambient temperature (21° to 30°C) a distance of 1080 miles from NARADCOM to Industrial Bio-Test for the wholesomeness study. In each case, the mode of transportation was via commercial truck. During storage at Industrial Bio-Test, Inc., the FC beef was held frozen at −29°C and the TP and GAM beef were held at ambient temperature (21° to 30°C). The feeding of the beef from each of the seven productions extended over a fifteen-month period.

Inspection of Cans of Beef

Container closure evaluation of the cans of beef was performed in accordance with the procedures of the National Canners Association and The Food Processors Institute (Doyle and Mercer 1975). The integrity of the cans of beef was determined at Oscar Mayer & Co. by making the necessary adjustments on the 2-can closing machines whenever the seam formation deviated from established tolerances and by performing complete seam measurements on a fixed schedule, i.e., removing 6 consecutive cans at the beginning of a production run, and every 2 h thereafter. In addition, visual seam inspections were made on a 100% basis just before the packing of the cans of beef into the fiberboard boxes.

After the irradiation processing of the frozen cans of beef at NARADCOM, they were thawed, dried to remove the surface moisture, and then tested on 100% basis for pressure with a "flip-tester" that had been calibrated against a standard pressure gauge. Rejected cans of GAM beef were subjected to integrity tests including double seam measurements, scanning electron microscopic examination, and headspace gas analysis.

The inspection and pressure testing of the FC, TP, and GAM beef were performed at Industrial Bio-Test just before the preparation of the diet used for one day's feeding. This inspection entailed (a) visual examination of the cans of beef for external damage and for the presence of concavity of the lids as an indication of pressure in the cans of beef and (b) a pressure test of the cans of beef with a calibrated puncture gauge.

Integrity of End-Sealing Compound During Production

Nondestructive pressure testing of 2400 cans of GAM beef from Production 5 (1200 cans) and from Production 6 (1200 cans) was performed (a) 24 and 48 h after vacuum sealing at Oscar Mayer & Co. — temperature of can of beef was 2° to 3°C; (b) just before irradiation of the frozen cans of beef at NARADCOM — temperature of can of beef

was −35° ± 5°C; and (c) after the irradiation processing, thawing and drying of the cans of beef at NARADCOM — temperature of cans of beef was 21°C. Pressure measurements were made with a flip-tester that had been calibrated against a standard pressure gauge.

Periodic Examination of Cans of Beef From Production 5

Emptied cans from Production 5 were subjected to an initial examination 5 weeks after irradiation and again after 3, 6, 12 and 24 months additional storage. Tests performed were concerned with the condition of end-sealing compound, external rust and corrosion, internal discoloration from product, sulfide corrosion, detinning, and enamel quality.

RESULTS AND DISCUSSION

There are several reasons for evacuating cans of meats for irradiation processing. These include the maintenance of container ends in a concave position during normal storage; the reduction of oxygen to minimize reaction of the oxygen with the food; and the prevention of permanent distortion of the container ends because of the irradiation produced gases. Based on the experiments of Pratt *et al.* (1967) the criterion for acceptance was that the can of GAM beef has a pressure not greater than 64.1 kPa corrected to standard conditions. Table 1 shows the results for the number of cans of beef that were rejected during the 7 productions. The percent defective was 2.0 or 793 critical defects for the 100% inspection of the 39,108 cans of GAM beef. Table 2 lists those areas considered as plausible for rejecting the cans of beef. While it was recognized that the volatiles are produced during the irradiation treatment and that these volatiles did increase the pressure within the cans of beef, this increase in pressure was not, of itself, of the order of magnitude to be a cause for rejection of a can of beef. The four areas listed in Table 2 were investigated as potential causes for rejection of cans of beef.

Incomplete Removal of Air During Can Closing Operation: Occluded Air Attributed to Beef Roll

The cans of beef were held at a chamber pressure of 7.2 kPa at 8°C for 2 seconds before sealing to ensure that the internal pressure of a can of GAM beef after the irradiation treatment did not exceed the stipulated pressure of 64.1 kPa at 21°C. The acceptable range of pressures at

Table 1. Rejected cans of GAM beef

Production No.	Irradiated	Cans Rejected	
		Number	Percent
1	4,788	68	1.4
2	4,711	215	4.6
3	5,322	128	2.4
4	4,607	131	2.8
5	6,840	123	1.8
6	6,440	103	1.6
7	6,396	25	0.4
TOTAL	39,108	793	2.0

Table 2. Possible reasons for rejection of cans of beef

1. Incomplete removal of air during can closing operation
2. Double seam defect
3. Permeation of air during shipment
4. Permeation of air or nitrogen during irradiation

each stage of production are listed in Table 3. These pressures were met for the cans of beef in which the beef roll (a) contained very few voids within the interior of the beef roll; (b) had a weight of 1249 g ± 14.2 g; (c) had the proper diameter (9.5 cm) to fit loosely in the can; and (d) had the proper height (15.9 cm) to insure that the distance between the top edge of the beef roll and top edge of the can was at least 1.27 cm.

Table 3. Pressure data for cans of beef

	kPa
24 hours after sealing, 4°C	13.9 to 20.5
After transit, frozen, —30°C	7.2 to 13.9
After irradiation and thawing, 21°C	37.3 to 64.1

The cans of beef that were rejected after the irradiation treatment because the pressure exceeded 64.1 kPa were (a) analyzed for the composition of the major components of the headspace gas and (b) opened and examined with respect to the tightness of fit of the beef roll within

the can, the disposition of the slices of beef that were added to the can either on top of the beef roll or along the side of the beef roll to bring the weight to the proper level, and the number and size of voids within the beef roll itself. Each of these factors could have led to the incomplete removel of air during the can closing operation. Under these conditions, air was drawn mainly from the headspace and only partially from the beef roll itself. The volume of air entrapped within the beading of the 404 × 700 can could be as much as 80 ml with a tight fitting beef roll.

Typical data on the major components and quantities of the headspace gas from accepted and rejected cans of GAM beef and FC beef are shown in Table 4. Trace components were reported by Merritt (1972). The quantity of headspace gas in acceptable cans of GAM beef ranged between 70 ml and 90 ml. The quantity in rejected cans of GAM beef ranged between 170 ml and 250 ml. Based on the volume of the empty can and the volume occupied by the beef roll in the can, it was estimated that the volume of gas produced by irradiation was between 40 ml and 50 ml.

Table 4. Headspace gas analysis for GAM beef

Beef Can	Can Pressure (kPa)	Gas[a] (ml)	Percentage				
			N_2	O_2	CO_2	H_2	CH_4
Accepted	37.3	80	43	1	24	32	1
Rejected	64.1	170	73	1	7	17	2
b	20.5	24	83	7	10	0	0

[a]Collected at 21° C
[b]Frozen control

Examination of the beef rolls from accepted and rejected cans of GAM beef showed that conditions existed in the rejected cans of beef to cause the entrapment of air during the can closing operation. These were the presence of large voids in the interior of the beef roll, and the tightness of fit of the beef roll itself to prevent removal of air except for the headspace. The occluded air was found to be the cause for rejection of 410 cans of GAM beef during the 7 productions.

Occluded Air Attributed to Malfunction of Can Closing Machine

The can closing machine that was used for the can closing of the

GAM beef and FC beef was a manually operated, four-station machine. During the pressure testing of the cans of GAM beef from Production 2A, 183 cans did not meet the stipulated pressure requirement not to exceed 64.1 kPa after the irradiation treatment. Double seam measurements of the rejected cans conformed to the dimensional standards for the 404 × 700 can. Examination of the beef rolls showed that they contained very few voids to occlude air. The problem still persisted after a thin slice of beef had been cut longitudinally from the beef roll to permit removal of air during the can closing operation. Headspace gas analysis of the rejected cans of beef indicated that air was not completely removed during the can closing operation. Thus, prior to the canning of GAM beef for Production 2B, the four stations of the machine were scrutinized for a possible defect in one of them. Pressure tests on 100 cans of water that were vacuum-sealed on each station of the machine showed that 3 stations operated satisfactorily to give the stipulated pressure. The cans from the other station had erratic, high pressures that did not meet the stipulated pressure requirement. Examination of the closure surface of this station showed slight pitting over a small area. By eliminating the defective station during the can closing operations of Productions 3 through 7, no further problem was encountered in rejecting cans of GAM beef for the reason of "occluded air attributed to the malfunction of the can closing machine."

Double-Seam Damage

Double-seam damage was the cause of rejection of 200 cans of GAM beef after the irradiation treatment. Each can was found to have a micro-leak. Fifty-seven cans were rejected because of damage that occurred during shipment from Oscar Mayer to NARADCOM and/or during handling before and after the irradiation processing at NARADCOM. The remaining 143 cans were rejected because of double-seam damage that occurred during the can closing operation. It was classified as "external double-seam damage" and "double-seam damage of the cover hook." The former was observed to be a moderate to severe droop at the side seam lap or in the seam at various points away from the lap. A droop is defined as a smooth projection of the double seam below the bottom of the normal seam. The latter damage was visible only by separating the body and cover hook of the finished double seam by the stripping method (Doyle and Mercer 1975). Inspection of the cover hook showed that it was not only short and uneven, but also had a fracture at the side seam lap area. Figure 1 shows the magnification of the fracture in scanning electronmicrographs at 50X, 100X, 200X and 500X.

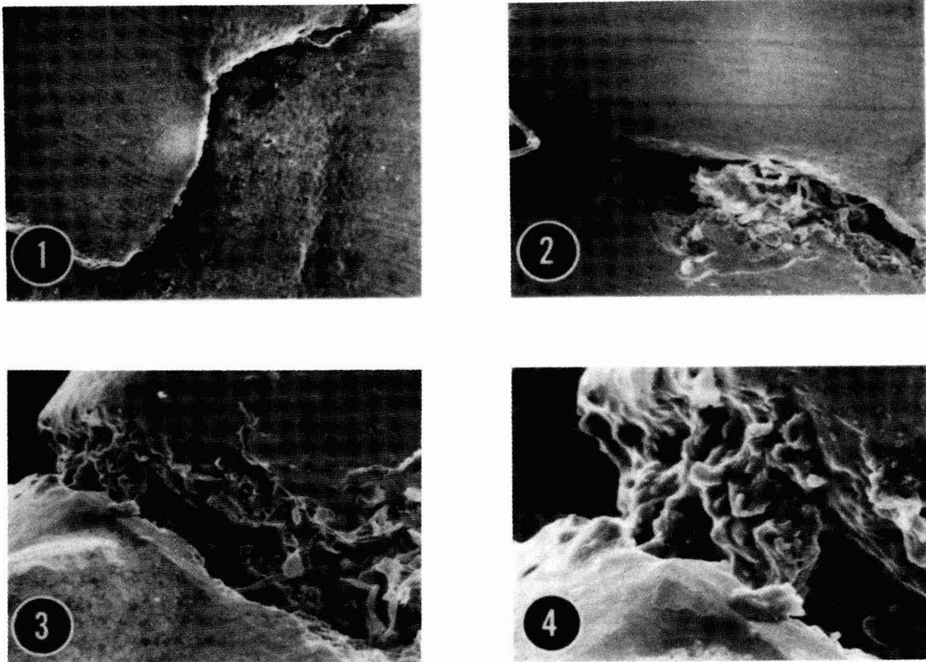

FIG. 1. SCANNING ELECTRON MICROGRAPHS OF FRACTURE AT SIDE SEAM LAP AREA

Integrity of End-Sealing Compound

The rubbery end-sealing compound (blend of cured and uncured isobutylene-isoprene copolymer) that was selected for the double seam of cans has a second order transition point of —65°C. The question was raised as to whether air could permeate the double seam of the cans of beef maintained at —20°C during transportation from Oscar Mayer & Co. to NARADCOM and also during storage at —40°C prior to irradiation. In addition, the question arose as to whether nitrogen would permeate the double seam during the irradiation of the cans of beef at —30°C. Under the conditions of transportation, storage and irradiation, the end-sealing compound stiffened and any movement or flexing of this compound, especially during transportation, could lead to microleaks that would permit entrance of air and/or nitrogen. Thus, two experiments were performed during the canning, storage, and post irradiation stages of production to monitor the pressure of 2,400 cans of beef randomly selected from Productions 5 and 6.

Table 5 lists the number of rejected cans and the cause of rejection

Table 5. Cans of beef rejected during production, transportation, and storage[a]

Cause for Rejection	Number of Rejected Cans			
	Storage: 24 Hours	Storage: 48 Hours	After Transit: Frozen	After Irradiation: Thawed
Pinhole in tinplate	1	0	0	0
Shipment damage	0	0	9	0
Cover hook	0	0	15	0
Occluded air	0	0	11	1
External seam	15	0	0	0
TOTAL	16	0	35	1

[a]2,400 cans from Productions 5 and 6

for the 2,400 cans of beef. Sixteen cans were rejected 24 h after storage, one for a pinhole in the tinplate and 15 for external double-seam damage attributed to the can closing machine. No cans were rejected after the 48-hour storage. Thirty-five cans were rejected after transit while frozen from Oscar Mayer & Co. to NARADCOM. Nine cans were rejected for shipment damage, 15 for microleak of cover hook, and 11 for occluded air. After irradiation and thawing, one can was rejected. Cause of rejection was attributed to occluded air. The total number of rejected cans was 52 or a percent defective of 2.2. Considering that 43 cans were rejected for causes (pinhole, cover hook, occluded air, external seam) that can be remedied under adequate production conditions, the conclusion may be drawn that the end-sealing compound, the blend of cured and uncured isobutylene-isoprene copolymer, does maintain the integrity of the cans of beef during shipment while frozen and during the irradiation of these cans at $-40° \pm 5°C$ to $-14° \pm 5°C$. In addition, these experiments showed that in those cans that were rejected because of occlusion of air within the beef roll itself or the beading area of the 404 × 700 can, the air diffuses very slowly in attaining a state of equilibrium within the can. This is shown in Table 5. Eleven cans were rejected "after transit-frozen" and one can "after irradiation-thawed." Thus, the pressure test for frozen cans of meat just before irradiation offers a means to remove cans of beef that otherwise would be rejected after the irradiation treatment.

Nitrogen-Argon Ratio

The frozen cans of beef were maintained at the −40°C set point

during gamma ray irradiation by the introduction of liquid nitrogen under controlled conditions (MacDonald 1976). Thus, the presence of a nitrogen atmosphere during the irradiation processing offered a means to acquire additional evidence to support the integrity of the end-sealing compound. Argon is present in air in the amount of 0.9%. If nitrogen permeated the end-sealing compound during the irradiation processing of the cans of beef, then the nitrogen-argon ratio of the headspace gas should increase, approaching as a limit the nitrogen-argon ratio of the liquid nitrogen. The nitrogen-argon ratios for air, liquid nitrogen, the headspace gas of three swollen cans of GAM beef, and the headspace gas of one can of GAM beef, that met the pressure requirements of Table 3 are listed in Table 6. These ratios were obtained from mass spectral analyses. The data show that there is no difference within the tolerance of the analysis between the nitrogen-argon ratio of air and nitrogen-argon ratio of a can of beef. The nitrogen-argon ratio of the three swollen cans of beef from Productions 2, 3, and 4, and the acceptable can of beef are considered to be identical within the tolerance of the analysis. The conclusion is that the nitrogen did not permeate the cans of beef during the irradiation processing.

Table 6. Headspace gas: Nitrogen/argon ratio

Sample	Nitrogen/argon Ratio[b]
Liquid nitrogen	88
Air	58
GAM beef, Production 2	54[b]
GAM beef, Production 3	55[b]
GAM beef, Production 4	55[b]
GAM beef, Production 4	57[c]

[a]Mass: nitrogen 28, argon 40
[b]Swollen can of beef
[c]Acceptable can of beef

Classification of Defective Cans of GAM Beef

The stipulated pressure of the cans of irradiation sterilized beef was not achieved because of incomplete removal of air during the can closing operation and double-seam damage. The incomplete removal of air during the can closing operation was attributed to: (1) the presence of occluded air within the voids of the beef roll and/or occluded air within the beading of the can because of the tight fit of the beef roll or

because of the slices of beef that were stuffed alongside the beef roll to achieve the proper weight; and (2) to a malfunction in one station of the can closing machine during Production 2. Double-seam damage was attributed to malfunctioning of the seaming mechanism of the can closing machine, to shipment of cans of beef while frozen from Oscar Mayer to NARADCOM and to handling of the frozen cans of beef before and after the irradiation processing.

Table 7 summarizes the critical defects attributed to the incomplete removal of air and double-seam damage for each production. The percent defective was 2.0 or 793 critical defects based on 100% inspection of 39,108 cans of GAM beef. The question was raised as to whether the GAM beef in the 404 X 700 tinplate can be reliably prepared under production conditions with a percent defective of 0.1% or one critical defective can of GAM beef per 1000 cans of GAM beef. By dividing the causes of rejection into assignable and non-assignable causes of rejection, and, furthermore, by assuming that the assignable causes of rejection can be remedied under improved production conditions, then a prediction can be made with regard to the percent defective during the commercial production.

Table 7. Critical defects in production of beef

	Incomplete Removal of Air		Double-Seam Damage	
Production Number	Occluded Air	Sealing Machine	Shipment and Handling	Sealing Machine
1	57	0	8	3
2	21	183	4	7
3	107	0	13	8
4	109	0	11	11
5	93	0	14	16
6	4	0	4	95
7	19	0	3	3
TOTAL	410	183	57	143
Percent Defective	1.05	0.45	0.14	0.36

Incomplete removal of air during the can closing operation and double-seam damage attributed to the sealing machine may be classified as assignable causes for rejection that can be remedied under improved production conditions. In the case of the occluded air within the beef

roll and/or the can itself, adequate production conditions are those described previously in this paper. It should be noted that only 4 cans of GAM beef were rejected during Production 6. In the case of the incomplete removal of air because of a malfunction in one station of the can closing machine, 183 (0.45%) cans were rejected during Production 2. This type of malfunction is a rare occurrence in a commercial plant and can be remedied by adherence to standard inspection procedures.

The double-seam damage attributed to the seaming mechanism of the can closing machine may be classified as an assignable cause of rejection of the cans of GAM beef and can be remedied during a preproduction run in which (1) the seaming stations of the can closing machine were adjusted for the 404 × 700 cans to give seam dimensions within the tolerances of the standard double seam dimensions; (2) 100% inspection of the cans after the sealing operation; and (3) inspection of cans for defects by the stripping method to expose the internal components of the seams. Thereafter, the quality control procedure would be one of auditing the production line of the sealed cans of beef, in accordance with the procedures required by the National Canners Association (Doyle and Mercer 1975).

The double-seam damage attributed to shipment and handling of the frozen beef may be classified as a non-assignable cause for rejection of the cans of GAM beef even though adequate packing protection was given to the frozen cans of beef during shipment and handling of the fiberboard boxes. The adequate protection entailed the use of a weather-resistant fiberboard box (V3c) with a liner, top and bottom pads, and partitions to prevent contact between any two cans of beef. The percent defective attributed to shipment and handling was 0.14.

The food irradiation plants of the future will be designed for the preparation and canning of the enzyme inactivated beef (or any other meat product) and subsequent irradiation of the frozen cans of beef at the same site, thus eliminating shipment of frozen cans of beef, and requiring much less handling of the frozen cans of beef.

Based on the evidence presented in regard to assignable and non-assignable causes for rejection, and, furthermore, on the assumption that the stated assignable causes for rejection may be remedied under improved production conditions, the percent defective for the seven productions of radappertized beef was 0.14. All the defects, considered as a non-assignable cause for rejection, were attributed to double-seam damage that occurred during shipment and handling of the frozen cans of beef — accounting for 7.2 percent of the total number of defects.

In the shipment of the thawed cans of beef after irradiation process-

ing from NARADCOM to Industrial Bio-Test (1080 miles), four cans out of 37,790 cans of GAM beef were rejected during the entire feeding study. Here again, rejection was attributed to double-seam damage that occurred during handling and shipment. The percent defective was 0.016.

Integrity of TP and FC Cans of Beef

The stipulated pressure requirement for the cans of TP beef and FC beef, that were used on a daily basis at Industrial Bio-Test, Inc. for preparation of the animal diets, was that the pressure did not exceed 80.9 kPa. Pressure tests on cans of TP beef between April 1971 and March 1974 showed that the pressures for the cans of beef ranged between 37.3 kPa and 28.1 kPa. Industrial Bio-Test, Inc. rejected only 3 cans of TP beef during this period. The 3 cans were from Production 1. During the same period, the pressures taken on cans of FC beef were not less than 20.5 kPa. Industrial Bio-Test, Inc. rejected 6 cans that did not meet the pressure requirement. Each can had double-seam damage.

Periodic Examination of Empty Cans From Production 5

The TP and GAM cans of beef had been stored at 20° to 30°C over the 2-year period. Internal discoloration from product, sulfide corrosion and detinning was minimal and comparable for the FC, TP and GAM beef cans. Underfilm product staining was confined to side-seams where no enamel was present to cover the stain. Detinning was rare. It occurred primarily at sideseam folds and notch areas. The enamel for FC, TP and GAM beef cans showed no softening, blistering, flaking, or adhesion loss over the 2-year storage period. Compared to FC cans, slight fading of enamel color occurred for the TP and GAM cans. The needle probe test indicated that there was softening of the end-sealing compound from the GAM cans, but not for the FC and TP cans. The softening of the end-sealing occurred during the irradiation processing because of the slight degradation of the isobutylene-isoprene copolymer. Slight external corrosion of the GAM, FC and TP cans occurred during the storage period. This corrosion was equivalent for the GAM, FC, and TP cans.

Judging from the observations and tests performed on empty cans during the 2-year storage period, the gamma irradiation had no detrimental effect on the performance of the tinplate can that was used to package the GAM beef for the wholesomeness study.

CONCLUSION

Review and analysis of the production data show that adequate information exists to assure the acceptability of the commercial tinplate can for packaging radappertized beef. Specifically, the data show that it is feasible to reliably prepare cans of beef on a production scale, transport the frozen cans of beef packed in fiberboard boxes on commercial equipment, irradiate the frozen cans of beef packed in fiberboard boxes, transport the radappertized cans of beef at ambient temperature to a destination point, and store these cans over a 2-year storage period without refrigeration.

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RELIABILITY OF FLEXIBLE PACKAGING OF RADAPPERTIZED BEEF UNDER PRODUCTION CONDITIONS

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ABSTRACT

The flexible package for radappertized food must have the physical and chemical properties to withstand irradiation processing while frozen and retain its protective qualities during transportation and storage. In providing electron irradiated beef for wholesomeness studies, a concomitant study was performed to determine the reliability of the flexible package as a container for radappertized beef over a significant production and storage period. The data showed that it is feasible to fabricate preformed pouches on commercial equipment, to vacuum seal pouches of beef on a production scale, to irradiate and transport pouches of beef while frozen, and to store the radappertized beef over a 2-year period without refrigeration and without any adverse effects on the beef.

INTRODUCTION

The flexible package for radappertized foods must have the physical and chemical stability to withstand irradiation processing in the frozen state and to retain its protective qualities during storage of food without any adverse effects on the food contained therein. Research and development work was concerned with the physical and chemical changes resulting from the electron irradiation and long-term storage of polymeric films; food compatibility of these films as determined by extractives in the presence of food simulating solvents (Killoran 1972); and pack tests of pouches containing beef, ham, pork and chicken, to determine their microbial resistance after the rough handling in laboratory and field abuse treatment (Payne *et al.* 1969). Based on these studies, certain multilayer materials were developed and found to possess the essential properties to package radappertized meat and poultry products (Killoran and Wierbicki 1972). How the flexible

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package would perform under strict and definitive criteria during commercial production remained unanswered. In providing electron irradiated (ELE), gamma ray irradiated (GAM), thermally processed (TP), and frozen control (FC) beef for the wholesomeness study (Raica and Becker 1973), a concomitant study was performed to determine the reliability of the flexible package as a container for radappertized beef over a significant production and storage period.

EXPERIMENTAL

Packaging and Packing of Flexible Packages of Beef

The preparation of the ELE beef for the seven productions was described by Heiligman *et al.* 1976. The flexible package was a preformed multi-layer pouch (11.5 cm × 17.8 cm). The materials used for fabricating the pouches consisted of a tough, heat resistant outside layer of nylon 6 (25μ), a middle layer of aluminum foil (9μ), and an inside layer of the intermolecularly bonded polyethylene terephthalate-medium density polyethylene (62μ). The polyethylene was the food contacting layer. The adhesive between the outside layer and the aluminum foil was an epoxy modified polyester and, between the inside layer and the aluminum foil, an ethylene-acrylic acid copolymer. The roll stock and the preformed pouches with bottom and side seals were manufactured in two productions on commercial equipment at the Continental Can Co. plant in Mt. Vernon, Ohio. Each production consisted of 250,000 pouches.

The enzyme inactivated beef slices in rectangular form (11.4 cm × 7.6 cm × 1.3 cm) were filled into the pouches, and then were vacuum-sealed on the Swissvac machine, CVEP-100, at a rate of 32 pouches per minute. Special attention was given to having opposing seal surfaces flat during the sealing operation, thus avoiding wrinkling in the seal area. The pressure gauge of the machine was set at 7.2 kPa. Sixteen pouches of beef were packed flat in a paperboard box constructed in accordance with Fed. Spec. PPP-B-566, Boxes, Folding, Paperboard. In turn, eight filled paperboard boxes were packed in a fiberboard box constructed in accordance with Federal Specification PPP-B-636, Boxes, Shipping, Fiberboard. Figure 1 is a flow chart of the production operations.

Transportation and Storage of Pouches of Beef

The ELE beef was shipped 1200 miles while frozen (−25°C) from

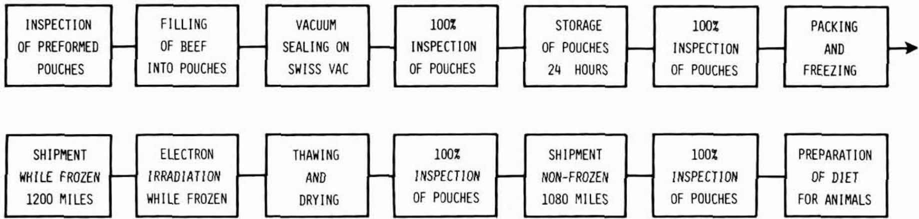

FIG. 1. FLOW CHART FOR PRODUCTION OF RADAPPERTIZED BEEF IN FLEXIBLE PACKAGES

Oscar Mayer & Co. to NARADCOM for the irradiation processing (Rees and Caspersen 1976). The irradiation processed beef was thawed and then shipped a distance of 1080 miles at ambient temperature from NARADCOM to Industrial Bio-Test for the wholesomeness study. Each shipment was via commercial truck. During storage at Industrial Bio-Test, the ELE beef was held at ambient temperature ranging between 21° and 30°C. The feeding of the beef from the 7 productions extended over a 15-month period.

Empty pouches from Production 5 were examined 5 weeks after irradiation and again after 3, 6, 15 and 24 months additional storage. Tests performed were concerned with condition of the seals, bonding between layers, internal discoloration from the beef, and visual aberrations such as heat creep, wrinkles in seal and body areas of pouches. One hundred pouches were used for each storage period.

Inspection Plan

Table 1 lists the tests and sampling plan for measuring the integrity of seals and the bonding between any two layers of the multilayer pouch throughout production of the preformed pouches and examination of pouches of beef after the vacuum sealing operation, after the irradiation processing, and prior to the preparation of the diets.

The preformed pouches were inspected in lots of 10,000 completed pouches. Each lot was examined for visual and dimensional defects, and tested for burst, seal, and bond strengths (Payne *et al.* 1969). The inspection sample for visual and dimensional defects was 80 randomly selected pouches for each inspection lot of 10,000 pouches. The acceptable quality level was that the lot be accepted with a maximum of 5 defects out of 80 pouches and the lot rejected with 6 or more defects out of 80 pouches. In testing of the pouches for burst, seal and bond strengths, the inspection sample was 20 randomly selected pouches for each inspection lot of 10,000. The acceptable quality level was that the

Table 1. Inspection plan for preformed pouches and pouches of beef during production and after irradiation

Sampling	Test	Number of Samples	Lot	Rejection Criterion
After pouch formation[1]	(a) Visual: dimensional	80	10,000	Accept on 5 Reject on 6
	(b) Burst, seal bond strengths	20 per test	10,000	Accept on 2 Reject on 3
After vacuum sealing	(a) Burst strength, top seal	5 consecutive, first hour, at 15 min interval	480	1
		5 consecutive per hour[2]	1,920	1
	(b) Visual	100%		All defective packages removed
After 24 h storage	Visual	100%		All defective packages removed
After irradiation processing	Visual	100%		All defective packages removed
Before diet preparation	Visual	100%		All defective packages removed

[1] Military Standard: MIL-STD-105D — Sampling procedures and tables for inspection by attributes
[2] Starting after second hour

lot be acceptable with a maximum of 2 failures out of 20 pouches and the lot be rejected with 3 or more failures out of 20 pouches. Basis for rejection was that burst strength was less than 1.4×10^5 Pa over a 30-s interval, with a maximum seal yield of 1.6 mm, the seal strength was less than 2.1×10^3 N/m, and the bond strength was less than 175 N/m.

The productions were monitored at startup during the first hour of operation by removing 5 consecutive pouches of beef after the vacuum sealing operation at 15-min intervals. These pouches were tested for burst strength of the top seal. Thereafter, 5 consecutive pouches of beef were removed from the production line after each hour of operation. These pouches were tested for burst strength of the top seal. Visual inspection of the pouches of beef for the presence of defects and the tightness of fit of the packaging material to the beef, as an indicator of residual air within the pouch, was performed on a 100% basis after the vacuum sealing operation and again after 24 h storage at 3°C. The 100% inspection was also performed after the electron irradiation and thawing of the pouches of beef from Productions 1, 2, 3, 6 and 7, and 25% inspection for Productions 4 and 5. The reduced inspection level was introduced because only one pouch out of 179,674 pouches was rejected in Productions 1, 2, and 3. The fourth 100% inspection was performed at Industrial Bio-Test just prior to the preparation of the diets for the animal feeding.

RESULTS AND DISCUSSION

There were several reasons for maintaining the low gauge pressure of the Swissvac machine during the vacuum sealing operation of the pouches of beef. These included: (1) the maintenance of a tight adherence of the multilayer material to the solid piece of beef to ensure that residual air within the pouch was minimal; (2) the reduction of oxygen in residual headspace gas to minimize reaction of the oxygen with the food prior to and/or during irradiation processing; and (3) to offset the effect of the small amount of headspace gas produced during irradiation processing. A gauge vacuum setting of 686 mm Hg was found to be satisfactory while maintaining the production rate of 32 pouches per minute. This gauge setting, corrected for the temperature of the meat at 4°C and the barometric pressure of 740 mm Hg, was calculated to be 54 mm or 7.2 kPa.

The inspection of the fiberboard boxes containing the frozen pouches of beef after shipment to the irradiation site and after the irradiation processing and thawing constituted the basis for the reliability calculations. The "shipment and handling of the frozen pouches of

beef" and "the irradiation of the frozen pouches of beef" were considered to be the major technical areas that had not been fully assessed on a commercial scale with regard to the integrity of the flexible container for packaging radappertized beef. Pouches of beef that were rejected during the other inspections were recorded to determine where production improvements were needed. Placement of pouches of beef on the sealing machine was a manual operation that required proper positioning of the pouches of beef onto the pouch holders. Otherwise, wrinkled seals resulted during the sealing operation. All pouches with wrinkled seals that were inspected after the sealing operation were rejected and the beef was repacked in new pouches. The percent defective for the pouches of beef inspected after the vacuum sealing operation was 0.03; percent defective for the pouches of beef inspected 24 h after the sealing operation was 0.01; and percent defective for the radappertized pouches of beef inspected just before the preparation of diets was less than 0.01.

Inspection After Irradiation Processing

The results for the number of pouches of beef rejected during the seven productions after the irradiation processing are shown in Table 2. The percent defective was 0.04 or 185 critical defects based on the 100% inspection of 441,470 pouches of beef. One hundred and twelve defects (0.16%) occurred in Production 6. The total number of critical defects for the other 6 productions was 73 or a percent defective of 0.012.

Table 2. Rejected pouches of electron irradiated beef

Production	Number of Pouches of Beef	Number Inspected Percent	Rejected[1]	
			Number	Percent
1	71,084	100	1	0.01
2	53,770	100	0	0.0
3	54,820	100	0	0.0
4	51,236	25	7(28)[2]	0.013(0.05)
5	74,458	25	8(32)[2]	0.01 (0.04)
6	69,590	100	112	0.16
7	66,512	100	12	0.02
TOTAL	441,470		140(185)[2]	0.03 (0.04)

[1] Inspected after irradiation processing
[2] Corrected to 100%

Classification of Defective Pouches of Beef

Major causes for the rejection of the pouches of beef were attributed to puncture in the body of the pouch (37.2%), wrinkle in the closure seal (25.0%), and side seal microleak (21.4%). In all, they accounted for 83.6% of the defects. The other defects were attributed to the presence of meat particles in the seal area (6.4%), extension of the side seam notch into the body of the pouch (2.9%), the presence of splice material in the body of the pouch (2.1%), the presence of an internal notch in the body of the pouch (1.4%), and pinholes in the aluminum foil (1.4%). These results are presented in Table 3 along with the cause of each defect.

Table 3. Cause for rejection of the pouches of beef

Defect Type	Cause	Number[1]	Percent
1. Puncture in body of pouch			
a. Handling after sealing	Packing into paperboard boxes	20(12)[2]	14.3
b. Sealing machine	Burrs on pouch holder	32(30)	22.9
2. Wrinkle in closure seal	Improper positioning onto pouch holder	35(33)	25.0
3. Side seal microleak	Incomplete fusion during fabrication	30(24)	21.4
4. Meat in seal area	Improper bottom-to-top fitting	9(4)	6.4
5. Extended notch	Folding of pouch during packing	4(3)	2.9
6. No closure seal	Human error during inspection	3(3)	2.1
7. Splice material	Improper manufacture of pouch	3(3)	2.1
8. Internal notch	Improper manufacture of pouch	2	1.4
9. Pinhole in aluminum foil	Imperfection in aluminum roll stock	2	1.4

[1] 7 productions
[2] Number of Production 6 rejections in parentheses

Puncture in the body of the pouch was attributed to the presence of burrs on one pouch holder of the sealing machine and to handling of the pouches of beef after sealing and during the packing of the pouches

of beef into the paperboard boxes. Both types of puncture were present as microleaks that did not indicate a "pouch failure" until after irradiation processing. Both types of puncture were classified as assignable causes for rejection. It should be noted that 30 of the 32 punctures caused by burrs on the pouch holder of the machine were from Production 6.

Wrinkles in the closure seals were caused by improper positioning of the pouches of beef onto the pouch holder. This operation was performed manually. It was a tedious operation requiring that the top opposing seal surfaces were flat and parallel. This cause for rejection was classified as an assignable cause for rejection that can be reduced by the use of taut tensioning by means of clamps or grippers, spring-loaded tensioning devices, or other mechanical means.

Seal contamination by meat particles occurred during the manual filling of meat slices into the pouches. Stainless steel filling tubes were used for this operation. The use of pouch carriers of formed guards that swing down into the package opening at the moment of filling to protect the inner seal surfaces offers a means to prevent seal contamination (Duxbury *et al.* 1970). Thus, seal contamination during the filling operation was classified as an assignable cause for rejection that can be improved by the use of the recently developed filling apparatus.

The other causes for rejection of the pouches of beef were attributed to defects that occurred during the manufacture of the pouches. These defects were the side seal microleak, extended notch, splice material, internal notch, and pinhole in aluminum foil. Each defect was classified as an assignable cause for rejection that can be reduced by improving the techniques used to manufacture the pouches and/or by tightening the inspection procedures for acceptance of the preformed pouches.

Integrity of ELE Pouches of Beef During Transportation and Storage

The inspection of the pouches of beef after irradiation processing at NARADCOM showed that the shipment of the beef, a distance of 1200 miles while frozen, caused no damage to the pouches of beef. Inspection of the pouches of beef after shipment, a distance of 1050 miles at ambient temperature, to Industrial Bio-Test showed that 11 pouches were damaged. Cause of rejection was attributed to puncture in the body of the pouch. Six of the rejected pouches were from Production 4, one each from Productions 2 and 3, and 3 from Production 6.

These observations indicate that the packing of 16 pouches of beef in the paperboard boxes, that were in turn packed in fiberboard boxes,

gave adequate protection to the pouches of beef during shipment, either while frozen or at ambient temperatures.

Visual examination of empty pouches from Production 5 in which the ELE beef had been stored 5 weeks and 3, 6, 15, and 24 months at 21° to 30°C, showed that the pouches were in excellent condition. These pouches were opened on the bottom seal to remove the beef in order to keep the top seal intact for bond strength and burst strength tests. One hundred pouches were tested at each storage period. There was no discoloration of the food contacting layer. Heat creep was not present in either the seal or body areas of the pouches. There was no evidence of major wrinkles. The effect of the irradiation treatment and storage over the 24-month period on bond strength and burst strength is shown in Table 4.

Table 4. Effect of irradiation and storage on bond strength and burst strength

	Bond Strength[1] N/m	Burst Strength[1] Pa
Before irradiation	178	1.9×10^5
After irradiation	535	2.4×10^5
Storage: 24 months[2]	533	2.4×10^5

[1] Test method: Payne *et al.* 1969
[2] 21° to 30°C

Based on previous research and development work (Killoran 1972), the criteria for high performance bond strength and burst strength was that the bond strength was not less than 178 N/m and the burst strength not less than 1.4×10^5 Pa with a maximum seal yield of 1.6 mm over a 30-s interval. The data show that the pouches from Production 5 not only exceed these requirements, but also show an increase in bond strength and burst strength after irradiation processing (Killoran and Wierbicki 1972). All pouches tested maintained bond strength and seal strength near their "after irradiation" values throughout the 24-month storage period. The constancy of the bond strength during storage is indicative of no fat permeation through the food contacting layer to cause delamination between this layer and the aluminum foil. This barrier to fat permeation was attributed to the presence of the polyethylene terephthalate that is intermolecularly bonded to the polyethylene food contactant. Overall, the data indicate that the multilayer

pouch performed satisfactorily as a container for the ELE beef during irradiation processing, transportation, and storage.

CONCLUSION

Review and evaluation of the production data show that adequate information exists to assure the acceptability of the flexible package as a container for radappertized beef. Specifically, the data show that it is feasible to fabricate preformed pouches on commercial equipment, to reliably prepare pouches of beef on a production scale, to transport the frozen pouches of beef packed in paperboard boxes, to transport the radappertized pouches of beef while nonfrozen to a destination point, and to store these pouches of beef over a 2-year period without refrigeration and without any adverse effects on the food contained within the flexible container. To be sure, defects in pouches will be greatly reduced or eliminated by modification of the design features of equipment to provide automated pouch filling stations, apparatus to closely shape and position the filled pouches before vacuumizing and sealing, and techniques for the elimination of the manual handling of pouches during the packing operation.

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EXPERIENCES IN THE OPERATION OF FOOD IRRADIATION FACILITIES

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ABSTRACT

At the Federal Research Centre for Nutrition, Institute of Radiation Technology, Karlsruhe, several different radiation facilities of semi-industrial type have been used in recent time for irradiation of foods (Diehl 1973; Grünewald 1973). While these are machine sources (electrons, X-rays), some experience has also been gained with a mobile Cs-137 source, with a stationary Co-60 source and with an X-ray machine installed aboard a fishing vessel. Experiences concerning reliability in operation, dose distribution, throughput capacity etc. are reported.

EXPERIENCES

Experiences With a Linear Accelerator

For laboratory scale research a linear accelerator is available with an energy range from 3 to 22 MeV with a maximum beam power of 6 kW, at 10 MeV. The best stabilization is obtained at this energy with a beam power of 3.2 kW. The theoretical throughput is 1,150 t • krad/h but the efficiency is limited by the conveyor system and the limitations for the ratio of the D_{max}/D_{min}.

For the irradiation of products in a large volume with a low dose (e.g. potatoes or onions) the efficiency is only 3%. All potatoes have to be loaded by hand onto trays with a dimension of 40 to 40 cm, and they are irradiated in one layer. The design of the conveyor system requires a certain minimum distance between two following trays and this reduces the efficiency of beam utilization. For much low-dose treatments the speed of the conveyor is at its maximum (about 35 cm/s) and there is only one passage of the goods through the irradiation field.

For radiation sterilization doses in the range of some Mrads a number of passages (e.g. 28 passages for 3.5 Mrad) at a speed of 2.8 cm/s is

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needed. The samples on the trays are moved to and fro in the irradiation field and in this way an efficiency of about 50% is achieved.

The problems of the dose distribution in the depth, that is in the beam direction, are well known and are the same for all electron accelerators. But the low scanning frequency of 10 Hz and the beam pulsing of the linear accelerator produce problems of homogeneity of dose distribution on the surface of the sample and in all other planes vertical to the radiation beam. Therefore there are some critical speeds for the conveyor system if one irradiation pulse should be arranged exactly beside the next one or if it is required that every point of the product should be hit by the same number of pulses, e.g. by 10 ± 1 pulses.

At a scanning frequency of 10 Hz and a pulse frequency of 170 Hz, 17 pulses are arranged on one line, one beside the next one over a length of 40 cm. If we want to have 10 radiation pulses on every point, the sample has to be moved in 1 s by a distance which corresponds to the diameter of the beam. The critical speed v_n and the critical pulse frequency can be calculated from beam diameter d, scanning with A, and scanning frequency f_A by the following equations (Grünewald 1966):

$$v_n = \frac{1.64 \cdot d \cdot f_A}{2^{n+1}}$$

$$f_p = \frac{2^{n+2} \cdot A}{1.64 \cdot d} \cdot f_A$$

for all $n \geqslant 0$.

Under these circumstances it is advantageous to keep the speed of the conveyor system and the pulse frequency constant if possible and to vary the dose only by changing the beam current or the number of passages through the radiation field.

With respect to dose rate a problem of definition arises. Depending on whether one considers the period for one pulse, for one scanning, for one passage or for the total irradiation time including the dead time needed for transporting the samples out of the radiation field, the dose rate is quite different (Grünewald and Rudolf 1975). In a single pulse the dose rate is 10 exp 10 rad/s. Considering the total irradiation process the dose rate is about 10 exp 3 rad/s.

Using a tungsten target, the electron beam can be converted to X-rays. The efficiency of converting depends on the primary energy. Normally 10 MeV X-rays are produced with a dose rate of 10 exp 7

rad/s. The theoretical throughput is about 10 t • krad/h and the practical throughput is 4.8 t • krad/h. The efficiency of beam utilization is higher than in electron irradiation because the penetration value of the X-rays is higher and the goods (e.g. potatoes) can be irradiated in more than one layer.

Experiences With a Mobile Cs-137 Source

For a number of years, laboratories in 5 countries of the European Community have collaborated in a joint program of research on irradiation of potatoes. In the course of this project, which was sponsored by the Bureau EURISOTOP, Brussels, we tested the suitability of irradiated potatoes for production of chips. Fifty tons of potatoes were irradiated in a warehouse of the chips' manufacturer. They were stored in 1 m^3 boxes for periods up to 8 months before being processed. Chips, produced from irradiated potatoes were compared with chips produced from potatoes treated with chemical sprout inhibitors and stored in the same warehouse under the same conditions. This was to reproduce the industrial conditions for irradiation, storage and processing of potatoes (Grünewald *et al.* 1976).

For the irradiation we used the mobile irradiator of the French firm Conservatome, which had to be transported from Paris to Neunburg in the Northeast of Bavaria. Numerous administrative difficulties had to be overcome before permission was obtained to transport the irradiator across the French-German border.

The facility's Cs-137 source has 175 kCi and the maximum theoretical throughput is 230 t • krad/h. But the conveyor system and the limitations for the D_{max}/D_{min} rate lower the efficiency. The facility has 22 containers which are moved periodically from one of the 24 positions on the conveyor system to the next one. The applied dose depends on the resting time of the containers on the positions. With the shortest resting time a dose of about 10 krad can be applied. Therefore the conveyor system has to work at the highest speed for sprout inhibiting irradiate of potatoes. It is impossible to apply a lower dose, e.g. to irradiate onions for sprout inhibition. This is a disadvantage of some food irradiation facilities.

In a continuous run over 8 h per day and over 5 days, 1 to 1.2 t of potatoes per hour were irradiated with a dose between 10 and 15 krad. This corresponds to an efficiency of beam utilization of 7%. This low efficiency was due to a reduction of the volume of the irradiation containers. Normally they have a volume of 54 l but only 13 l could be used for the product to get an acceptable dose homogeneity and a ratio D_{max}/D_{min} lower than 1.5. The reduction of the volume has been achieved by styrofoam.

The dose and the dose distribution were controlled by Fricke dosimeters in vials. More than 200 dosimeters used during the irradiation period demonstrated that all dose values were higher than the required minimum dose of 10 krad and lower than the permitted maximum dose of 15 krad. The ratio D_{max}/D_{min} over all measured values was 13.67/10.41 = 1.31.

The facility is best suited for radiation in a dose range between 100 krad and some Mrads. At lower doses the conveyor system has to be operated at high speed, resulting in more frequent mechanical breakdowns. Only the range for loading and unloading is accessible for repair without removing the source in the shielded container. For maintaining and repairing the other parts of the conveyor system, the source has to be moved down into the shielding and locked. When there were troubles with the conveyor the total charge in the loaded containers had to be discarded because the applied dose was not exactly definable. The dust which adhered to the potatoes caused additional difficulties in the conveyor system and at the end of the irradiation period the frequency of troubles increased.

Experiences With a Co-60 Facility

Preliminary experiments on the radurization and radicidation of brown shrimp had shown that an irradiation process can satisfactorily be incorporated into the usual procedures of landing, home peeling and production of retail items. The experiments had been restricted to laboratory scale due to the very low throughput of the only available irradiator. A repetition of the experiment on a larger scale was therefore desirable. It should be noted that brown shrimp from the North-Sea coast (*Crangon crangon*) are quite different, with respect to flavor and shelf life, from brown shrimp caught off the North-American continent (*Panaeus aztecus*). Available data from mostly U.S. American research could therefore not be readily transferred to the situation prevailing on the North-Sea coast.

Under coordination of EURISOTOP and in collaboration with the Instituut voor visserijprodukten, TNO (Institute for Fishery Products) in Ijmuiden and the Proefbedrijf voedselbestraling (Ulmann 1967 and 1969) (Experimental Station for Food Irradiation) in Wageningen (The Netherlands, both) fresh shrimp of known initial quality were obtained, transported and irradiated under controlled temperature.

The choice of irradiator parameters was made according to the expected dose distribution (Chadwick *et al.* 1977) for the product geometry used:

The irradiator boxes were coated inside with styrofoam. Alternate

layers of crushed ice and of plastic pouches containing 1 kg of unpeeled shrimp and 1.1 kg of shrimp meat respectively were put into the insulated irradiation boxes, always with a bottom and top layer of ice. From landing until irradiation the shrimp were kept under crushed ice and the temperature of the samples was held at 0°C during the irradiation process. Including the transit time the boxes emerged after 45 min from the irradiator. For the purpose of dosimetry the mixture of shrimp and ice can be considered as homogeneous. From the dose distributions established by the staff of the irradiation facility the irradiator parameters were set to obtain the desired minimum dose throughout the product at a 99% confidence probability. The dose was monitored by ferrous-cupric-sulfate-dosimeters in the expected positions of the minimum and the maximum dose values. The minimal observed dose values of 133 krad for unpeeled shrimp and of 130 for shrimp meat, 12 measurements each, are on the safe side. A minimal dose of 100 krad is generally accepted to be effective for the radurization of brown shrimp. On the other hand the maximal observed dose values of 146 krad for unpeeled shrimp and of 148 krad for shrimp meat were well below the dose limit of 200 krad above which the occurrence of off-flavors has to be feared.

The radurization treatment of the shrimp meat resulted in a reduction of the total bacterial load by up to four orders of magnitude; the contamination during home peeling by possibly harmful organisms was effectively eliminated (Ehlermann and Münzner 1976). Shelf life — until the initial microbial count was reached again — was 18 days for the irradiated shrimp meat. Sensory evaluation and chemical freshness indices gave similar shelf life. From these results radiation preservation of brown shrimp is technologically feasible. Evaluation of economic aspects, especially under local conditions, have shown that the process is also commercially feasible (Ehlermann and Diehl 1977).

Irradiators like the one of the Proefbedrijf voedselbestraling are suitable for the large scale treatment of brown shrimp. About 200 kg were treated in the present experiment and the full throughput capacity of the irradiator was not used. Experiments with larger quantities of this relatively expensive material have to be postponed until at least a provisional clearance for test marketing of irradiated shrimp is obtained.

Experiences With a Shipboard X-ray Facility

Under the circumstances prevailing for the German fisheries the deep sea trawlers use about one third of their time to reach the fishing grounds, one third to stay there, and one third to return to the harbor. The maximum shelf life of well iced fish in the holds of a trawler is

about 14 days to obtain highest proceeds at auction. Consequently the time of the first catch of a trawler determines the departure from the fishing grounds. The yield of the fishing grounds accessible to the German fleet has decreased during the years. The trawlers return more and more frequently with incompletely used holds and the returns decrease. Due to the limited shelf life of the fish, the time spent for fishing cannot be extended to catch more fish and to fill the hold further. A technique to extend the shelf life of iced fish would therefore be extremely interesting for the German fisheries (Ehlermann 1973). However, further investigations have demonstrated the limitations of the process (Ehlermann 1978).

The German fishery research vessel "Anton Dohrn" (ex "Walter Herwig") has been equipped with a 200 kV/150 mA X-ray source (Hoffman *et al.* 1968). The decision for this type of irradiator was made primarily on the fact that the shielding of an X-ray machine is much less than for isotope sources. The costs for remodeling a ship to bear an irradiator have to take into account the weight of the shielding. Despite initial fears regarding the reliability of on board operation, the high voltage system and the conveyor mechanisms proved to be reliable (Feldt 1973). The guaranteed dose distribution within the 20 cm in diameter and 80 cm long tubelike irradiation containers was achieved. The theoretical throughput of the unit is 70 kg of round fish per hour at a dose of 100 krad. It is equivalent to about 70% of beam energy utilization. The irradiation boxes can hold only five large redfish, a species primarily caught by German trawlers. The dose distribution for the irradiation of fish in the round has been followed up by thermoluminescent dosimetry (Feldt 1973). Results are in conformity with the measurements of the manufacturer during commissioning: an average dose of 100 krad to the goods results in a minimum dose value of 65 krad and a maximum of 170 krad. Irradiations of several species of fish (redfish, cod, haddock, saithe) have been conducted during several cruises and the facility operated reliably; the above given dose values were always achieved. However the microbiological and the sensory evaluation lead to the conclusion that the broad dose distribution with a lower limit of 65 krad does not reduce the microbial load sufficiently, while the upper dose limit of 170 krad may, under certain conditions, affect the flavor of redfish which has a relatively high fat content (Karnop *et al.* 1976). During further cruises the used diameter of the irradiation tubes was reduced to 10 cm, resulting in a ratio of maximum to minimum dose values of 1.4. Such a uniformity ratio seems to be acceptable. For an effective treatment at 100 krad minimum dose it results in a maximum dose of only 140 krad, which does not affect the fish flavor. However, under these conditions the throughput of

originally 70 kg round fish per hour at an average dose of 100 krad is reduced to 12 kg round fish per hour at a minimum dose of 100 krad.

CONCLUSIONS

Our experiences with a linear accelerator, a mobile Cs-137 source, a Co-60 facility and a shipboard X-ray facility and the irradiation of quite different kinds of foods as potatoes, onions, shrimps, fish etc. in the ranges of low and high doses show, that compromises have to be made with respect to the efficiency of beam utilization, the dose distribution, the D_{max} /D_{min} ratio or the throughput, if the universal containers and the universal conveyor systems of the facilities are used. The irradiation of a specific food product on a commercial scale seems to be economically efficient only if the irradiation facility is equipped with a special conveyor system and with special irradiation containers for the kind of food to be irradiated.

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THE COMBINED EFFECT OF ELECTRICAL STIMULATION AND BLADE TENDERIZATION ON SOME BOVINE MUSCLES

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ABSTRACT

The electrical stimulation (ES) period was a very important factor determining the tenderness of three bovine muscles: Biceps femoris (BF), Longissimus dorsi (LD) and Semimembranosus (SM). Tenderness (shear force values) increased in most cases with increasing the ES period. An ES period of 15 min was optimal for obtaining a tenderness equal to that obtained by conventional processing of beef. The shortest ES period used (1 min) was adequate in most cases to set the muscles in rigor mortis and in preventing cold shortening. The tenderness of blade tenderized muscles was in most cases independent from the ES period. Blade tenderization was very effective to tenderize the BF muscle followed in decreasing order of efficacy by the LD and SM muscles. The cooking times and cooking losses of the three muscles were not affected by either the ES period or by blade tenderization.

INTRODUCTION

Tenderness is one of the most important quality attributes of meat. A number of procedures have been developed to improve meat tenderness. The alteration of carcass suspension or mechanical restraint of muscles was found to improve tenderness (Smith *et al.* 1971; Hostetler *et al.* 1970; Buege and Stouffer 1974). Environmental treatments such as storage of carcasses at elevated pre-rigor (Smith *et al.* 1973; Fields *et al.* 1976) and post-rigor temperatures (Culp *et al.* 1973) improved tenderness. Beef can be tenderized as well by proteolytic enzymes (Kang and Warner 1974). It has been shown that electrically stimulated meat samples were as or more tender than those boned from sides chilled conventionally for 24 h (Savell *et al.* 1976; Gilbert *et al.* 1976; Pierce and Henrickson 1978).

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Recently studies have been reported on blade tenderization of beef which showed that the treatment ensured acceptable tenderness, equalized tenderness in portioned items which contain two or more muscles of differing tenderness and last but not least upgraded cuts not previously utilized for steaks. Davis *et al.* (1976) demonstrated that blade tenderization provided an increase in meat tenderness greater than that achieved by cooler aging. These results were confirmed by Campbell *et al.* (1976) using both a sensory panel and shear force values. Seideman *et al.* (1977) using blade tenderization equalized the tenderness (measured by shear force values) of the Semitendinosus to that of the Psoas major.

The purpose of this work was to study the combined effect of electrical stimulation and blade tenderization on the tenderness and the cooking times and cooking losses of three bovine muscles.

MATERIALS AND METHODS

Beef Carcasses

Sixteen carcasses from commercial Angus and Hereford steers in the weight range of 271 to 302 Kg were used.

Electrical Stimulation

The electrical stimulation (a square wave pulse of 300V, 400 cpm with a duration of 0.5 msec and a current of 1.7 amp) of beef sides started at 30 min postmortem and continued for 1, 2, 5, and 15 min. Both sides of the same animal, electrically stimulated and control (non-stimulated), were held at 16°C for 1.5 h (including the stimulation period) after which the selected muscles were boned.

Boning and Storage

The Semimembranosus (SM), Biceps femoris (BF) and Longissimus dorsi (LD), (from the end of the Ilium to the 5th thoracic vertebrae), were hot boned from the stimulated side and cut at the center of their long axis. This group of muscles was designated "hot boned hot." The unstimulated side was stored (1.1°C for 22–24 h) and served as a control (conventional boning). Half of each hot boned muscle was chilled (1.1°C, 15 h) and designated "hot boned chilled" then sampled for various measurements.

Blade Tenderization

One half of each muscle was blade tenderized twice (top and bottom) using a Hollymatic AMT 625A Blade Tenderizer (Hollymatic Corp., Park Forest, Illinois). The other half of each muscle was kept as a control.

Heat Transfer Studies

Steaks (5.08 cm thick) were sampled from each half muscle at a location adjacent to the center of the whole muscle. The steaks (about 250 g each) were squared and heat treated in a 163°C convection oven (The G. S. Blodgett Co., Inc., Burlington, VA) to an internal temperature of 68.3°C (measured with a meat thermometer). Cooking time, and cooking weight losses were measured, and expressed as min/kg and (%) respectively.

Shear Force Measurements

The cooked steaks were cooled for 40 min at room temperature (22–25°C) and chilled for 22–24 h at 1.1°C to provide adequate firmness and uniform cores. Three cores (each 2.5 cm in diameter) were sampled from each steak using a mechanical borer. Each core was sheared 3 times at 3 different locations using a Warner Bratzler shear (J. Chatillon & Sons, New York, New York). The cores were sheared perpendicular to the muscle fibers.

Fat Analysis

The cooking loss drip was examined for its fat content according to the method of AOAC (1965).

Statistical Analysis

The results were subjected to the analysis of variance and to the multiple F and range tests (Steel 1960).

RESULTS AND DISCUSSION

Increasing the electrical stimulation (ES) period from 1 to 15 min in most cases significantly ($P < 0.05$) decreased the shear force values (SFV) of the hot boned hot nontenderized samples of the BF (16.3 to

13.2 Kg/2.5 cm) and SM (16.4 to 12.4 Kg/2.5 cm) muscles (Tables 1 and 2). This trend applied to the hot boned hot nontenderized and tenderized LD samples (Table 3) but was found only when the ES period was increased from 2 to 15 min (13.6 to 11.7 and 13.3 to 10.1 Kg/2.5 cm respectively).

Blade tenderization of the hot boned hot BF and SM muscles (Tables 1 and 2) abolished the effect of the ES period on the tenderness (shear force values). The exceptions were when the ES period of either the tenderized BF or SM muscles increased from 2 to 5 and from 5 to 15 min respectively, the SFV significantly ($P < 0.05$) decreased. Both the hot boned hot tenderized BF and SM muscles stimulated for 1 min had SFV almost identical to those stimulated for 15 min. These results point out that the ES period is an important factor and should be taken into consideration for obtaining a satisfactory tender product. The high SFV obtained with the short ES periods may suggest that an inadequate ES was given to the muscles resulting with an insufficient acceleration of the postmortem metabolism leading to an inferior tenderness (high SFV) when compared to that obtained using a 15 min ES period.

All the hot boned hot nontenderized samples of the BF and SM muscles (except for those ES 15 min resulting with no significant difference) had significantly ($P < 0.05$) higher SFV than the nontenderized conventionally processed (control) samples (Tables 1 and 2). The hot boned hot nontenderized LD samples did not significantly ($P < 0.05$) differ from the nontenderized control samples (except for those samples ES for 5 min) (Table 3). In this respect the LD was different than either the BF or the SM muscle. Probably the positioning of the LD muscle (in line with the electrodes and might have received more stimulation than the other muscles), the stretching it received when the carcass was suspended and stimulated and last but not least the lower amount of connective tissue it contained may have positively contributed to the tenderness of this muscle. The results obtained with the hot boned hot nontenderized muscles suggest that an ES period of 15 min was optimal for obtaining a tenderness (expressed as SFV) comparable to that of the same muscles from conventionally processed carcasses. Any ES period used (except 2 min) was adequate for obtaining hot boned hot nontenderized LD samples with a tenderness comparable to their control.

Blade tenderization of the hot boned hot BF muscle ES for 1, 2, and 5 min significantly ($P < 0.05$) lowered the SFV as compared to the nontenderized muscles (Table 1). This was true for the SM and LD muscles when ES for 1 and 5 min respectively (Tables 2 and 3). Tenderization of the hot boned hot BF and LD muscles equalized their

Table 1. Tenderness (shear force value) of electrically stimulated blade tenderized Biceps femoris muscle

Treatment	Stimulation Period (min) 1	2	5	15
		(Kg/2.5 cm)[1]		
Hot boned hot				
NT[3]	16.3 ± 4.7^{a} [2]	18.2 ± 4.8	16.5 ± 5.8^{a}	13.2 ± 4.4^{tv}
T	11.0 ± 3.2^{bcno}	12.9 ± 4.3^{bdq}	10.9 ± 3.5^{cs}	11.2 ± 1.6^{cdtw}
Hot boned chilled (1.1°C, 15 h)				
NT	11.7 ± 2.4^{efm}	12.6 ± 3.2^{ep}	14.1 ± 4.3	12.2 ± 4.9^{fuv}
T	10.0 ± 3.2^{ghimn}	12.0 ± 3.3^{gjpqr}	9.1 ± 2.3^{hs}	11.4 ± 3.4^{ijuw}
Control[4]				
NT	13.1 ± 2.9^{k}	14.5 ± 4.9^{k}	12.9 ± 2.9^{k}	13.0 ± 2.9^{kv}
T	9.2 ± 1.9^{lo}	9.9 ± 1.8^{lr}	9.9 ± 2.9^{ls}	10.3 ± 2.8^{lw}

[1] Each shear force value is the average of 36 measurements
[2] Values bearing identical superscript are not significantly ($P < 0.05$) different
[3] NT = Nontenderized, T = Tenderized
[4] Not electrically stimulated

Table 2. Tenderness (shear force value) of electrically stimulated blade tenderized Semimembranosus muscle

Treatment	Stimulation Period (min) 1	2	5	15
	(Kg/2.5 cm)[1]			
Hot boned hot				
NT[2]	16.4 ± 4.0^{ab} [3]	15.0 ± 3.9^{alm}	17.0 ± 3.4^{bn}	12.4 ± 4.0^{o}
T	13.3 ± 3.8^{ck}	13.9 ± 3.5^{cdl}	15.4 ± 3.8^{dn}	13.0 ± 3.5^{co}
Hot boned chilled (1.1°C, 15 h)				
NT	12.6 ± 3.3^{e}	15.7 ± 3.2^{fm}	15.4 ± 2.9^{fn}	12.5 ± 3.0^{eo}
T	12.3 ± 2.4^{egk}	10.7 ± 3.0^{gh}	12.3 ± 2.3^{g}	9.4 ± 2.5^{h}
Control[4]				
NT	12.5 ± 2.8^{i}	12.9 ± 3.5^{i}	13.6 ± 2.6^{i}	13.5 ± 2.4^{io}
T	10.5 ± 3.6^{j}	10.7 ± 1.7^{j}	10.5 ± 2.4^{j}	11.2 ± 3.5^{j}

[1] Each shear force value is the average of 36 measurements
[2] NT = Nontenderized, T = Tenderized
[3] Values bearing identical superscript are not significantly ($P < 0.05$) different
[4] Not electrically stimulated

Table 3. Tenderness (shear force value) of electrically stimulated blade tenderized Longissimus dorsi muscle

Treatment	Stimulation Period (min) 1	2	5	15
		(Kg/2.5 cm)[1]		
Hot boned hot				
NT[3]	12.9 ± 2.9^{abln} [2]	13.6 ± 5.4^{apq}	13.0 ± 4.4^{ac}	11.7 ± 3.0^{bcuwx}
T	12.1 ± 2.7^{delo}	13.3 ± 4.7^{dpr}	10.4 ± 2.1^{eft}	10.1 ± 2.4^{fuyz}
Hot boned chilled (1.1°C, 15 h)				
NT	11.4 ± 1.9^{gmn}	15.5 ± 3.2	10.7 ± 3.6^{gs}	11.3 ± 2.9^{gvw}
T	10.9 ± 2.4^{himo}	12.4 ± 4.4^{hr}	10.3 ± 3.3^{ist}	10.1 ± 2.7^{ivy}
Control[4]				
NT	12.7 ± 5.2^{jn}	12.5 ± 3.5^{jq}	11.2 ± 2.3^{j}	12.2 ± 3.8^{jx}
T	8.9 ± 2.6^{k}	9.1 ± 2.6^{k}	9.0 ± 1.5^{kt}	9.6 ± 2.5^{kz}

[1] Each shear force value is the average of 36 measurements
[2] Values bearing identical superscript are not significantly ($P < 0.05$) different
[3] NT = Nontenderized, T = Tenderized
[4] Not electrically stimulated

tenderness (SFV) (except for the BF and LD muscles stimulated for 2 min and 1 and 2 min respectively) to that obtained with the tenderized control (Tables 1 and 3). The SFV of the hot boned hot tenderized SM muscle were significantly ($P < 0.05$) higher in all instances than their tenderized control (Table 2). These results show that different muscles are not equally tenderized using blade tenderization. Blade tenderization of the hot boned hot BF muscle gave the best results followed in decreasing order of efficacy by the LD and SM muscles. The SM was not significantly ($P < 0.05$) tenderized.

Chilling the hot boned nontenderized BF muscles ES for 1, 2, and 5 min significantly ($P < 0.05$) decreased their SFV as compared to the hot boned nonchilled muscles (Table 1). Similar results were obtained with the nontenderized LD except that the chilled samples ES for 2 min had significantly ($P < 0.05$) higher SFV when compared to the hot boned hot samples (15.5 versus 13.6 Kg/2.5 cm) (Table 3). The SFV of the tenderized chilled BF and LD samples were not significantly ($P < 0.05$) different than those of the tenderized hot boned hot samples (Tables 1 and 3). In all instances (except for an ES of 1 min) the tenderized chilled SM samples had significantly ($P < 0.05$) lower SFV than the hot boned hot tenderized samples. These results show that an ES as short as 1 min was adequate in most cases to set the muscles in rigor before their temperature fell below 10°C thus cold shortening was prevented (Locker and Hagyard 1963). Chilling of the muscles did not increase their SFV (except for NT LD ES for 2 min) and even decreased them in some cases. These results have a significant practical importance which shows that muscles ES for a period as short as 1 min can be chilled without impairing their tenderness (measured by SFV). In practice hot boned muscles will be chilled before being consumed. It is postulated that the ES period required for preventing cold shortening is different than that required to assure acceptable tenderness (as compared to the tenderness of muscles from conventionally processed carcasses). As pointed out earlier, an ES period of 15 min was optimal in most cases for obtaining acceptable tenderness while 1 min was adequate in most cases to set the muscles in rigor mortis before their temperature fell below 10°C.

In most cases the cooking times of the 3 muscles examined were not significantly ($P < 0.05$) affected by either the ES period or the blade tenderization (Tables 4, 5 and 6). The cooking time ranged from 76.1 to 121.1, 67.4 to 113.6, and 72.3 to 112.5 min/Kg for the BF, LD, and SM muscles respectively. Similar results were obtained with the cooking losses (Tables 7, 8 and 9) with 2 exceptions. The first was with the tenderized hot boned hot BF sample ES for 5 min, and the second with

Table 4. Cooking time of electrically stimulated blade tenderized Biceps femoris muscle

Treatment	Stimulation Period (min)			
	1	2	5	15
	(Min/Kg)[1]			
Hot boned hot				
NT[2]	80.3 ± 9.0[a] [4]	95.7 ± 11.7[a]	102.0 ± 22.5[a]	76.1 ± 28.0[a]
T	91.0 ± 13.8[a]	88.0 ± 13.8[a]	93.0 ± 11.7[a]	88.3 ± 28.8[a]
Hot boned chilled) (1.1°C, 15 h)				
NT	97.5 ± 11.2[a]	114.5 ± 24.6[a]	101.5 ± 14.4[a]	114.4 ± 13.4[a]
T	99.8 ± 13.4[a]	109.7 ± 29.8[a]	112.2 ± 7.1[a]	121.1 ± 14.6[a]
Control[3]				
NT	93.8 ± 9.8[a]	97.0 ± 6.8[a]	95.7 ± 28.8[a]	103.5 ± 11.5[a]
T	90.4 ± 12.2[a]	92.7 ± 17.7[a]	92.7 ± 24.1[a]	98.5 ± 23.2[a]

[1] Each value is an average of 4 replicas
[2] NT = Nontenderized, T = Tenderized
[3] Not electrically stimulated
[4] Values bearing identical superscript are not significantly ($P < 0.05$) different

Table 5. Cooking time of electrically stimulated blade tenderized Semimembranosus muscle

Treatment	Stimulation Period (min)			
	1	2	5	15
	(Min/Kg)[1]			
Hot boned hot				
NT[2]	81.6 ± 18.4^{ab}[4]	95.0 ± 12.9^{ab}	93.1 ± 10.2^{ab}	92.0 ± 32.1^{ab}
T	72.3 ± 7.1^{ab}	84.8 ± 16.6^{ab}	91.2 ± 13.2^{a}	92.0 ± 39.6^{ab}
Hot boned chilled (1.1°C, 15 h)				
NT	82.1 ± 14.4^{ab}	112.5 ± 19.0^{ab}	112.0 ± 25.8^{ab}	112.3 ± 13.0^{ab}
T	74.6 ± 8.9^{b}	109.5 ± 18.7^{a}	89.2 ± 10.3^{ab}	100.3 ± 10.4^{ab}
Control[3]				
NT	90.8 ± 7.0^{ab}	102.9 ± 9.8^{ab}	96.8 ± 31.8^{ab}	103.6 ± 8.6^{ab}
T	82.4 ± 10.9^{ab}	99.6 ± 16.2^{ab}	93.0 ± 23.1^{ab}	108.0 ± 15.9^{ab}

[1] Each value is an average of 4 replicas
[2] NT = Nontenderized, T = Tenderized
[3] Not electrically stimulated
[4] Values bearing identical superscript are not significantly ($P < 0.05$) different

Table 6. Cooking time of electrically stimulated blade tenderized Longissimus dorsi muscle

Treatment	Stimulation Period (min) 1	2	5	15
	(Min/Kg)[1]			
Hot boned hot				
NT[2]	78.8 ± 11.8^{bcde}[3]	85.8 ± 10.3^{abcde}	96.8 ± 11.0^{abcde}	90.8 ± 32.5^{abce}
T	85.4 ± 11.9^{abcde}	86.0 ± 12.7^{ade}	86.7 ± 14.5^{abcde}	67.4 ± 13.0^{abc}
Hot boned chilled (1.1°C, 15 h)				
NT	103.8 ± 20.1^{af}	101.2 ± 14.2^{abcde}	105.5 ± 6.5^{abcde}	112.4 ± 15.0^{abce}
T	95.6 ± 14.7^{abcde}	113.6 ± 19.2^{cf}	102.8 ± 13.8^{abcde}	100.1 ± 16.8^{def}
Control[4]				
NT	83.5 ± 12.3^{abcde}	87.7 ± 6.0^{abcde}	89.7 ± 22.9^{abcde}	98.5 ± 10.9^{abcde}
T	88.6 ± 8.3^{abcde}	94.0 ± 6.8^{abcde}	94.8 ± 8.3^{abcde}	109.2 ± 10.9^{ef}

[1] Each value is an average of 4 replicas
[2] NT = Nontenderized, T = Tenderized
[3] Values bearing identical superscript are not significantly ($P < 0.05$) different
[4] Not electrically simulated

Table 7. Cooking loss of electrically stimulated blade tenderized Biceps femoris muscle

Treatment	Stimulation Period (min) 1	2	5	15
		(%)[1]		
Hot boned hot				
NT[2]	23.5 ± 2.2^{abcde}[3]	27.8 ± 2.3^{abcde}	24.8 ± 3.0^{abcde}	23.6 ± 3.2^{bde}
T	26.3 ± 13.5^{abcde}	30.8 ± 6.8^{abcde}	32.6 ± 1.2^{abcde}	34.1 ± 2.1^{ac}
Hot boned chilled (1.1°C, 15 h)				
NT	31.0 ± 4.6^{abcde}	29.0 ± 5.3^{abcde}	23.3 ± 4.0^{abd}	28.0 ± 1.3^{abcde}
T	27.7 ± 10.6^{abcde}	28.2 ± 11.4^{abcde}	34.5 ± 4.1^{ce}	32.3 ± 1.8^{abcde}
Control[4]				
NT	27.4 ± 1.6^{abcde}	28.3 ± 3.1^{abcde}	31.0 ± 2.0^{abcde}	26.9 ± 3.0^{abcde}
T	30.7 ± 3.6^{abcde}	35.8 ± 3.9^{abcde}	34.5 ± 2.8^{abcde}	29.3 ± 6.5^{abcde}

[1] Each value is an average of 4 replicas
[2] NT = Nontenderized, T = Tenderized
[3] Values bearing identical superscript are not significantly ($P < 0.05$) different
[4] Not electrically stimulated

Table 8. Cooking loss of electrically stimulated blade tenderized Semimembranosus muscle

Treatment	Stimulation Period (min) 1	2	5	15
	(%)[1]			
Hot boned hot				
NT[2]	26.9 ± 2.3[a][4]	26.9 ± 1.3[a]	27.7 ± 1.8[a]	27.9 ± 1.7[a]
T	29.5 ± 2.4[a]	31.7 ± 1.3[a]	32.2 ± 5.6[a]	30.6 ± 6.7[a]
Hot boned chilled (1.1°C, 15 h)				
NT	26.7 ± 4.0[a]	28.2 ± 4.1[a]	28.1 ± 4.2[a]	27.7 ± 2.8[a]
T	31.7 ± 10.4[a]	32.3 ± 0.9[a]	30.1 ± 2.9[a]	31.5 ± 5.2[a]
Control[3]				
NT	29.4 ± 4.3[a]	29.2 ± 3.9[a]	31.1 ± 2.7[a]	27.7 ± 3.6[a]
T	26.6 ± 2.8[a]	32.2 ± 2.2[a]	31.9 ± 2.0[a]	32.3 ± 2.1[a]

[1] Each value is an average of 4 replicas
[2] NT = Nontenderized, T = Tenderized
[3] Not electrically stimulated
[4] Values bearing identical superscript are not significantly ($P < 0.05$) different

Table 9. Cooking loss of electrically stimulated blade tenderized Longissimus dorsi muscle

Treatment	Stimulation Period (min)			
	1	2	5	15
	(%)[1]			
Hot boned hot				
NT[2]	29.0 ± 9.0[a] [4]	23.4 ± 2.2[a]	23.8 ± 1.0[a]	25.8 ± 0.9[a]
T	29.4 ± 0.9[a]	25.5 ± 4.3[a]	26.0 ± 2.4[a]	26.9 ± 5.0[a]
Hot boned chilled				
(1.1°C, 15 h)	26.1 ± 1.2[a]	23.5 ± 2.2[a]	22.3 ± 2.5[a]	24.4 ± 0.7[a]
NT	26.8 ± 3.6[a]	28.0 ± 2.6[a]	25.8 ± 1.6[a]	26.6 ± 3.5[a]
Control[3]				
NT	28.3 ± 12.7[a]	23.8 ± 2.7[a]	26.3 ± 3.0[a]	23.8 ± 0.8[a]
	26.2 ± 1.9[a]	29.1 ± 1.5[a]	28.5 ± 2.2	27.9 ± 3.0[a]

[1] Each value is an average of 4 replicas
[2] NT = Nontenderized, T = Tenderized
[3] Not electrically stimulated
[4] Values bearing identical superscript are not significantly ($P < 0.05$) different

the tenderized hot boned chilled BF sample ES for 5 min. Both had significantly ($P < 0.05$) higher cooking losses than these of the non-tenderized samples (34.1 versus 23.6% and 34.5 versus 23.3% respectively) (Table 7). The cooking losses ranged from 23.3 to 35.8, 22.3 to 29.4 and 26.6 to 32.3% for the BF, LD, and SM muscles respectively. The cooking drip ranged from 2.9 to 6.4% and contained 0.4 to 6.7% fat of the drip with no significant ($P < 0.05$) difference among the 3 muscles examined. These results point out the fact that a combination of electrical stimulation and blade tenderization did not affect either the cooking time or the cooking loss and the economical advantage is self evident. Probably the ES did not impair the electrical properties of the meat proteins thus did not affect the protein-water interaction (Hamm 1960) as expressed by no significant change in the cooking loss.

This study has shown the combined effects of electrical stimulation and blade tenderization on the tenderness and the cooking times and cooking losses of three muscles. Other research is required to elucidate the effect of the ES period on postmortem metabolism in order to establish a procedure which will lead to a better product.

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MEMBRANE FILTRATION FOR ISOLATION, FRACTIONATION AND PURIFICATION OF FOOD- AND FEED-GRADE PROTEINS FROM PASTURE HERBAGE

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ABSTRACT

In 3 trials a comparison of the membrane filtration (ultrafiltration — UF) efficiency of 3 different ultrafiltration systems (ABCOR, ROMICON AND DDS) has been made with an evaluation of the feasibility of the procedure for its application for protein recovery from juices extracted from pasture herbage as an alternative to heat and acid precipitation.

The ABCOR system was the most convenient for a batch system of herbage juices within a wide range of dry matter content (4 to 11% DM). The Romicon hollow fiber system had a much higher ultrafiltration efficiency compared to the ABCOR and DDS UF units but was subject to spontaneous mechanical failure — blockage and breakage of the hollow fibers.

Five protein fractions were observed in pasture herbage juices with the major 3 being separated routinely in practice. Protein fractions concentrated by the ultrafiltration after spray drying were still water soluble (dispersable). This compares with an insoluble product obtained as a result of heat precipitation.

The ultrafiltration conditions (dry matter and protein content, temperature, recirculation rate, pressure, pH, size of plant) are critical for the efficiency of DM and protein concentration in the final product recovered from herbage juice in a plant scale operation so there is a need for their careful standardization and optimization.

INTRODUCTION

In the system of protein extraction from pasture herbage one of the factors restricting the efficiency of protein recovery during the extraction process is the method of protein separation from the herbage juice

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(Ostrowski 1975). The most commonly used method to isolate protein from plant extract is that based on heat and/or acid precipitation (Pirie 1971). The treatment of juice with heat or acid, however, causes the denaturation of proteins which become water insoluble. In such form they are not favored in modern food and feed technology. Thus, looking at the different methods and techniques available, membrane filtration — ultrafiltration (UF) was chosen as a possible alternative to the precipitation procedure for protein recovery from herbage juice.

The ultrafiltration technique has already been reported by Singh *et al.* (1974) and Knuckles *et al.* (1975) as useful in concentrating alfalfa proteins by removing 80 to 90% of the water and non-protein components. The final product — dried protein concentrate was still water-soluble.

In recent years when suitable commercial ultrafiltration membranes appeared on the market, (ABCOR, ROMICON, DDS, IOPOR-CIP and others) ultrafiltration became one of the cheapest protein isolation and concentration methods (Payne *et al.* 1973). Thus, the ultrafiltration technique was introduced for protein isolation, fractionation and concentration as an alternative method to heat (at 55 and/or 85°C) or acid coagulation for separating proteins from herbage juices processed within the system of protein extraction from pasture described in detail earlier (Ostrowski 1976).

MATERIALS AND METHODS

The experiment was conducted using the juice extracted from a pasture herbage which was based on white clover (approximately 60%) and perennial (approximately 40%). It grew on a stand of permanent pasture of an average herbage dry matter productivity 1.8×10^4 kg ha^{-1} yr^{-1}. The pasture and the system of herbage sampling, processing and juice separation from the fibrous fraction, have earlier been described in detail (Ostrowski 1976).

Trial 1

Ten samples each of 100 l bulk juice of chemical composition as in Table 1, after being filtered through a double muslin cloth followed by cooling in a QVF glass heat exchanger to 0°C, were stored at this temperature until being processed in a herbage juice processing line (Fig. 1) using one of the 3 different commercial type ultrafiltration modules: ABCOR, DDS and ROMICON (Table 2).

Table 1. Dry matter (DM), total and protein nitrogen concentration in the grass juices used in Trial 1

Juice Number	Juice DM	Total Nitrogen	Protein Nitrogen
1	10.49	.493	.425
2	9.74	.469	.413
3	8.48	.423	.369
4	7.42	.406	.355
5	6.71	.366	.334
6	6.03	.360	.325
7	5.85	.347	.315
8	5.49	.337	.312
9	5.12	.323	.305
10	4.84	.315	.280
$\bar{x}$	7.017	0.3839	0.3441
S.D.	1.968	0.0615	0.0460

(a) Four ABCOR HFA-1801 ultrafiltration tubes (in line) having membranes with 2 X 10^4 MW cut off each of 0.11 m^2 UF area, were used. Juice at 28°C was recirculated at 60 l/min through the tubes, the inlet pressures being 170 K N/m^2 with drop in pressure along the 4 tubes 20 KPA. The samples of 40:1 grass juice were concentrated to approximately 18—22% DM. Operation conditions and membranes' management were similar to those described by Horton (1973).

(b) Laboratory DDS module with 20 DDS-500 ultrafiltration membranes each of 0.038 m^2 area was used with a nominal 6.8 X 10^4 MW cut off. The ultrafiltration conditions were similar as in (a) with the flow rate of concentrated juice 8 l/min. Samples of 10:1 of grass juice were processed. Operation conditions were similar to those described by Bundgaard *et al.* (1972).

(c) ROMICON — HF 30—20 XM50 hollow fiber cartridge with 2.7 m^2 membrane area, having a nominal 5 X 10^4 MW cut off was used. Samples of 40 l of juice were processed at 28°C, 170 KN/m^2 on inlet to the cartridge by feed flow rate 60 l/min. Operation conditions and membranes' management were similar to those described by Blatt (1972).

In all UF systems (a,b,c), the concentrates were recirculated so that each sample passed in a closed circuit many times over the membranes (Fig. 2) and permeate flow rates were measured at 15 min intervals throughout the period of each experiment with simultaneous concentrate sampling for dry matter (DM), total (TN) and protein nitrogen (PN) analysis.

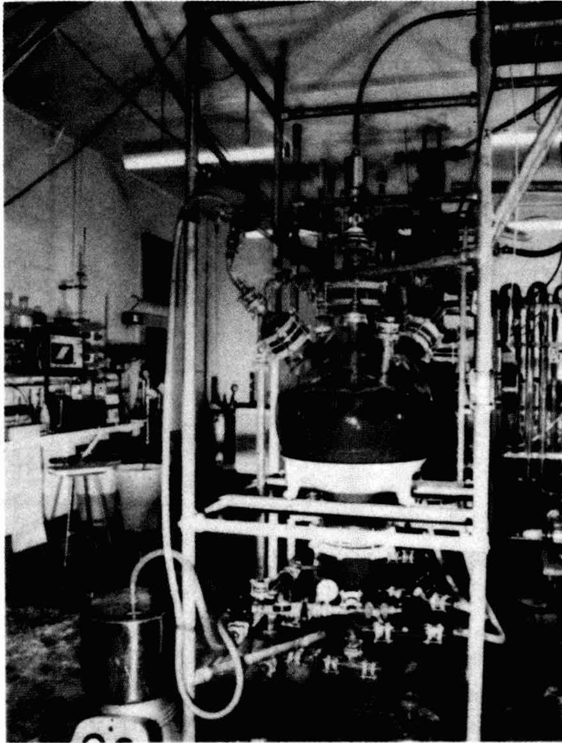


FIG. 1. THE PILOT SCALE MEMBRANE FILTRATION (UF) PLANT USED AS AN ALTERNATIVE PROCEDURE TO HEAT COAGULATION FOR PROTEIN ISOLATION FROM HERBAGE JUICE WITH THREE TYPES OF UF SYSTEMS

ABCOR — a ten HFA-180 ultrafiltration tube plant (on the right): ROMICON — PM-50 or PM-10 hollow fiber cartridges (rear center) and laboratory DDS unit (rear left).

Trial 2

A schematic diagram of the trial as a part of the protein extraction system from pasture is given in Fig. 3.

On 2 occasions, 2 bulk pasture grasses each of approximately 500 kg representing different DM content were processed each time and the juice from each of the 4 grasses, after cooling was stored at 0°C. Portions of 40 liters of juice were processed as follows:

(a) After acidification to pH 3.5 using concentrated formic acid followed by heat precipitation at 85° using a low pressure steam

Table 2. Characteristics of ultrafiltration membranes used in the plant-scale protein concentration and fractionation of proteins from pasture herbage

Membrane	Type	Nominal MW cut-off	
(ABCOR) (	HFA-180 (polyethylene)	2×10^4	Tubular membrane
(DDS 500 ((	DDS-500 plate membrane (cellulose acetate)	6.5×10^4	Plate membrane
(ROMICON (	HF 30-20-XM-50 (polysulfone)	5×10^4	Hollow fiber cartridge
(ROMICON (	HF 30-20-XM-10 (polysulfone)	1×10^4	Hollow fiber cartridge

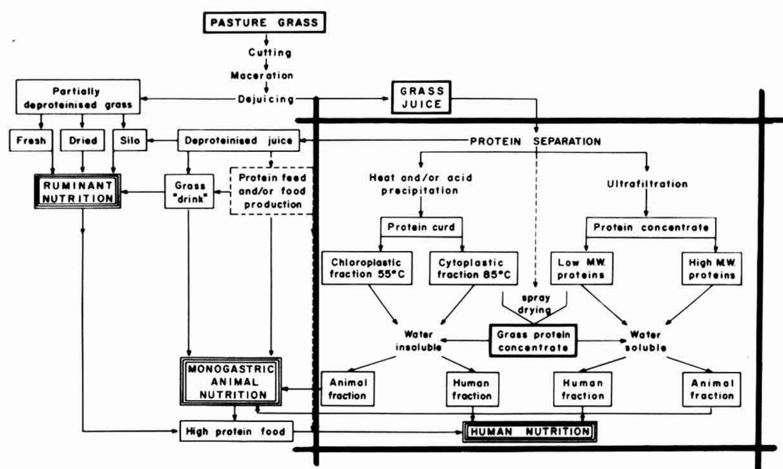


FIG. 2. DIAGRAM OF A PROTEIN EXTRACTION SYSTEM FROM PASTURE HERBAGE WITH ULTRAFILTRATION BEING ALTERNATIVE TO HEAT COAGULATION USED FOR ISOLATION OF PROTEINS FROM HERBAGE AND FRACTIONATION INTO "HUMAN" AND "ANIMAL" FRACTIONS (FRAMED PART OF THE PROCESS)

coagulation chamber the juice was cooled and centrifuged using a self desludging centrifuge (Westphalia SA-7-06-076).

(b) Heat precipitation, without acidification, at 55°C using coagulum (chloroplastic fraction) separation as in (a).

(c) Supernatant (centrate) from procedure (b) was precipitated by steam injection at 85°C followed by coagulum (cytoplastic fraction) separation as in procedures (a) and (b).

(d) Ultrafiltration of the whole juice using ABCOR membranes (4

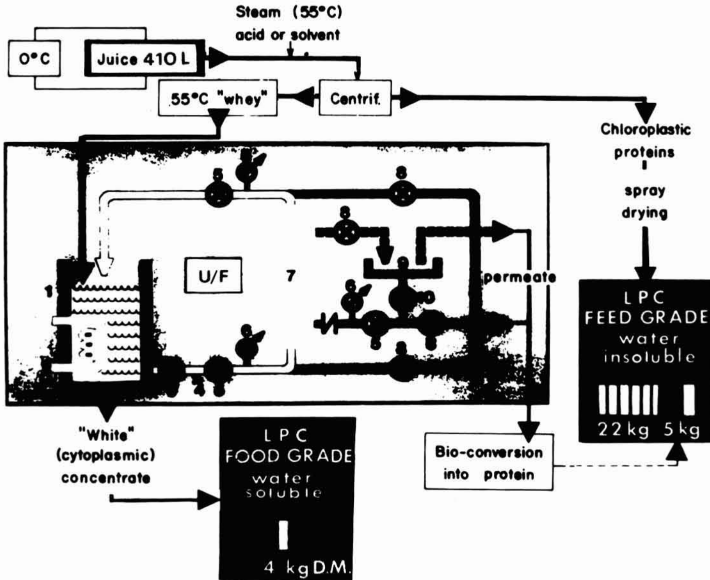


FIG. 3. SCHEMATIC DIAGRAM OF PROCESSING JUICE EXTRACTED FROM PASTURE HERBAGE AND ITS FRACTIONATION INTO CHLOROPLASTIC — FEED GRADE AND CYTOPLASMIC — FOOD GRADE PROTEIN FRACTIONS USING STEAM/ACID COAGULATION FOLLOWED BY ULTRAFILTRATION

Framed part of the process represents "batch-type" ultrafiltration system: (1) Process tank; (2) Heat exchanger; (3) Process pump; (4) Strainer; (5) Pressure control valve; (6) Pressure gauge; (7) Ultrafiltration module; (8) Ball Valve; (9) Permeate tank; (10) Permeate pump. Diagram indicates use of permeate remaining after ultrafiltration in nitrogen bio-conversion into process for feed-grade protein production (single cell protein and yeast).

tubes) according to the procedure given in Trial 1.

(e) Supernatant after procedure (b) was ultrafiltrated at 40°C, 170 KPA and 60 l recirculation rate using an ABCOR HFA-180 membrane.

(f) Supernatant from the procedure (b) was ultra-filtrated using ROMICON PM-10 hollow fiber cartridge in conditions as used in procedure (e).

Protein concentrates obtained from recovery procedures were spray dried in a small laboratory spray drier at temperatures of 95°C followed by the chemical analysis of the LPC samples.

Trial 3

The following factors affecting the efficiency of the ultra-filtration process were investigated using the 4 tube HFA-180 ABCOR plant:

Ultrafiltration temperature — either 20 or 38°C

Flow of the recirculation rate (30 or 60 l min^{-1})

Pressure during filtration (85 or 170 KPA)

Degree of juice concentration (4- or 6-fold), pH of juice (pH 6.2 or 5.5)

Size of UF plant (4 or 10 tubes connected in line)

Permeate flow rates were measured at 15 min intervals. Both concentrates and permeates were then samples for DM, TN and PN determination.

Electrophoretic Study

On 3 occasions the juice used for ultrafiltration with DDS-500 membranes was further used for the electrophoretic protein fraction separation. The Gradipore gel electrophoresis technique on a multi-sample cell with an increasing polyacrylamide concentration (linear gradient 25—27%) and with a 4—6% gradient of cross-linkage in the direction of the electrophoretic flow (Margolis 1973) was used. Separations at 100 volts were made in phosphate buffer (pH 8.5) during 45 min followed by staining with the use of an amido black dye. After destaining, the gel plates were scanned in the Helena Auto Scanner with the filter 585 nm.

When the total volume of juice was reduced from 10 l to 2 l then the concentrate and supernatant were sampled for the same procedure. The ultrafiltrate permeate was further ultrafiltered using the procedure with the identical DDS-600 system which represents nominal 6×10^3 molecular weight cut off membranes. After a 16-fold volume concentration, concentrate and permeate were further sampled for electrophoretic procedures. Supernatant obtained from the centrifugation of the original juice samples previously heated either to 55 or 85°C (which is the common procedure for separation of the chloroplastic from cytoplastic protein fraction) or to 85°C were also sampled accordingly. Supernatants obtained from the 85°C precipitation and permeates from the ultrafiltration using a DDS-600 module were analyzed for free amino acids using CPK Zip Zone electrophoretic system with Titan 111 cellulose acetate plates.

Analysis

Analytical procedures were identical to those previously described (Ostrowski 1976).

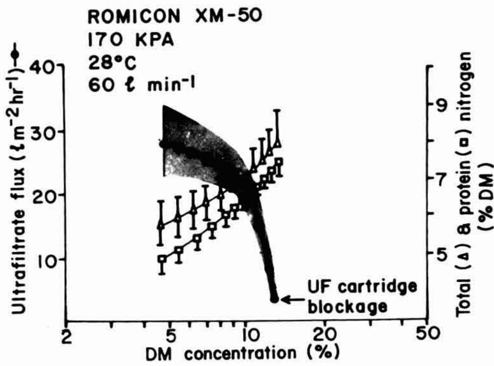
RESULTS AND DISCUSSION

Comparison of the ABCOR, ROMICON and DDS Ultrafiltration Systems for Protein Concentrations in Raw Herbage Juice Without Fractionation

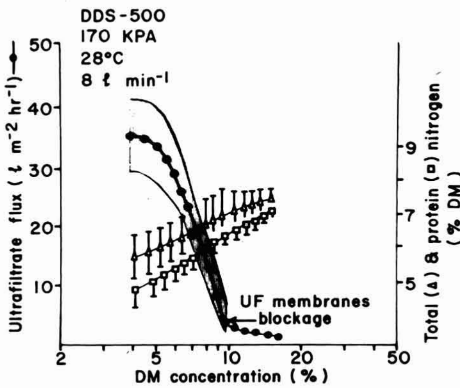
Comparison of the raw juice ultrafiltration efficiency measured by the ultrafiltrate flux (permeation rate) as a result of the increase in juice DM content, showed the Romicon PM-50 hollow fiber system as the most effective at low juice DM concentrations (Fig. 4a) with notably lower ultrafiltrate fluxes for the DDS-500 UF module (Fig. 4b) and lowest fluxes recorded with the ABCOR HFA 180 module (Fig. 4c). With an increase in juice DM to approximately 5% with a DDS module and to approximately 8% with a Romicon hollow fiber cartridge a rapid decrease in the permeation rate occurred which on 3 occasions was finished with unrecoverable blockage (clogging) of both of the modules at ranges of 9.7 and 12% DM respectively. The ABCOR tubular membrane was not subject to the blockage and the gradual decrease in permeate fluxes was recorded up to approximately 25% DM, i.e. until ultrafiltrated concentrate stopped recirculating in the system due to juice thickening.

The degree of both total and protein nitrogen concentrations which were related to both DM content in the concentrated grass juices and to the ultrafiltrate flux (Fig. 4a, b and c), showed, despite the UF system, a gradual and steady increase until approximately 6% TN in concentrated DM. In using the ABCOR system in the range of approximately 17 to 24% DM during the ultrafiltration process a rapid increase in TN and PN concentration was observed when the other 2 systems were already blocked. The concentration of the TN above the limit of 6% DM was below the permeation rate acceptable as satisfactory in plant scale operation. With the juice concentration process, notable changes occurred in the ratio of TN to PN. More protein nitrogen remained in the ultrafiltrated concentrate with the nonprotein nitrogen being removed with the permeate from the concentrate during the UF procedure. The chemical composition and yields of the protein concentrates obtained as a result of juice concentration to the extent when permeate fluxes decreased below 10:1 m^{-2} h^{-1} is given in Table 3.

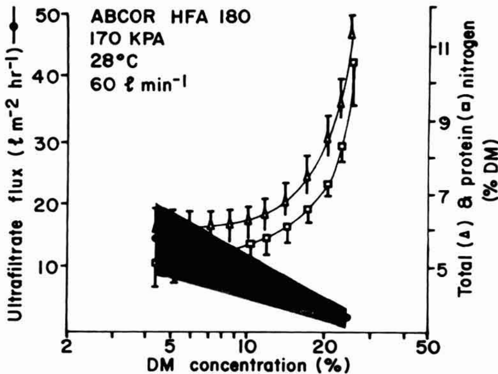
Results as reported here are in agreement with those published by Knuckles *et al.* (1975) who showed that a level of 5.5% protein in alfalfa juice as critical for the ultrafiltration efficiency, exceeding this level means a rapid decrease in the permeation rate with a simultaneous decrease in the protein concentration. Singh *et al.* (1974) studying ultrafiltrations of alfalfa proteins showed the level of 7—8% DM as critical for permeate flux rate. This seems to be similar to that determined in the present experiment with the DDS UF process with the



(A)



(B)



(C)

FIG. 4. VARIATION IN ULTRAFILTRATE FLUXES WITH THE CONCENTRATION OF HERBAGE JUICES (MEAN ± S.E.) AND GRADIENT OF TOTAL AND PROTEIN NITROGEN CONCENTRATION USING:

(a) ROMICON DM-50 hollow fiber cartridge (5×10^4 MW nominal cut off). (b) DDS-500 membranes (6.8×10^4 MW nominal cut off). (c) ABCOR HFA-180 tubular membrane (2×10^4 MW nominal cut off).

Table 3. Chemical composition of the herbage protein concentrates obtained by ultrafiltration procedure with the use of the three ultrafiltration systems

Ultrafiltration System (membrane type)	Yield of LPC from 100 l of Juice[1] (kg DM)	Composition (% DM)[2]			
		Total Nitrogen	Protein Nitrogen	Total Ash	Soluble Sugars
ABCOR (2×10^4 MW)	7.72a[3]	6.16b	5.49bB	24.1aA	19.3aA
ROMICON (5×10^4 MW)	7.24a	6.57ab	6.08abAB	18.5bAB	12.8bB
DDS (6.5×10^4 MW)	7.01a	7.53a	7.06aA	12.3aA	11.4bB

[1] Juice of the average composition: DM = 7.02; TN = 0.384 and PN = 0.344

[2] The composition of the concentrate as recovered after juice concentration to the degree in which permeate fluxes decreased below 10 l m^2 h^{-1}

[3] Values with unlike letters differ statistically ($P < 0.05$ — small letters and $P < 0.01$ — capital letters) according to multiple Duncan's test.

three different systems studied and may be ascribed to a number of factors reported intensively in the literature (Horton 1973; Jackson *et al.* 1972; Payne *et al.* 1973; Singh *et al.* 1974). The most obvious factors reported by Porter and Michaelis (1971) are the increase in viscosity of the concentrated proteinaceous material and protein-gel formation on the membrane's surface.

The protein nitrogen content was almost 10% on a DM basis when grass juices were concentrated 16-fold by using the ABCOR UF system. This is comparable with the results as reported by Knuckles *et al.* (1975). However, in the UF procedure above approximately 12% DM was very slow - too slow to be recommended for commercial use.

It appears from the results presented in this trial that the most efficient ultrafiltration procedure takes place with juices of low DM content. Thus, to obtain a high degree of protein nitrogen concentration and the high protein concentration rate it is necessary to use the "hold-up volume" UF system with a constant volume of low DM juice recirculating through the UF plant from which sugars and ash would be gradually rejected causing concentration of proteins. Horton (1973) reported that using this system, the crude protein concentration in ultrafiltered whey was as high as 80—82% in concentrate of 30% DM. With the addition of water to equate permeate being passed through the membrane, the Romicon hollow fiber cartridge could be used for efficient protein concentration and purification from sugars and ash followed by secondary dewatering in the ABCOR UF tubular system, up to approximately 20% DM content, before the concentrate being dried. In such a system the DDS membrane module and the Romicon hollow fiber system would be prevented against clogging (blockage) which occurred in these two systems in higher range of DM.

Ultrafiltration as an Alternative to Acid and/or Steam Coagulation Procedure for Grass Protein Concentration and Fractionation

Ultrafiltration of the whole grass juice resulted in slightly higher yield of GPC recovered from 100 l of raw juice as compared to heat and acid coagulation at 85°C (Table 4). Acid and heat coagulated protein concentrate — leaf protein concentrate (LPC) which was water insoluble, contained slightly more total nitrogen and less ash and soluble sugars as opposed to the UF LPC which was water soluble (dispersable).

Of the total recoverable proteins approximately 1/4 was represented by cytoplastic protein fraction (white cream one) which has been shown by Pirie (1971), Oke (1973) and Parrish *et al.* (1974) to be suitable for human nutrition. There were no consistent differences in

Table 4. Efficiency of protein separation procedure and chemical composition of the LPC's obtained by different methods used for protein separation from herbage juice

Protein Separation Method[1]	Protein Fraction	Yield of LPC (kg DM) From 100 l of Juice	Total Nitrogen (% DM)	Total Ash (% DM)	Soluble Sugars (% DM)	Water Solubility (dispersity)
Acid (pH 3.5) and Heat (85° C)	chloro-plastic and cyto-plasmic	9.72 (0.52)	7.41 (0.47)	12.2 (0.62)	7.3 (0.54)	—
ABCOR 2 × 10^4 MW (whole juice ultrafiltration at 39° C)	chloro-plastic and cyto-plasmic	9.57 (0.64)	7.10 (0.68)	15.5 (0.56)	11.7 (0.66)	+
Heat (55° C)	chloro-plastic	6.60 (0.38)	7.23 (0.36)	12.9 (0.46)	6.4 (0.43)	—
Heat (55/85° C)	cyto-plasmic	1.91 (0.12)	8.90 (0.51)	8.9 (0.41)	11.1 (0.58)	—
ABCOR 2 × 10^4 MW membrane (55° C filtrate processing at 39° C)	cyto-plasmic	1.70 (0.21)	9.90 (0.72)	12.5 (0.63)	7.5 (0.39)	+
ROMICON 1 × 10^4 MW membrane (55° C filtrate processing at 39° C)	cyto-plasmic	1.87 (0.14)	10.33 (0.55)	11.6 (0.44)	8.2 (0.35)	+

[1] Each value represents mean of the four recovery procedures (± standard deviation)

yield and chemical composition due to the system used in its separation, i.e. Romicon UF or acid/heat coagulation. There was, however, a notable difference in yield of the UF cytoplastic fraction as obtained by the ABCOR HFA 180 or Romicon PM-10 system. The latter gave a high yield of LPC of higher nitrogen concentration than the ABCOR system.

The Romicon PM-10 hollow fiber system appeared to be much more efficient for cytoplastic protein fraction concentration and separation from the chloroplastic-free supernatant obtained from centrifugation of the raw juice heated up to 55°C (Fig. 5). Also, nitrogen concentration in the cytoplastic fraction occurred much faster with the Romicon PM-10 unit than with the ABCOR HFA-180 unit (Fig. 6). These 2 UF units have had different characteristics which can explain the differences in the UF efficiency, while the Romicon cartridge had a nominal 1×10^4 MW cut off, the ABCOR membranes had 2×10^4 MW as a nominal cut off level. Singh *et al.* (1974) and Knuckles *et al.* (1975) who used membranes with different molecular weight cut off values for cytoplastic proteins isolation and concentration also showed higher retention values for membranes with the lower MW cut off figures.

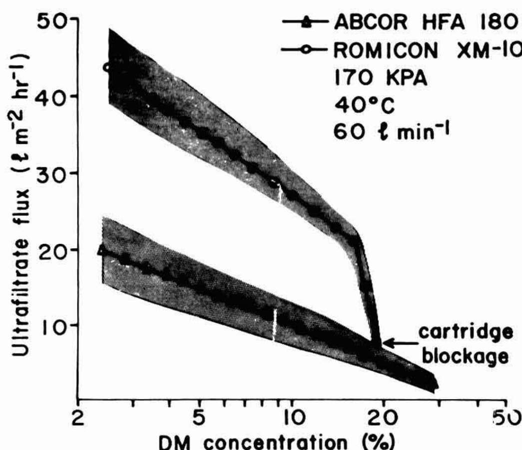


FIG. 5. VARIATION IN FLUX RATES WITH DRY MATTER CONCENTRATION IN CHLOROPLAST-FREE HERBAGE JUICE (CENTRATE FROM HEAT PRECIPITATION AT 55°C) USING EITHER ABCOR HFA-180 MEMBRANE OR ROMICON PM-10 HOLLOW FIBER CARTRIDGE

The reason for high performance with chloroplast free juice of the Romicon PM-10 hollow fiber system as compared to Romicon PM-50

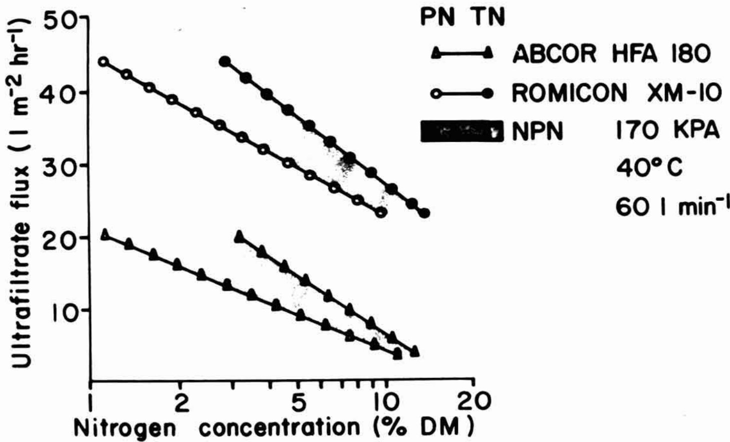


FIG. 6. RELATIONSHIP BETWEEN ULTRAFILTRATE FLUXES AND THE DEGREE OF TOTAL AND PROTEIN NITROGEN CONCENTRATION IN CHLOROPLAST-FREE HERBAGE JUICE USING EITHER ABCOR HFA-180 MEMBRANES OR ROMICON PM-10 HOLLOW FIBER CARTRIDGE

(used in Trial 1) for raw (whole) juice ultrafiltration was the low DM content in the original chloroplast-free juice (2.6 to 3.5%) which did not cause a clogging problem (even by 19% DM) as opposed to the whole juice generally of a much higher DM content (4—11%).

No attempt has been made to determine the economics of the ultrafiltration procedure either in its application to the whole grass processing or to the cytoplastic protein fraction concentration. At this stage, it is extremely difficult to conclude whether the UF procedure would be a viable means of concentrating and purifying grass proteins in large scale operations for protein concentrates production. Much research has yet to be done on a variety of grass juices obtained from different grasses during the different seasons of the year. It has been observed, e.g. that juice extracted from Tama ryegrass in the winter season is of very high DM content (13 to 14%) and high viscosity processed. Extremely low permeates flux rates (2—5 l m^2/h) occurred when the grass was processed fresh. However, after juice pretreatment (freezing and thawing, mixing with water, pH changes, etc.) the ultrafiltration rate was similar to that observed with low DM (5—6%) juices.

Ultrafiltration of the chloroplast-free protein fraction appeared to be a very efficient process despite the UF system used, with Romicon PM-10 hollow fiber system being superior to the ABCOR HFA-180 one. The spray-dried product obtained from UF procedure was water soluble

(dispersable) and reconstitutes easily and readily. Knuckles *et al.* (1975), Singh *et al.* (1974) and Tragardh (1974) also found the UF technique to be the most interesting with regard to cytoplastic protein separation and concentration as an unconventional protein source for human consumption.

There are however, a number of factors affecting the ultrafiltration process (Horton 1973; Payne *et al.* 1973; Singh *et al.* 1974), which may alter efficiency of the commercial ultrafiltration procedure with the grass juice on the interaction basis. Thus, some of these factors were investigated in relation to the grass juice.

Some Factors Affecting Grass Juice Ultrafiltration Efficiency in ABCOR UF System

The effect of some factors which alter the efficiency of protein extraction was investigated in batch concentration procedures in relation to average permeate flow rate, volume, DM and total nitrogen concentration factors as well as efficiency of protein (protein N $\times$ 6.25) recovery.

Temperature

There was a highly significant increase in the speed of ultrafiltration as measured by the permeate flow rate with the rise in average temperature of the UF grass juice from 20 to 38°C (Table 5). The increase in the temperature of the UF is generally known as a factor improving the rate of the UF procedure (Horton 1973; Payne *et al.* 1973) and in this experiment it was shown that by the ultrafiltration of the juice at 38°C, just below the protein precipitation range, nearly twice as much juice could be concentrated as compared to the procedure running at 20°C.

Recirculation Rate

An increase in the recirculation rate from 30 to 60 l min^{-1} (without increasing the pressure) resulted in a significant rise in the average permeate rate and protein recovery efficiency (Table 6) without affecting either the DM and/or the TN concentration in the final product. As seen in Table 6, the permeate flux rate increased as the recirculation rate increased. This has been established as due to an influence on concentration polarization and the gel layer formation on the UF membrane surface (Payne 1973). Knuckles *et al.* (1975) also showed an increase in UF efficiency with increased recirculation when applied to alfalfa juice.

Table 5. Concentration of herbage proteins using 4 HFA-180 ABCOR UF membranes as an effect of UF temperature (Feed flow rate : 60 l/min; and pressure : 170 KPA)

Measurement	Average Permeate Flow Rate ($l\ m^2\ h^{-1}$)	Volume Concentration Factor	Dry Matter (%) Feed (juice)	Dry Matter (%) Concentrate	DM Concentration Ratio	Total Nitrogen (% DM) Feed (juice)	Total Nitrogen (% DM) Concentrate	Protein Recovery Efficiency[1] (%)
Temperature 20°C	12	4.3	6.1	20.4	3.3	5.76	6.07	24.8
40°C	21	4.3		21.3	3.5		6.62	24.4

[1] In this and subsequent tables: Protein Recovery Efficiency (%) = $\frac{\text{(protein nitrogen in juice x 100)}}{\text{(protein nitrogen in final concentrate)}}$

Table 6. Concentration of herbage proteins using 4 HFA-180 ABCOR UF membranes as an effect of recirculation rate (temperature : 28°C; pressure : 170 KPA)

Measurement	Average Permeate Flow Rate ($l\ m^2\ h^{-1}$)	Volume Concentration Factor	Dry Matter (%)		DM Concentration Ratio	Total Nitrogen (% DM)		Protein Recovery Efficiency[1] (%)
			Feed (juice)	Concentrate		Feed (juice)	Concentrate	
Recirculation Rate (1/min)								
30	27	2.7	5.8	13.0	2	5.44	5.95	11.6
60	48.3	2.7		12.8	2		5.97	14.8

[1] See Table 5.

Pressure

An increase in pressure during the UF procedure resulted in an increased permeation flow rate with the simultaneous increase in both DM concentration and protein recovery, with the TN concentration being unaffected by the pressure (Table 7). The increase in UF efficiency, due to higher pressure, during the UF procedure, was similar to that reported by Horton (1973), Payne *et al.* (1973) and Singh *et al.* (1974). This is also connected with the problem of the protein gel layer or "cake" on the membrane surface thickening with the increase in pressure. Thus, it is important to adjust the recirculation rate in parallel with the pressure applied and according to Horton (1973) it is necessary to optimalize both the recirculation and pressure which are the functions of the changeable viscosity of the gradually concentrated material and thus are variable in every state of the UF procedure.

Degree of Volume Concentration

The permeate flow rate was significantly reduced (Table 8) together with simultaneous significant increases in DM, TN concentration and protein recovery efficiency the higher the volume concentration. Similar tendency was reported by Singh *et al.* (1974) in reference to alfalfa juice.

pH

A slight decrease in the grass juice pH — from an original 6.2 to 5.5— meant a significant increase in the rate of the UF process, a simultaneous lowering in the degree of DM concentration, a significant increase in the degree of TN concentration and efficiency of protein recovery (Table 9). It was also reported by Horton (1973) that a change in the pH of the UF material (whey) increased flux rate significantly without any other real benefits experienced elsewhere. This does not confirm the results obtained in this trial. Horton (1973) underlined however, that the most variable and peculiar process to whey was pH and that its effect on the UF procedure can be quite dramatic (Horton — personal communication).

Size of the UF Plant

An increase in the number of UF tubes connected on line from 4 to 10 did not result in any significant change in the efficiency of the UF process (Table 10) as calculated on the standard UF section size basis. Thus the size of the plant was linearly related to the volume of the juice in a batch being processed within the standard time unit.

Table 7. Concentration of herbage proteins using 4 HFA-180 ABCOR UF membranes as an effect of average pressure in the UF tubes (temperature : 28°C; flow rate : 60 l/min)

Measurement	Average Permeate Flow Rate ($l\ m^2\ h^{-1}$)	Volume Concentration Factor	Dry Matter (%)		DM Concentration Ratio	Total Nitrogen (% DM)		Protein Recovery Efficiency[1] (%)
			Feed (juice)	Concentrate		Feed (juice)	Concentrate	
Pressure (KPA)								
85	23	1.9	8.3	13.4	1	5.28	5.77	12.7
170	31	1.9		14.2	1		5.81	15.3

[1] See Table 5.

Table 8. Concentration of herbage proteins using 4 HFA-180 ABCOR UF membranes as an effect of degree of concentration temperature : 28° C; flow rate : 60 l/min; pressure : 170 KPA)

Measurement	Average Permeate Flow Rate ($l\ m^2\ h^{-1}$)	Volume Concentration Factor	Dry Matter (%)		DM Total Nitrogen (% DM)			Protein Recovery Efficiency[1] (%)
			Feed (juice)	Concentrate	Concentration Ratio	Feed (juice)	Concentrate	
Volume Concentration								
4-fold	21	4.3		21.3	3.5		6.62	24.4
			6.1			5.76		
6-fold	14	5.8		24.6	4.3		7.40	33.1

[1] See Table 5.

Table 9. Concentration of herbage proteins using 4 HFA-180 ABCOR UF membranes as an effect of juice pH (temperature : 28°C; flow rate : 60 l/min; pressure : 170 KPA)

Measurement		Average Permeate Flow Rate ($l\ m^2\ h^{-1}$)	Volume Concentration Factor	Dry Matter (%)		DM Concentration Ratio	Total Nitrogen (% DM)		Protein Recovery Efficiency[1] (%)
				Feed (juice)	Concentrate		Feed (juice)	Concentrate	
pH	6.2	31	1.9	8.3	14.2	1	5.28	5.81	15.3
	5.5	45	1.9		13.4	1		6.12	19.6

[1] See Table 5.

Table 10. Concentration of membrane protein using 4 HFA-180 ABCOR UF membranes as an effect of the size of the UF plant (temperature : 28°C; recirculation rate : 60 l/min; pressure : 170 KPA)

<table>
<tr><th rowspan="2">Measurement</th><th rowspan="2">Average Permeate Flow Rate (l m^2 h^{-1})</th><th rowspan="2">Volume Concentration Factor</th><th colspan="2">Dry Matter (%)</th><th rowspan="2">DM Concentration Ratio</th><th colspan="2">Total Nitrogen (%) DM</th><th rowspan="2">Protein Recovery Efficiency[1] (%)</th></tr>
<tr><th>Feed (juice)</th><th>Concentrate</th><th>Feed (juice)</th><th>Concentrate</th></tr>
<tr><td>Size of the Plant (number of tubes)</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>4</td><td>48.3</td><td>2.7</td><td rowspan="2">5.8</td><td>12.8</td><td>2</td><td rowspan="2">5.44</td><td>5.97</td><td>14.8</td></tr>
<tr><td>10</td><td>46.1</td><td>2.7</td><td>13.2</td><td>2</td><td>5.93</td><td>14.4</td></tr>
</table>

[1] See Table 5.

Electrophoretic Study on Protein Fractions as Separated in Different Procedures

ABCOR membranes allowed retention in the concentrate particles of above 2×10^4 MW while the Romicon and the DDS UF membranes retained particles above 5×10^4 and 6.8×10^4 MW respectively. Thus the ABCOR concentrate being a mixture of proteins of MW above 5.10^4 also contains those proteins which characterize with the MW of 2×10^4 to 5×10^4 and which are normally recoverable in the fractionation procedure together with the cytoplastic proteins (Singh *et al.* 1974).

When the permeate obtained from the ultrafiltration process with the use of DDS-500 membrane, with a nominal 6.8×10^4 MW cut off level was ultrafiltrated again using the DDS-600 membrane, with a nominal 6×10^3 MW cut off level, then 3 major fractions were separated from grass juice, the largest being above a molecular weight of 6.8×10^4 (approximately 75—80% of total protein) and the smallest being below 6×10^3 (approximately 5% of the total). In addition, to precipitable proteins the fraction below 6000 MW contained 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 was after concentration cream white in color and tasteless and was comparable with the cytoplastic fraction separated from juice using fractionated steam coagulation. The distribution of the protein fractions in concentrates and permeates separated in the two stage ultrafiltration procedure, with the two DDS membranes of 6.8×10^3 MW cut off levels, in reference to the whole juice and supernatants obtained from fractionation by heat coagulation at 55 and 85°C, is demonstrated in the polyacrylamide gel electrochromatograms shown in Fig. 7.

Five proteins were observed on the gel to which a sample of grass juice was added. Proteins labelled as 4 and 5 and a small portion of the 3, were separated from the juice with the permeate using an ultrafiltration membrane of molecular weight cut-off 6.8×10^4. Ultrafiltered juice concentrate contained proteins labelled as 1, 2 and 3 with traces of 4 and 5 which indicate that a majority of proteins present in the grass juice are those of high molecular weight one. Similarly, electrochromatograms of the UF permeate and supernatant from 55°C precipitation (during which chloroplastic protein fraction was separated) indicated that the chloroplastic fraction is not a homogenous protein but a mixture of proteins representing high molecular weight proteins above 6.8×10^4 MW.

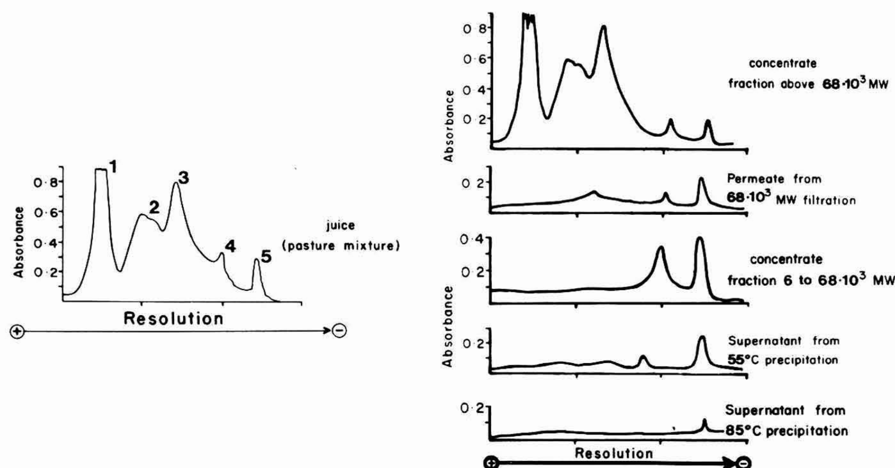


FIG. 7. PROTEIN FRACTIONS AS DETERMINED BY POLYACRYLAMIDE GEL ELECTROPHORESIS IN CONCENTRATES AND PERMEATES FROM TWO PROCEDURES AND COMPARISON OF JUICE WITH FRACTIONS IN SUPERNATANTS OBTAINED BY PROTEIN FRACTIONATION USING HEAT PRECIPITATION (55 AND 85°)

Further ultrafiltration of the permeate from the 6.8×10^4 MW process using a membrane with 6×10^3 MW cut off level resulted in the separation of the concentrate composed from 2 proteins labelled as 4 and 5 representing MW in the range between 6×10^3 and 6.8×10^4. Comparing the permeate from the DDS-600 membrane and supernatant from the heat precipitation (85°C) it may be concluded that the so-called cytoplastic fraction commonly recoverable in several protein extraction procedures as a human protein fraction represents the mixture of 2 proteins exhibiting a molecular weight below 6.8×10^4.

In the supernatant obtained from heat precipitation at 85°C there was much less free amino acids such as lysine, glycine and methionine as compared to permeate obtained from the ultrafiltration procedure of the same juice (Fig. 8). This may be due to the endogenous proteolytic and oxidative enzymes which may to some extent impair the recovery of protein during the two 3-h ultrafiltration process. Knuckles *et al.* (1975) reported a decrease in the protein content during the ultrafiltration process due to proteolytic activity which may explain the slightly lower yields of ultrafiltrated LPC as compared to the heat precipitation procedure (Table 4).

Free and Satterlee (1975), studying the biochemical properties of alfalfa protein concentrate using polyacrylamide gel electrophoresis indicated 7 proteins in chloroplast-free juice and 6 in the dialyzed one.

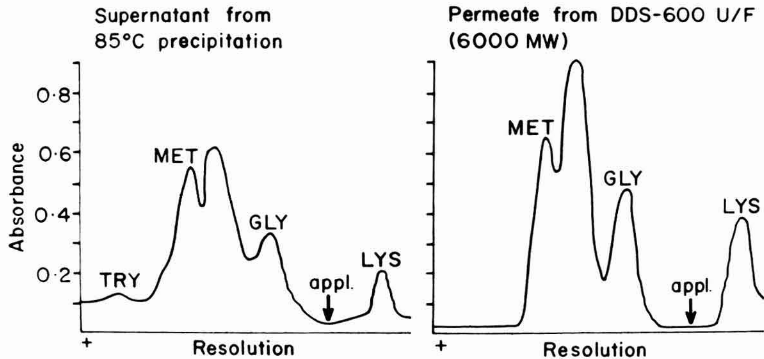


FIG. 8. SOME OF THE ESSENTIAL AMINO ACIDS IN DEPROTEINIZED HERBAGE JUICE OBTAINED AS A RESULT OF HEAT COAGULATION (85°C) OR ULTRAFILTRATION (6×10^3 MW CUT OFF) PROCEDURES

This has not been confirmed in the results obtained in this study which may be due to differences in the plant material (alfalfa and pasture grass mixture instead of alfalfa monoculture) since protein fractions distribution is different in the juices of the various plants. Electrophoretic studies indicate a substantial difference in protein fractions between perennial grasses and legumes (Ostrowski — unpublished data). Further experiments are in progress to determine the differences in protein recovery in relation to different herbage species growing in pasture grass mixture and/or pure legumes and other crops growing as monocultures.

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ERRATA

The paper, MICROSTRUCTURE OF FREEZE DRIED EMULSIONS, published in Volume 2, Number 3, of the *Journal of Food Processing and Preservation* contained an error in the caption of Figure 11. The corrected caption is given below.

Page 219. The caption below Fig. 11 should read:
OPTICAL MICROSCOPE VIEW OF SAME FIELD AS IN FIG. 10 (400X)

MEETING

Sept. 28, 1979: PRACTICAL APPLICATIONS OF MICROWAVE ENERGY SYMPOSIUM. K-State Union, Kansas State University, Manhattan, Kansas. Contact Dr. D. Y. C. Fung, Chairman, or Dr. F. E. Cunningham, Co-chairman, Call-Hall, Kansas State University, Manhattan, Kansas 66506. Telephone: 913-532-5654.

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