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Effects of calcium pre-treatments and freezing rates on fluid loss from plantain products

A. O. OLORUNDA* AND M. A. TUNG†‡

Summary

Ripe plantain slices treated with calcium chloride (0, 1360 or 2700 ppm Ca) solutions were packed one layer thick in flexible pouches prior to freezing in still air at −18 to −20°C, liquid immersion at −18 to −20°C, and cryogenically in liquid nitrogen. A new technique in which the extent of fluid loss from thawed plantain slices could be associated with tissue damage was used to assess quality of the thawed plantain slices. Plantain frozen in liquid nitrogen had the least fluid loss followed by liquid immersion and still air freezing. Treating with calcium ions prior to freezing significantly reduced fluid loss in all freezing methods used. In another experiment the treated plantain slices were frozen in still air −18 to −20°C followed by sensory evaluation of the thawed slices after frying in hot peanut oil. Calcium also significantly increased firmness in the treated slices compared to the control and there were no adverse effects on flavour and appearance. The techno-economic implications of the study are discussed.

Introduction

Plantain fruit (*Musa paradisiaca*) is an important staple starchy food in many tropical countries (Simmonds, 1966; Olorunda, 1976; Peleg & Gomez Brito, 1977). Both the green and the ripe fruits are utilized as food, mainly in the cooked or fried form, although there is a general preference for the ripe fruit. In Nigeria and most of West Africa the ripe forms are usually cut radially or diagonally into slices about 2–3 cm thick prior to frying in palm or peanut oil. The resulting food which is called 'dodo' is very popular, particularly in Nigeria.

The fruit may also be utilized as an industrial raw material for products such as plantain chips, flour, purée, etc. (Peleg & Gomez Brito, 1977). Unfortunately, plantain is a seasonal crop and its period of availability is limited by a relatively short shelf life under conditions which prevail in many tropical countries. As yet there is no satisfactory method of extending the shelf life of fresh produce for any reasonable length of time, thus post-harvest losses are high (Olorunda, 1976; Olorunda & Aboaba, 1978). Air freezing in domestic deep freezers is used as a preservation method for the pulp in many homes in Nigeria but the texture of the thawed product is very often unsatisfactory compared with the fresh produce. Plantain slices tend to lose their firmness even after frying.

It has been widely recognized that the location and size of ice crystals formed in frozen tissue is associated with the rate of freezing. In general, slow freezing results in the formation of large ice crystals located in the extracellular spaces, while rapid

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freezing results in small ice crystals in both intra- and extracellular spaces (Fennema, 1975). Formation of large ice crystals will damage the cell structure and thus the food material, upon thawing, has a poorer texture (Fennema & Powrie, 1964). It is also well known that fruit tissue can be firmed to some extent by treatment with calcium salts prior to freezing (Ponting, Jackson & Watters, 1971; Fennema, 1975). The firmness could result from divalent calcium ions forming crosslinks between negatively charged polymers and pectic substances.

The objective of the present work is to investigate the effects of varying calcium chloride concentrations and the rate of freezing on textural quality of thawed frozen plantain. It is hoped that the study will lead eventually to the development of a freezing process which is not only technically sound but commercially feasible.

Materials and methods

Plantain fruits were purchased at a wholesale outlet in Vancouver at various times during the study. The fruits were brought to the laboratory at a green unripe stage and were left to ripen at a room temperature of 22–25°C. Fruits were used for the study when their colour was about 4 on the ripening chart of the United Fruit Sales Corp. (1964). Previous work by Olorunda and co-workers (unpublished) has indicated that the behaviour of plantain is similar to that of bananas and that the colour chart could be adapted to plantain for the purposes of determining the ripening stage.

The plantain was peeled, and the pulp and peel weighed separately in order to determine the pulp/peel ratio. The pulp was then sliced in the radial direction into cylindrical pieces between 4 and 5 cm in length. The average diameter of the plantain pieces was 3.5 cm, four to five pieces were obtained from one plantain fruit and forty fruits or fingers were used at any one time. The pieces were immersed in water containing 1% NaCl to prevent browning on the cut surface. Following this, the slices were blanched in boiling water for 3 min. Previous studies by Olorunda & Aworh (unpublished) have shown that a blanching time of 3 min was adequate to inhibit all enzymes. From this point the treatment of the slices diverged according to the pre-treatments to be examined.

Prior to freezing the plantain slices were dipped in NaCl solutions containing 0, 1360 and 2700 ppm Ca for 5 min at 22°C. The 5 min dipping time was chosen following a series of preliminary investigations prior to the main experiment. The plantain slices were vacuum packed one layer thick in 15.2 × 23.4 cm flexible pouches constructed of 12 μm polyester/9 μm aluminum foil/76 μm polyolefin (Continental Can Co., Chicago, IL). The freezing methods used were: (i) still air freezing at −18 to −20°C, (ii) immersion in 60% ethylene glycol at −18 to −20°C, and (iii) immersion in liquid nitrogen at −196°C. The freezing rates of the different freezing methods used were determined by the use of a needle-type Ecklund copper-constantan thermocouple (O.F. Ecklund Ltd, Cape Coral, FL). The thermocouple was inserted into the pack in such a way that the tip was embedded in the geometric centre of one of the cylindrical pieces. The insertion was done from the circular end of the plantain slice.

Temperatures in the centre of the pouch pack as well as that of the freezing medium were recorded using a Digitec data logger (United Systems Corp., Dayton, OH) at 1, 2 or 10 min intervals. These data were used to evaluate the freezing times to cool the material to −10°C and −18°C and also to determine the time taken to cross the zone of maximum ice crystal formation (−1 to −5°C).

Textural quality and tissue damage were indirectly assessed by means of a new

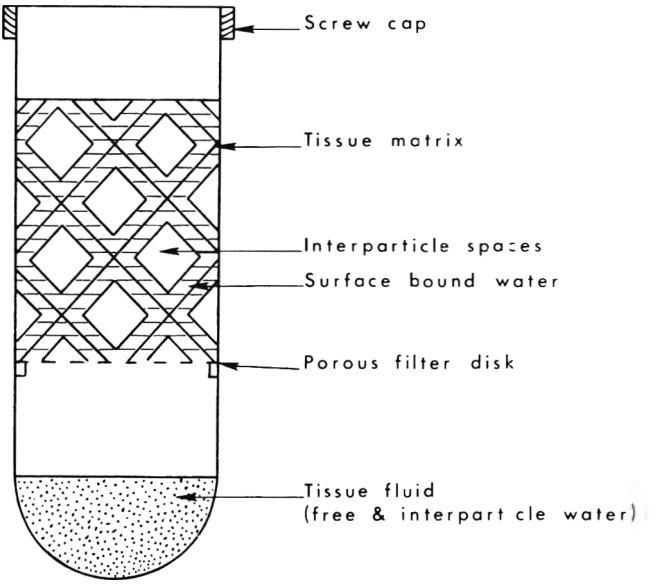

Figure 1. Diagram showing a filtrafugation tube and a schematic relationship between tissue bound and free water.

technique, first used in this laboratory by Lapsley (1980), which measures the cellular fluid loss from tissue resting on a porous support and subjected to centrifugal force. Intact fruit and vegetable tissues do not exhibit fluid loss as a result of this treatment, whereas tissues with cell wall and membrane damage will lose fluids, presumably in proportion to the extent of damage. In this experiment, the frozen plantain samples were thawed overnight in a refrigerator at 5–8°C. A known quantity of tissue (10–15 g) was placed on top of a Millipore filter disk (0.22 mm pore size) supported on a screen (Millipore xx62 025 50) within a polycarbonate filtering centrifuge tube. A diagram of such a tube, called a filtrafugation tube, is shown in Fig. 1. The filtrafugation tube was placed in a 250 ml screw-cap adaptor centrifuge bottle and centrifuged for 30 min at $3000 \times g$. The residue on the filter disk was weighed and this value was divided by the original tissue weight to obtan the filtrafugation index, which was taken as a measure of tissue damage in the thawed plantain. More extensive tissue damage would be expected to result in a greater loss of fluids, and be reflected in a lower filtrafugation index. Tissue damage will also affect texture, and hence the filtrafugation index may be used as an indication of textural quality.

In a follow-up experiment at the Department of Food Technology, University of Ibadan, sensory evaluations of thawed plantain slices were carried out using a method for sensory evaluation of food described by Larmond (1977). A multiple comparison difference analysis test was used with a rating scale in which 1 and 9 were equated to 'dislike extremely' and 'like extremely', respectively. Prior to freezing in still air at −18 to −18°C the plantain slices were dipped as in previous experiments in $CaCl_2$ solutions containing 0, 1360 or 2700 ppm Ca. The freezing was carried out in still air because this freezing method has more promise from a developing country's standpoint. The plantain used were approximately at the same stage of ripeness as those used in Vancouver. The thawed plantain slices were prepared into dodo before evaluation. Dodo was prepared by frying the plantain slices at 185°C in a restaurant-type deep fat

frier (T.I. Catering Equipment Ltd, Leeds, England) containing about 28 litres of peanut oil. Small samples of not more than 1 kg were fried at a time to minimize temperature drop during the frying process. All lots were fried until they were just brown which required a frying time of approximately 4 min. Dodo prepared from fresh plantain slices at approximately the same stage of ripeness as the experimental samples served as a reference on the rating scale at a value of 5. Seven panellists evaluated treated products with the reference, and the evaluations were replicated.

Results and discussion

The mean pulp/peel ratio of forty plantain fruits used at the colour index of 4 was 1.82 ± 0.16. Typical freezing curves for the different freezing methods are presented in Fig. 2. In this work freezing time was defined as the time taken to cross the zone of

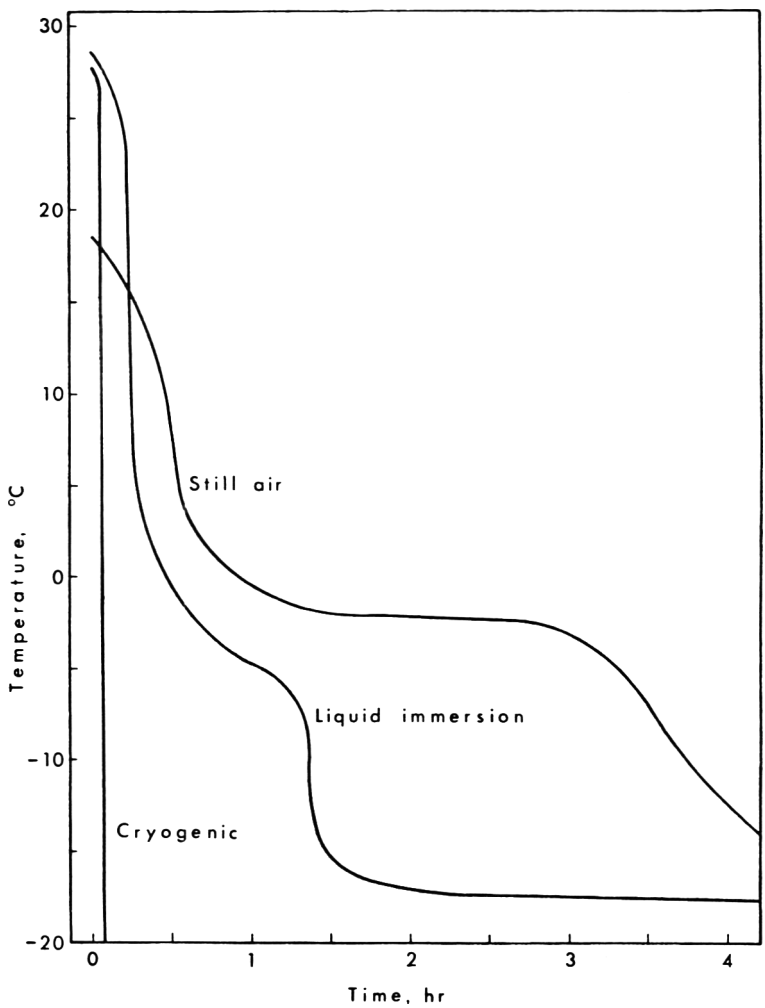

Figure 2. Typical freezing curves for plantain slices vacuum packed in flexible pouches and exposed to still air at −18 to −20°C, liquid immersion at −18 to −20°C or liquid nitrogen.

maximum ice crystal formation which is in the range of −1 to −5°C. The average freezing time for the plantain in still air was 2.1 hr, samples in ethylene glycol froze in 0.60 hr and those frozen in liquid nitrogen required 0.16 hr. It was observed that the plantain tissue frozen in liquid nitrogen tended to crack or fracture on thawing; however, air and liquid immersion frozen samples remained intact on thawing.

Samples for the filtrafugation test were obtained from three packs sampled per freezing treatment and from the values obtained the means were calculated. The effects of the pre-freezing treatment on the filtrafugation index of the thawed plantain products are presented in Table 1.

Table 1. Effects of pre-freezing treatment on the filtrafugation index of thawed plantain

Freezing method	Calcium content of blanch (ppm)	Filtrafugation index* (n = 3)	Average (n = 9)
Still air	0	0.756a	0.810x
	1360	0.823c	
	2700	0.850d	
Liquid immersion	0	0.787b	0.838y
	1360	0.849d	
	2700	0.877e	
Cryogenic	0	0.902f	0.943z
	1360	0.917g	
	2700	0.978h	

*Mean values in a column sharing the same letter do not differ by Duncan's multiple range test ($P > 0.05$).

Analysis of variance of the results showed that filtrafugation index was significantly ($P < 0.05$) affected by the freezing methods used as well as by the calcium pre-treatment. Thawed plantain tissue obtained from slices frozen in liquid nitrogen, which also had the most rapid freezing rate, had the highest filtrafugation index of 0.943, thus indicating that the destructive effect of freezing on this tissue was not as pronounced as those frozen in still air for which the filtrafugation index was 0.810. The results also clearly showed that calcium pre-treatments were effective in reducing tissue fluid loss and that higher calcium concentrations significantly ($P < 0.05$) increased the filtrafugation index.

The analysis of variance of panellist scores for the effect of calcium treatment on the sensory quality of fried plantain slices is presented in Table 2.

Increasing calcium concentrations improved sensory texture as measured by the degree of tissue firmness. Flavour, appearance and overall acceptability of the fired plantain slices were not adversely affected by the calcium treatment. The role of calcium ion in reducing the effect of freezing on tissue damage is not completely understood. Part of its effect could be due to the fact that it forms crosslinks between negatively charged polymers and pectic substances in the plantain tissue (Fennema, 1975) or it could be that the calcium salt acts as a solute to reduce the fraction of water in the tissue available for freezing.

As commonly observed in many types of plant tissue, rapid freezing of plantain had

Table 2. Effects of pre-freezing treatment on sensory attributes of fried thawed plantain

Quality attributes	Calcium content of blanch (ppm)	Sensory score
Texture	0	2.86b
	1360	5.00a
	2700	5.71a
Flavour	0	3.21a
	1360	3.64a
	2700	3.50a
Appearance	0	3.42a
	1360	3.93a
	2700	4.64a
Overall aceptability	0	3.29a
	1360	4.00a
	2700	3.00a

*Means within a column for each attribute followed by the same letter do not differ by Tukey's test ($P > 0.05$). Higher values indicate greater preference.

some quality advantages over slower freezing. This was indicated by the filtrafugation index which clearly showed that plantain frozen in liquid nitrogen has the least fluid loss and hence less disruption in cellular structure. As pointed out in the foregoing section, the tissue of the material frozen by this method tended to fracture on thawing as has been pointed out by Reeves & Brown (1966). These surface cracks may present quality problems if liquid nitrogen is used in its present form; however, this may be overcome by using evaporated liquid nitrogen gas to pre-cool the product prior to spraying with liquid nitrogen. Such a freezing method would be very expensive for a commodity of low unit value such as plantain. Furthermore it is also known that the quality advantages initially apparent in rapidly frozen foods, as compared to slowly frozen foods, tend to disappear with frozen storage (Gutschmidt, 1968; Fennema, 1975). Thus, it does not appear as if liquid nitrogen would be an appropriate method. Immersion techniques may be promising as indicated in this work; however this method has not been commercialized. From all indications freezing in still air with calcium pre-treatment appears to be the method of choice. Further work aimed at evaluating the economic feasibility of freezing preservation and the reduction of refrigeration load by partial concentration of the plantain slices prior to freezing are currently receiving our attention.

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Effect of ice and cold storage on the chemical and technological characteristics of Egyptian crab meat

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Summary

Effect of ice and cold storage at 4°C on the chemical and technological characteristics of one of red local Egyptian crab (*Carcinus meanas*) has been studied. Such studies involved the determinations of: pH values, moisture, total nitrogen, protein nitrogen, ether extract, acid value, thiobarbituric acid value (TBA) and total bacterial count (TC). It has been found that the shelf life of ice stored crabs was 2 days. Raw crabs stored at 4°C can be kept without any deterioration for 8 days. Dipping of raw crab in 20 ppm chlorotetracycline (CTC) for 20 min raised the keeping period at 4°C up to 11 days. Cooked crabs can be stored at 4°C without any spoilage for 14 days. However, dipping of cooked crabs in 20 ppm of CTC for 20 min increased the keeping period to 17 days.

Introduction

It is well known that after death the fish passes through three stages as follows: rigor mortis, autolysis and bacterial decomposition. The physical and biochemical changes occurring in fish tissues after killing will affect the quality of fresh as well as of cooled fish (Tarr, 1962, 1969). Such changes may occur also in crab meat as reported by Kurtzman, Snyder & Wilson (1960), Gangal & Magar (1963), Zaitsev *et al.* (1969), Salem *et al.* (1970), Allen & Woodburn (1972), Spinelli, Lehman & Wieg (1974) and Melnikova & Petrov (1976). Many antibiotics have been used in the U.S.A., Canada, U.K. and Japan to prolong the storage life of fresh fish and shellfish (Tarr, Southcott & Bissett, 1950, 1952; Tomiyama *et al.*, 1957). Later on, in Egypt, similar work was done by Hamed & Elias (1971) and Thabet (1973). Antibiotics of the 'broad-spectrum' type, i.e. chlorotetracycline (aureomycin) and oxytetracycline (terramycin), have been used either as a dip or in ice.

The aim of this work was to study the effect of the commercial storage with ice or at 4°C during handling and marketing of the local red crab under Egyptian conditions on the chemical and technological characteristics. Also, the storage life of the red crab stored under such conditions had been limited.

Materials and methods

Materials

The red Crabs (*Carcinus meanas*) were obtained from Suez and transported alive to

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the laboratory. Fresh crab meat was immediately analysed as control. Traditional cooking of crabs was carried out in 4% sodium chloride at about 100–105°C for 15 min. Meat extracted from both raw and cooked crab was minced by passing through a meat grinder to obtain homogeneous samples. The samples were kept in tightly closed glass jars until analysed. Ice storage of raw crab was carried out using 1 kg crushed ice : 1 kg of product and stored at room temperature until spoilage actually started. Cold storage of both raw and cooked crabs at 4°C was applied. It should be stated here that such conditions represent the conditions of commercial storage of fresh crab during handling and marketing in Egypt. There is no well developed cold chain. It is realized that the raw crabs were not at 0°C during ice storage, since there was no regular replenishment of the ice during storage period. Raw and cooked crabs were treated also with or without dipping in solution of 20 ppm CTC for 20 min before its storage at 4°C until spoilage actually started.

Methods

Sensory evaluation. The degree of spoilage of crab preserved by icing or at 4°C was determined by sensory evaluation after the samples had been cooked in boiling water containing 4% NaCl for 15 min. Such evaluation was carried out daily, while the chemical and microbiological determinations were conducted at a regular interval of 3 days during 18 days (maximum cold storage period of crab under Egyptian conditions). The exception was the first 3 days through which the sensory evaluation, chemical and microbiological determinations had been done daily due to the short storage life of ice stored crab at room temperature. Cooked samples were evaluated by a ten member panel on the basis of appearance, aroma, taste, colour and texture. Panellists were familiarized with reference samples (cooked fresh crab) and the judging procedure as described by Kramer & Twigg (1973). The hedonic scale was in the form of the following description was used: 'liked definitely', 'liked mildly', 'neither liked nor disliked', 'disliked mildly', and 'disliked definitely'. The description of 'disliked mildly' means the start of spoilage, while that of 'disliked definitely' indicates spoiled samples. Samples having the judgment of 'disliked mildly' and 'disliked definitely' evaluated by not less than eight panellists were rejected while those having both 'liked mildly' and 'liked definitely' determined by at least eight panellists were accepted.

Chemical methods

Moisture content. The moisture content was determined according to the method recommended by the AOAC (1975) using air drying oven at 100–102°C for 16–18 hr.

pH. The pH value of the crab meat was measured according to the method described by Krilova & Liskovskaia (1961) using a Beckman pH meter with glass electrode.

Total nitrogen content. The total nitrogen content of crab meat was determined using the micro-Kjeldahl method described in AOAC (1975).

Protein nitrogen. The protein nitrogen was determined in all samples of crab after its extraction with cold phosphate buffer at pH 7.2 and ionic strength of 0.5 to obtain the total proteins as described by King (1966). The precipitation of the extracted proteins was carried out by 5% solution of trichloroacetic acid. The protein nitrogen was

determined in the precipitate by the micro-Kjeldahl method described in AOAC (1975).

Ether extract. The ether extract of crab was determined according to AOAC (1975).

Acid value. The acid value in the form of mg KOH/1 gm ether extract was determined as recommended by AOAC (1975).

Thiobarbituric acid value (TBA). The TBA value was determined as optical density measured by 538 nm/1 kg of crab meat as described by Pearson (1975) using a sample of 2 gm.

Microbiological methods

Total bacterial count (TC). The plate count method using glucose yeast extract agar medium (El-Nasr Pharmaceutical Chemical Co.) was used. The medium used had the following composition (as g/litre of distilled water):

Peptone:	5.0 g	Yeast extract:	2.5 g
Dextrose:	1.0 g	Agar (purified):	13.0 g

The pH of the medium was 7. The medium was sterilized at 121°C for 15 min. One ml from each dilution was plated in the above medium in replicates and incubated at 37°C for 48 hr. The count was calculated as number of colonies per 1 g of crab meat as described by Frazier & Foster (1950). A temperature of 37°C represents the optimum temperature for growth of the mesophiles, which includes human pathogenic organisms as reported by Zapatka & Bartolomeo (1973) and Byran (1974). It is worth stating here that the aerobic plate count (APC) was determined at room temperature (26 ± 3°C) and at 35 ± 1°C as decribed by Slabyj, Martin & Gordon (1981).

Results and discussion

Results in Table 1 show a slight decrease in the moisture content of skulled crab meat kept at 4°C. This may be due to a partial evaporation of water from crabs during storage. On the other hand, there was a marked increase in water content in samples stored with ice at room temperature, in all probability due to the absorption of ice water by the tissues. On the basis of sensory evaluation of all studied samples after cooking in boiling water containing 4% NaCl for 15 min and according to the results of Table 1 it can be seen that:

1 Raw crabs stored with ice at room temperature remained acceptable for 2 days and were definitely spoiled after 3 days.
2 Raw crabs stored at 4°C remained acceptable for 8 days and were definitely spoiled after 9 days.
3 Raw crabs treated with 20 ppm CTC and stored at 4°C remained acceptable for 11 days and were definitely spoiled after 12 days.
4 Cooked crabs stored at 4°C remained acceptable for 14 days and were definitely spoiled after 15 days.
5 Cooked crabs treated with 20 ppm CTC and stored at 4°C remained acceptable for 17 days and were definitely spoiled after 18 days.

Table 1. Percent moisture content of Egyptian skulled crab meat during ice and cold storage

Storage period (in days)	Technological treatments				
	Raw crabs			Cooked crabs	
	Ice storage at room temperature	Cold storage at 4°C	Cold storage at 4°C after treating with CTC	Cooled storage at 4°C	Stored at 4°C after treating with CTC
0	79.0	79.0	79.2	76.6	76.6
1	80.5	78.7	79.0	76.3	76.2
2	81.2	78.3	78.7	76.0	76.0
3	81.3*	78.1	78.7	75.9	75.8
6	—	78.1	78.3	75.9	75.8
9	—	78.1*	78.2	75.9	75.7
12	—	—	78.1*	75.8	75.7
15	—	—	—	75.8*	75.7
18	—	—	—	—	75.4*

*Spoiled samples.

Table 2. pH values of Egyptian skulled crab meat during ice and cold storage

Storage period (in days)	Technological treatments				
	Raw crabs			Cooked crabs	
	Ice storage at room temperature	Cold storage at 4°C	Cold storage at 4°C after treating with CTC	Cold storage at 4°C	Stored at 4°C after treating with CTC
0	6.70	6.70	6.65	7.10	7.15
1	6.50	6.60	6.55	7.00	7.00
2	6.45	6.50	6.40	6.80	6.85
3	7.20*	6.40	6.28	6.78	6.80
6	—	6.35	6.27	6.70	6.75
9	—	7.01*	6.25	6.70	6.72
12	—	—	7.00*	6.65	6.70
15	—	—	—	7.20*	6.65
18	—	—	—	—	7.10*

*Spoiled samples.

Slight decrease in the pH value of raw crab meat stored with ice or at 4°C (Table 2) may be due to the breakdown of glycogen with the formation of lactic acid, especially at the stage of rigor mortis. The resolution of rigor mortis followed by the stage of microbial decomposition of certain main components such as proteins caused an increase in the pH value as shown in Table 2. Moreover, CTC-treated samples stored at 4°C showed lower pH values compared with untreated one, indicating the less pronounced microbial growth. Results of Table 2 showed that cooking caused an increase in the value of pH, which may be due to the action of heat treatment on some main components such as proteins yielding alkaline reaction products. These findings were in agreement with those obtained by Aman (1976). Spoiled samples of crabs

Table 3. Total nitrogen content of Egyptian skulled crab meat during ice and cold storage (% on dry weight basis)

Storage period (in days)	Technological treatments				
	Raw crabs			Cooked crabs	
	Ice storage at room temperature	Cold storage at 4°C	Cold storage at 4°C after treating with CTC	Cold storage at 4°C	Stored at 4°C after treating with CTC
0	11.8	11.8	11.8	11.9	12.0
1	11.8	11.8	11.8	11.9	12.0
2	11.8	11.8	11.8	11.9	12.0
3	11.9*	11.8	11.8	12.0	12.0
6	—	11.9	11.8	12.0	12.1
9	—	11.9*	11.9	12.1	12.2
12	—	—	12.0*	12.1	12.3
15	—	—	—	12.2*	12.3
18	—	—	—	—	12.4*

*Spoiled samples.

Table 4. Protein nitrogen content of Egyptian skulled crab meat during ice and cold storage (% on dry weight basis)

Storage period (in days)	Technological treatments				
	Raw crabs			Cooked crabs	
	Ice storage at room temperature	Cold storage at 4°C	Cold storage at 4°C after treating with CTC	Cold storage at 4°C	Stored at 4°C after treating with CTC
0	9.9	9.9	9.9	8.4	8.5
1	9.0	9.6	9.8	8.4	8.4
2	8.5	9.6	9.7	8.3	8.4
3	8.1*	9.3	9.6	8.2	8.3
6	—	9.0	9.2	8.0	8.1
9	—	8.8*	9.0	7.5	7.7
12	—	—	8.7*	7.1	7.4
15	—	—	—	7.0*	7.1
18	—	—	—	—	6.9*

*Spoiled samples.

showed higher values of pH ensuring the progressive increment of microbial growth at prolonged storage (Table 2).

The total nitrogen content of raw crab meat amounted to 11.8% (on dry weight basis) and did not change markedly during ice and cold storage at 4°C for both raw and cooked samples as shown in Table 3, while the protein nitrogen content of raw crab meat was found to be 9.9% (on a dry weight basis) as shown in Table 4. Such a quantity was noticeably decreased during ice and cold storage at 4°C. This may be due to the enzymatic or microbial breakdown of proteins during storage. Also, cooking caused a

Table 5. Ether extract content of Egyptian skulled crab meat during ice and cold storage (% on dry weight basis)

Storage period (in days)	Technological treatments				
	Raw crabs			Cooked crabs	
	Ice storage at room temperature	Cold storage at 4°C	Cold storage at 4°C after treating with CTC	Cold storage at 4°C	Stored at 4°C after treating with CTC
0	12.0	12.1	12.0	12.3	12.3
1	12.0	12.0	12.1	12.3	12.3
2	12.0	12.2	12.2	12.3	12.3
3	12.0*	12.2	12.2	12.3	12.3
6	—	12.3	12.2	12.4	12.3
9	—	12.3*	12.3	12.4	12.3
12	—	—	12.3*	12.4	12.4
15	—	—	—	12.5*	12.4
18	—	—	—	—	12.6*

*Spoiled samples.

Table 6. Acid value (Mg KOH/1 g ether extract) of Egyptian skulled crab meat during ice and cold storage

Storage period (in days)	Technological treatments				
	Raw crabs			Cooked crabs	
	Ice storage at room temperature	Cold storage at 4°C	Cold storage at 4°C after treating with CTC	Cold storage at 4°C	Stored at 4°C after treating with CTC
0	1.7	1.7	1.7	2.3	2.3
1	2.5	2.0	1.8	2.4	2.3
2	3.1	2.4	1.9	2.4	2.4
3	3.5*	2.5	2.0	2.5	2.4
6	—	2.8	2.3	2.5	2.4
9	—	3.1*	2.5	2.7	2.5
12	—	—	3.0*	2.7	2.6
15	—	—	—	3.0*	2.6
18	—	—	—	—	3.3*

*Spoiled samples.

significant decrease in the content of protein nitrogen due to heat effect as presented in Table 4. These results were in agreement with those obtained by many authors (Gangal & Magar, 1963; Salem *et al.*, 1970; Aman, 1976).

The total ether extract of raw crab meat (12.0% on dry weight basis) did not change significantly during ice and cold storage at 4°C for both raw and cooked samples as indicated in Table 5. This means that the ether extract (mainly free lipids) did not change during this short period of storage as stated by Giddings & Hill (1975).

Data in Table 6 show the acid value of crab meat lipids as affected by different treatments during ice and cold storage at 4°C. The acid value of raw samples was 1.7 mg

Table 7. Thiobarbituric acid value (as OD 1t 538 nm/l kg) of Eryptian crab meat during ice and cold storage

	Technological treatments				
	Raw crabs			Cooked crabs	
Storage period (in days)	Ice storage at room temperature	Cold storage at 4°C	Cold storage at 4°C after treating with CTC	Cold storage at 4°C	Stored at 4°C after treating with CTC
0	0.015	0.015	0.010	0.005	0.005
1	0.025	0.020	0.015	0.025	0.020
2	0.050	0.040	0.031	0.037	0.030
3	0.075*	0.050	0.040	0.046	0.035
6	—	0.062	0.045	0.058	0.044
9	—	0.080*	0.055	0.073	0.060
12	—	—	0.066*	0.089	0.072
15	—	—	—	0.150*	0.085
18	—	—	—	—	0.095*

*Spoiled samples.

Table 8. Total bacterial count (count × 10^6/1 g) of Egyptian skulled crab meat during ice and cold storage

	Technological treatments				
	Raw crabs			Cooked crabs	
Storage period (in days)	Ice storage at room temperature	Cold storage at 4°C	Cold storage at 4°C after treating with CTC	storage storage at 4°C	Stored at 4°C after treating with CTC
0	0.015	0.015	0.004	0 001	0.001
1	0.050	0.023	0.010	0 010	0.008
2	1.200	0.060	0.025	0 015	0.010
3	6.000*	0.075	0.042	0.025	0.020
6	—	0.082	0.055	0 033	0.030
9	—	5.400*	0.085	0 042	0.040
12	—	—	3.800*	0 068	0.057
15	—	—	—	3 100*	1.100
18	—	—	—	—	3.000*

*Spoiled samples.

KOH/1 g of ether extact. After cooking, the acid value rose to 2.3 mg KOH/1 g ether extract, which may be due to the effect of heat treatment on the breakdown of lipids and liberation of free fatty acids. During cold storage the acid value progressively increased as the time of storage elapsed, indicating both hycrolysis and oxidation of fat with a parallel release of more free fatty acids as reported by Krzeczkowski, Tenney & Hayes (1972) and Krzeczkowski & Stone (1974). The increase in acid value was more noticeable during the ice storage of raw samples compared with cold storage at 4°C, indicating more lipid changes in the former case, perhaps because of relatively higher temperatures of storage. The increase of acid value in CTC-treated samples (uncooked)

was lower as compared wih untreated ones, indicating the effect of CTC on reducing the microbial load (Zaitsev *et al.*, 1969).

The thiobarbituric acid values (TBA) of crab meat as affected by different treatments during ice and cold storage are presented in Table 7. It can be seen that the TBA value of raw samples was 0.015 (OD/1 kg). Soaking of cooked crabs in CTC solution (20 ppm for 20 min) and storing at 4°C reduced the value of TBA to about 0.005, which may probably be due to the leaching of some malonaldehyde from tissues at cooking. CTC treatment of raw and cooked samples reduced the TBA value during cold storage, which could be ascribed to the inhibition of microbes producing enzymes capable of hydrolysis and oxidation of lipids. Results in Table 7 indicate that the TBA values increased as the time of storage elapsed, indicating that oxidation of lipids. The increase was more pronounced during ice storage, perhaps because of the relatively high storage temperature. Shenikova (1975) reported that the thermal treatment accelerated the rate of lipid oxidation during storage of calmar. Similar results were obtained by Dessouki (1976) during storage of freeze dried raw and pre-cooked grey mullet fish. These findings were in agreement with those obtained during storage of cooked crab at 4°C as shown in Table 7.

The total bacterial count (count $\times 10^6$/1 g) of crab meat as affected by different treatments during ice and cold storage is presented in Table 8. From Table 8 it can be seen that the total bacterial count (TC) of raw crab amounted to 0.015×10^6/1 g. Dipping of raw and cooked crab in solution containing 20 ppm CTC for 20 min reduced noticeably the TC to 0.004 and 0.001×10^6/1 g for raw and cooked crab respectively. Results of Table 8 show that TC of crab meat dipped in CTC before storage was found to be very low compared with the untreated samples, especially at prolonged storage. Cooked samples always showed lower bactrial counts than the uncooked (raw) ones. The bacterial load of ice stored raw crab was very high compared with that stored at 4°C for the same period, indicating the short shelf-life storage of raw crab kept with ice alone under the commercial storage conditions of fresh crabs in Egypt. These findings were in agreement with those obtained by Benarde & Littleford (1957). In general, the TC of crab meat increased progressively as the time of storage elapsed in all treatments. It is interesting to note here that such increases did not happen suddenly but gradually over a period of about 3 days in all treatments except with that of ice storage at room temperature as shown in Table 8.

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Composition of shrimp by-catch fish from the Gulf of California and effects on the qualities of the dried salt fish cake product

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Summary

The gross chemical and physical characteristics of a range of common fish species found in shrimp by-catch from the Gulf of California have been studied. These fish represented about 90% by weight of all fish species found in the by-catch. The crude protein, fat, moisture and ash contents were all found to be within the normal ranges for demersal teleosts, though fat contents of several were as high as 6%. The heterogenous nature of the by-catch fish species was also apparent from a wide range in lengths and weights, and variation in the colours and textures of their flesh. These factors may necessitate pre-selection of fish types destined for specific food products. Dried salt fish cakes were prepared from two of these fish species that had been manually and acid eviscerated. Cakes from acid eviscerated fish minces to which 10–15% crude salt had been added dried well and were found to be as acceptable in taste panel trials as those from manually eviscerated fish minces prepared in the trad tional way with higher additions of crude salt.

Introduction

Shrimp by-catch from shrimping operations is an often large and under-utilized resource. In 1967 it was estimated that almost 600000 t of fish were taken in shrimping operations and discarded in the U.S. Gulf of Mexico fisheries (Bullis & Carpenter, 1968). More recently this estimate has been raised to about 1 million t (National Academy of Sciences, 1978). A more conservative estimate for annual losses of shrimp by-catch from Mexican waters has also been recently quoted at 400000 t, equivalent to over 250000 t of usable fish (Schafer *et al.*, 1982).

Generally the fish found in the Mexican shrimp by-catch are small demersal teleosts unsuitable for marketing in the normal way as wet fish. Only 2–5% of this shrimp by-catch is composed of fish of commercial grade (Chavez & Arvizu, 1972). With the advent of meat–bone separation techniques it has proved possible to process these small bony fish to yield deboned minces of high quality. In Mexico, various products have been developed into which these deboned minces have been incorporated. These include dried salt fish cakes (Del Valle & Nickerson, 1968; Young *et al.*, 1979), frozen breaded fish sticks and fish minces (Tableros & Young, 1981; Poulter, 1982a), canned fish pâtés, sausages and a mince/vegetable mixture (Poulter, 1982a; Poulter & Treviño, 1983). Plans for the commercial production of these products in Mexico have been

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made and several financial studies prepared (Street, Young & Crean, 1980; Young & Marter, 1981).

In the Gulf of California there exist a number of logistical problems associated with the recovery of the shrimp by-catch resource due especially to its variable nature. Studies have been conducted from which recommendations have arisen for the sorting, handling and retrieval of by-catch fish (Crean & Young, 1979; Poulter, 1982b), together with the financial incentives necessary for fishermen to bring the raw material ashore (Poulter, 1982b; Schafer *et al.*, 1982).

The heterogenous nature of shrimp by-catch is particularly evident from studies in which over eighty-seven species or groups of species of fish from forty-three families have been identified (Young & Romero, 1979; Mellado, 1980). The physical characteristics and chemical composition of these fish could clearly differ, one from another, and in turn could affect the way they have to be processed into the newly developed food products. Indeed, it has been established that the deboned minces prepared from a number of by-catch fish species vary considerably in terms of their colours and textures, and washing and acid soaking techniques are being developed in attempts to standardize these characteristics (Poulter & Treviño, 1982; N. H. Poulter & D. Mejia, unpublished data).

Little information is available, however, on the chemical composition of Gulf of California shrimp by-catch fish species. Thus, the fish species or groups of species which make up the bulk of the shrimp by-catch fish from this region have been analysed for their gross chemical composition. This data will then allow for the identification of any requirement for the selective sorting of individual fish species from the by-catch. Further, a study is described in which two fish species drawn from the by-catch which show different physical and chemical characteristics were processed into dried salt fish cakes. The effects of manual and acid evisceration and cleaning techniques applied to these fish on the chemical, sensory and drying qualities of cakes is discussed.

Materials

Raw materials

Shrimp by-catch fish were collected fresh from various voyages made in the northern sector of the Gulf of California during the latter half of the 1981/82 shrimping season (February–June). Quantities of the twenty most common fish species or groups of species were collected (Table 1). After being washed thoroughly in seawater the fish were packed in crushed ice overnight and subsequently frozen (−20°C) prior to analysis.

Commercially available iodized fine salt was used in the preparation of dried salt fish cakes. This salt was determined to contain 87.76% NaCl dry weight basis (d.w.b.) (AOAC, 1980). The acid used for the evisceration and cleaning of fish was glacial acetic acid (AR).

Methods

Proximate chemical composition

Whole fish were thawed overnight at +5°C, and skinless fillets prepared from ten individuals were then minced and mixed thoroughly prior to analysis. Crude protein (N×6.25) was determined by the micro-Kjeldahl method (Joslyn, 1970). Crude fat, moisture and ash contents were determined by standard procedures (AOAC, 1980). All analyses were carried out in duplicate.

Table 1. List of the most common fish species found in shrimp by-catch from the Gulf of California

No.	Scientific name	Common name	% (by weight) occurrence		
			Ref. 1	Ref. 2	Ref. 3
1	*Eucinostomus* spp.	Mojarras	16.04	26.29	12.09
2	*Orthopristis reddingi*	Rayadillo	32.74	5.46	11.40
3	*Citharichthys* spp. & *Etropus* spp.	Flatfishes	6.74	8.78	8.69
4	*Calamus brachysomus*	Pacific porgy	0.20	3.31	1.00
5	*Rhinobatos productos*	Guitarfishes	9.09	2.29	1.28
6	*Galeichtys caerulescens*	Catfish	5.06	5.55	3.50
7	*Porichthys* spp.	Midshipmen	0.12	2.00	4.17
8	*Mustelus* spp.	Sharks	0.39	3.92	0.15
9	*Diplectrum* spp.	Cabaicuchos	1.98	17.60	12.47
10	*Synodus scituliceps*	Lizardfish	2.00	4.10	9.57
11	*Bairdiella icistia*	Ronco	0.35	2.83	—
12	*Pseudupeneus grandisquamis*	Red goatfish	0.87	1.68	3.51
13	*Micropogonias altipinnis*	Chano	0.75	0.31	2.38
14	*Xenistius californiensis*	Salema	1.80	3.62	0.32
15	*Paralabrax maculatofasciatus*	Cabrilla	5.99	1.43	0.94
16	*Trachinotus* spp.	Pompanos	3.16	0.04	0.10
17	*Scorpaena* spp.	Scorpion fish	0.29	1.31	1.42
18	*Balistes polylepsis*	Trigger fish	0.27	1.49	0.92
19	*Cynoscion xanthulus*	Corvinas	0.50	0.92	1.16
20	*Myliobatis californicas*	Aguila ray	3.08	0.13	1.21
		Totals	91.42	93.15	76.28

Figures for % occurrence (by weight) of fish species calculated from Ref. 1: Poulter (1983), Ref. 2: Young & Romero (1979) and Ref. 3: Mellado (1980). These figures are calculated in relation to the total weight of the fish material found to occur in the catch and not to the total weight of unsorted shrimp by-catch.

Dried salt fish cakes

Deboned minces were prepared from both manually and acetic acid eviscerated fish. The two fish species used were red goat fish (*Pseudupeneus gradisquamis*) and cabrilla (*Paralabrax maculatofasciatus*). These two species of fish were chosen since the minces were found to exhibit differing fat contents and coloration.

Fish to be prepared manually (ME) were thawed overnight at +5°C, the heads and viscera removed and the carcasses then washed in ice cooled water ensuring removal of kidney and peritoneal tissue. Those eviscerated and cleaned using the acetic acid aided evisceration method (AE) (Poulter & Treviño, 1982), were also thawed and the fish nobbed. The carcases were then chopped transversely into 3 cm pieces. These chunks were then soaked in a 4% (v/v) solution of aqueous acetic acid for 30 min (fish/solution: 1:1). The material was then strained and rinsed in two separate volumes of ice cooled water.

Both ME and AE fish were deboned using a Paoli deboning system (Model 19-529). The resultant minces were divided into five 1 kg portions and mixed with various quantities of crude salt (87.76% NaCl d.w.b.) to give final crude salt additions to the minces ranging from 0 to 25% by weight. This was achieved by adding 0, 100, 150, 200 or 250 g of crude salt to the 1 kg portions of mince. For subsequent ease of identification these salted minces are referred to as minces to which 0, 10, 15, 20 or 25% crude salt

has been added. The salted mince was mixed for 5 min at a slow speed using an industrial Hobart mixer. Ten cakes of approximately 100 g each were then formed using petri dish moulds. These were placed on wire mesh trays for pre-cooking and drying as described by Young *et al.* (1979) (1 hr at 100°C followed by drying at 40°C), using a Proctor and Schwartz convection oven.

Individual cakes were weighed initially, after pre-cooking and at intervals during the drying period. Weight loss curves were prepared and the moisture and salt contents of 50 hr dried cakes determined. Moisture contents were determined by a standard procedure and the NaCl contents of cakes by the ammonium thiocyanate/silver nitrate method (AOAC, 1980).

Additionally, the mean diameter for each batch of dried salt fish cakes was determined and rehydration studies conducted in which the percentage weight gains of cakes due to the absorption of water after 30 min immersion were calculated.

Sensory evaluation

Cakes were desalted by soaking in fresh water for 30 min. Approximately 1 litre of water was used for two cakes. The soak water was replaced with fresh water which was heated to boiling. The cakes were then simmered for 15 min. The procedure used for the sensory evaluation was based on that described by Del Valle *et al.* (1973). Samples of cakes from each of the four fish species/evisceration treatment batches which showed the greatest weight loss on drying were prepared in this manner. These batches of cakes were chosen for evaluation because they had reacted optimally to the processing conditions used. As a result, the cakes were likely to have lower moisture contents and this, coupled with relatively high salt contents, would probably ensure a long shelf life at ambient tropical temperatures. The samples were served together in random order to a panel of ten assessors drawn from ITESM staff. Assessors were asked to compare and contrast the samples given them and then to score each on a scale of from 1 to 9 where 1 indicated the sample to be totally unacceptable, 5 acceptable and 9 excellent. Comments for each sample were also requested.

Results

Proximate chemical composition

The twenty most commonly occurring fish species or groups of species found in the shrimp by-catch, together with their average percentage occurrence by weight in the catch and chemical composition are given in Tables 1 and 2. Considerable variation was found to exist between the groups of fish. The moisture contents ranged between 73.7 and 82.3%, crude protein contents between 15.2 and 25.4%, crude fat contents between 1.2 and 6.1% and ash contents between 0.5 and 1.2%. However, the crude protein contents of the majority of the teleost fish species studied ranged only between 16.6 and 18.9% whereas the elasmobranch fish species showed levels as high as 25.4% due probably to high non-protein nitrogen (NPN) contents. The average crude fat content of the fish species studied was 2.72%, though several, notably red goat fish (*P. grandisquamis*) and pompanos (*Trachinotus* spp.), gave values of over twice this level.

Physical characteristics

The heterogenous nature of the most common shrimp by-catch fish in terms of their lengths and weights can be seen in Table 2. Mean weights and lengths were found to range from 20.8 to 1532.1 g and from 10.1 to 60.6 cm, respectively. However, the

majority of these fish had weights of less than 200 g and lengths of less than 25 cm.

The texture and colour of both raw and cooked fillets prepared from these fish were found to vary considerably. The texture of the muscular tissue from midshipmen (*Porichthys* spp.), for example, was extremely soft whilst that of finescale trigger fish (*Balistes polylepsis*) very hard. The colour of most of the fish fillets prepared was cream but certain had flesh colours that were dark grey, e.g. rayadillo (*Orthopristis reddingi*), and red goat fish (*P. grandisquamis*) fillets were orange-red.

Table 2. Weight/length data and proximate chemical composition of the most common fish species found in shrimp by-catch from the Gulf of California

Fish species No.	Mean weight (g)	Mean length (cm)	Moisture (%)	Crude protein* (%)	Lipid (%)	Ash (%)
1	20.75	10.06	79.21	17.58	2.67	1.22
2	77.06	16.56	73.66	17.79	3.56	1.13
3	105.06	21.50	79.37	16.92	2.34	0.81
4	74.60	12.50	77.40	18.90	2.21	1.43
5	645.13	60.55	74.19	25.23	1.76	0.99
6	150.49	21.51	78.38	16.68	3.79	0.66
7	75.97	17.76	82.27	15.22	1.23	0.76
8	168.94	32.75	76.97	21.65	1.98	1.36
9	55.31	14.94	78.87	17.84	5.26	1.07
10	185.07	27.56	76.38	20.80	2.51	0.87
11	210.32	19.57	78.95	17.67	2.71	1.34
12	70.43	17.40	74.01	20.22	6.13	1.09
13	421.98	35.65	78.94	16.74	1.52	0.82
14	170.50	19.90	75.76	17.75	3.21	1.27
15	76.59	16.87	78.66	18.33	2.55	0.64
16	81.38	13.13	75.86	17.58	4.59	0.67
17	274.66	21.01	78.01	18.83	1.78	0.53
18	1021.25	34.69	78.49	18.38	1.29	0.49
19	194.20	24.87	77.90	17.89	1.98	0.83
20	1532.11	25.67	74.43	25.40	1.31	0.74

*Crude protein = total N × 6.25.

Dried salt fish cakes

Weight loss curves. Weight loss curves for the four groups of dried salt fish cakes prepared (cabrilla ME and AE; red goat fish ME and AE) are given in Figs 1, 2, 3 and 4.

It is evident that the methods of evisceration used (ME and AE), the inherent differences in the composition of the fish species themselves (particularly fat content), as well as the amount of crude salt added to the minces (0–25%), affect the weight loss profiles obtained.

Fish cakes prepared with different amounts of crude salt from AE minces tended to have more uniform weight losses on pre-cooking and drying than those prepared from ME minces. In general, when crude salt was added to AE minces at levels lower than that previously recommended (20%, Young *et al.*, 1979), the cakes still lost weight rapidly. This was particularly so for cabrilla minces. For this species the addition of 10% crude salt to AE mince resulted in a drying rate equivalent to that for ME mince to which 20% crude salt had been added (Figs 1 and 2). The various additions of crude salt also affected the weight losses of cakes prepared form all types of minces during the pre-cooking stage. It was found that greater weight losses were achieved, the larger the

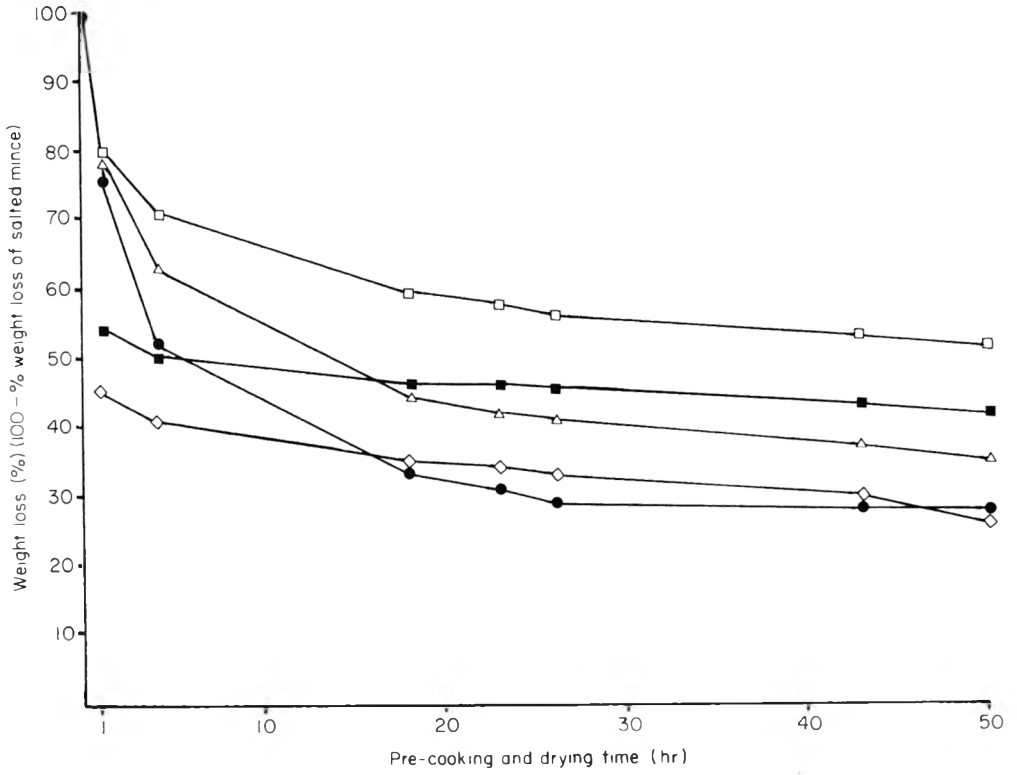

Figure 1. Weight loss curves for ME cabrilla fish cakes; percentage crude salt added: ●, 0%; △, 10%; □, 15%; ◇, 20%; ■, 25%

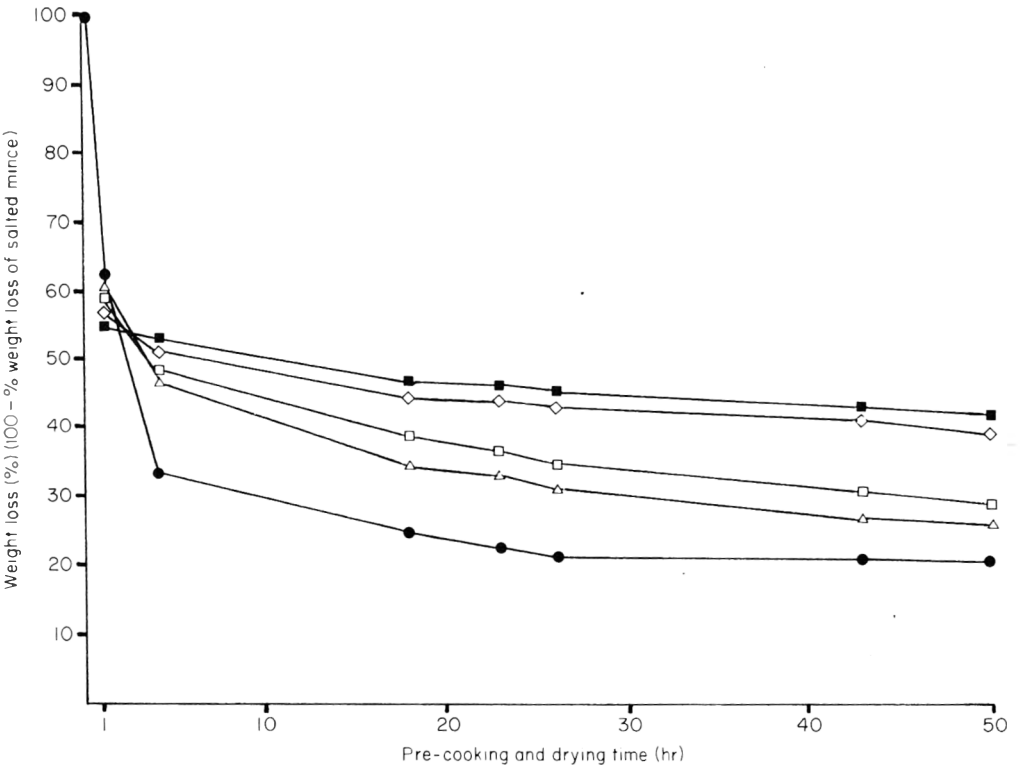

Figure 2. Weight loss curves for AE cabrilla fish cakes (symbols as in Fig. 1).

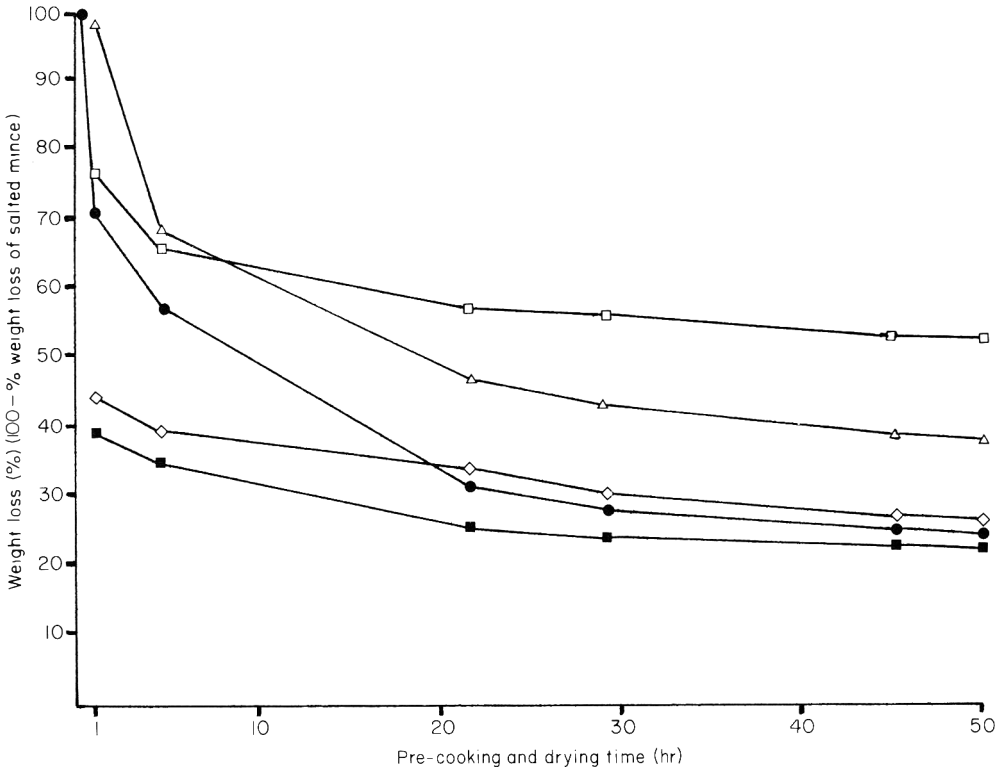

Figure 3. Weight loss curves for ME red goat fish cakes (symbols as in Fig. 1).

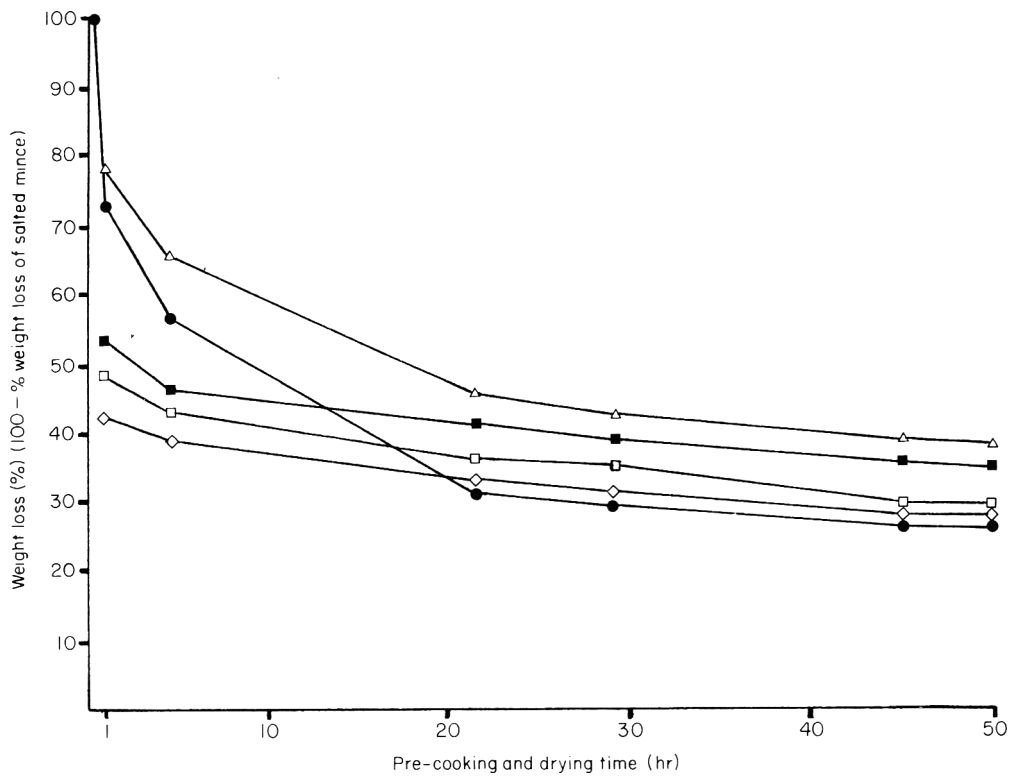

Figure 4. Weight loss curves for AE red goat fish cakes (symbols as in Fig. 1).

quantity of crude salt added. Cakes formed with minces from which salt was omitted pre-cooked the least well but then dried the most rapidly of all. This effect was more pronounced with AE minces than with ME minces from both the fish species studied. Furthermore, the treatment of the fish with acetic acid during the evisceration process resulted in different effects when crude salt was added to minces. Thus, AE minces from red goat fish required the addition of relatively more crude salt to obtain the same weight losses seen for AE minces from cabrilla after 50 hr of drying.

Moisture and salt contents. The moisture and salt contents of each batch of 50 hr dried salt fish cakes are given in Table 3. It was found that the levels of salt also varied considerably between cakes. Moreover, cakes to which lower quantities of crude salt had originally been added (i.e. 10 and 15%), contained disproportionally higher salt (NaCl) contents on a dry weight basis than those in which higher quantities of crude salt had originally been incorporated (i.e. 20 and 25%) (Table 3). This effect appeared to result from the different weight losses, due to drip exuded, seen during the pre-cooking stage.

Redydration. Data for the rehydration of cakes is given in Table 3. Rehydration of those cakes prepared without salt was low. Furthermore, there appeared to be an inverse relationship between the increase in weight on rehydration and the moisture content of the 50 hr dried cakes prepared with salt. However, the extent of rehydration could have been influenced by the area and thickess of the cakes. Indeed, the diameters

Table 3. Moisture and salt (NaCl) contents, % weight increases on rehydration and mean cake diameters of dried salt fish cakes prepared from two shrimp by-catch fish species

Fish species	Method of fish evisceration	% Crude salt added	% Moisture in 50 hr dried cakes	% Salt (NaCl) in 50 hr dried cakes	% Salt (NaCl) in 50 hr dried cakes d.w.b.	Cake rehydration (% increase in weight)	Mean cake diameter (mm)
Cabrilla (*P. maculato fasciatus*)	Manual (ME)	0	13.47	—	—	14.88	53.5
		10	25.94	22.55	30.45	6.55	60.5
		15	30.43	21.06	30.27	14.14	68.8
		20	21.85	20.77	26.58	34.36	63.0
		25	35.80	19.31	30.08	14.61	78.1
	Acid aided (AE)	0	9.32	—	—	50.48	60.5
		10	5.82	26.33	27.96	67.81	76.0
		15	7.43	31.44	33.96	39.71	82.5
		20	32.79	28.52	42.43	15.44	86.0
		25	33.05	23.99	35.83	19.44	87.3
Red goatfish (*P. grandisquamis*)	Manual (ME)	0	18.23	—	—	24.78	60.8
		10	23.51	24.61	32.18	8.35	67.5
		15	40.43	15.33	25.74	3.51	72.5
		20	8.33	23.06	25.16	59.28	69.0
		25	7.90	25.92	28.14	74.22	74.4
	Acid aided (AE)	0	16.92	—	—	20.92	64.3
		10	20.75	24.01	30.30	13.51	69.3
		15	13.47	22.73	26.27	45.42	66.4
		20	13.52	23.47	27.14	58.48	74.7
		25	21.40	25.01	31.82	81.46	84.0

of the cakes were found to increase with the addition of larger amounts of crude salt, particularly those prepared from AE minces (Table 3).

Sensory evaluation. Fish cakes exhibiting some of the largest weight losses over 50 hr from each of the four fish species/evisceration treatment groups were prepared for sensory evaluation. Mean scores obtained for overall acceptability were: cabrilla, ME 20%—6.8, AE 10%—6.5 and red goat fish, ME 25%—5.4. AE 15%—5.2. Thus, the method of evisceration used and the quantity of crude salt added to minces seemed to have little effect on the acceptability of the products. However, cakes prepared from red goat fish minces were found to be somewhat less acceptable than those prepared from cabrilla minces. This was probably due to the harder texture and grittier particle feel of the red goat fish cakes. No appreciable differences in colours of flavours were noted.

The appearance of dried salt fish cakes prepared from the two fish species which had been eviscerated and cleaned in two different manners and to which various quantities of crude salt had been added varied marginally. The large differences noted in the colours of the original deboned fish minces were not reflected in the finished dried salt cakes. Most cakes prepared with salt, irrespective of method of fish evisceration or fish species were cream-tan coloured. Those without salt were dark brown with heavily wrinkled surfaces. Cakes prepared from AE minces, however, tended to be larger and more irregularly shaped than those from ME minces.

Discussion

The fish from the shrimp by-catch from the Gulf of California have generally been considered to have fat contents of less than about 2%. However, it is clear that the fat contents of the more common fish species are a little higher than this.

Studies with by-catch from the Gulf of Mexico have showed that the fat content of fish varied seasonally, ranging from 1.2 to 14.5% (Thompson, 1958). This effect may also occur with by-catch fish from the Gulf of California. Indeed fish collected during the last few months of the 1981/82 shrimping season (May–June) were found to contain large amounts of roe indicating the beginning of the spawning cycle. This is consistent with increased water temperatures (November–April, 13–15°C; May–October, 25–30°C). The fact that some of the fish species found in the by-catch, which are generally considered to be lean demersal fish, can have fat contents rising to 5–6%, and possibly higher, may introduce a further problem in the quality control of developed food products. Such problems could possible include increased risk of rancidity development, reduced drying rates and softened textures.

Indeed, it has already been established that fat contents greater than about 5% may markedly lower the drying rates of fish minces (C. D. Wood, unpublished data, TDRI). Further, it has been found that treatment of fatty fish species in dilute acid solutions can reduce the fat content of resultant minces leading to accelerated drying rates (Wood, 1981).

The heterogenous nature of the fish from the shrimp by-catch is also evident from their wide range of weights and lengths. The evisceration and cleaning of these small and irregularly shaped fish by hand is very labour intensive and the variation in the physical characteristics of the flesh, especially colour and texture, must mean the pre-selection of fish types for specific food products will be necessary. However, the technique developed whereby fish are treated in an acid medium has been shown to hasten the process of evisceration and to reduce colour variation (Poulter & Treviño,

1983). The acid evisceration technique has also been found to confer certain advantages to products, such as dried salt fish cakes, which are prepared from the deboned fish minces. Weight loss curves obtained for cakes prepared from AE fish minces incorporating 10–15% crude salt were similar to those for ME minces to which the recommended 20% crude salt had been added (Young *et al.*, 1979). Furthermore, it has been found that due to lower weight losses on pre-cooking for cakes prepared from minces with lower initial crude salt contents, that the relative losses of salt in the cooking drip were reduced. Consequently cakes prepared, for example, with 10% crude salt showed levels of salt (NaCl) in the final dried product as high as those prepared with 20% crude salt (i.e. 25–30% NaCl d.w.b.). Thus, cakes prepared from AE minces containing initially lower levels of crude salt, dry rapidly and have similar final salt (NaCl) and moisture contents to those prepared in the traditional manner from ME minces. Cakes prepared from AE minces to which 10–15% crude salt had been added also rehydrated well and were found to be as acceptable to taste panellists as those prepared from ME minces to which 20–25% salt had been added.

Acknowledgments

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On-board handling and quality changes during storage of fish predominant in Mexican shrimp by-catch

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Summary

An important factor in the realization of the potential of shrimp by-catch relates to how it can be easily and economically retrieved. This study deals with this problem and proposes a system of handling and storage of by-catch fish at sea. Some fish representative of by-catch from the Gulf of California were investigated for their storage qualities when held in crushed ice and chilled seawater (CSW). It was determined, using various chemical and physical parameters, that high quality was retained for fish held in ice and CSW for up to 14 and 10 days, respectively. Such storage periods are compatible with the average duration of shrimper voyages. Use of CSW appears a more appropriate method since storage and off-loading would be less labour intensive than with iced storage and ice usage would be reduced. Incorporation of CSW tanks, taking into account existing shrimp boat designs, is proposed which would not affect present iced/frozen storage capacities.

Introduction

Mexican shrimp by-catch is a large and under-utilized resource (Shafer *et al.*, 1982). The fish component of the by-catch from the Gulf of California consists of many small demersal species (Young & Romero, 1979). This factor alone has, until recently, retarded the realization of its potential food uses due to lack of appropriate processing equipment.

With the advent of meat and bone separation techniques and processes for the evisceration of the small and irregularly shaped by-catch fish, high quality deboned minces may now be prepared suitable for incorporation into a range of products for human consumption (Poulter, 1982a). With the development of these technologies together with the reduced incomes and profits being made in the declining shrimp industry, shrimp by-catch has now become of greater relative commercial value. Indeed, it has been established that prices attractive to the shrimper may be paid for sorted by-catch fish from the Gulf of California (Street, Young & Crean, 1980; Young & Marter, 1981).

Nonetheless, the problem remains of how to handle and store large quantities of shrimp by-catch on board until the return of shrimp vessels to port. Little information is available on methods for the storage of by-catch and the effects of such storage on the qualities of the fish. However, most shrimp boats working in the Gulf of California have iced storage capacities sufficient to last for 10–20 day voyages. This capacity is rarely fully utilized at the present time due to low yields of shrimp and, consequently, space

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may be available for the storage of partially or fully sorted shrimp by-catch (Crean & Young, 1979).

The use of ice is one of the simplest methods for extending the storage life of fish, particularly fish caught in tropical waters (Disney, 1976). Generally, information is only available about the storage characteristics of large commercial fish species which are suitable for marketing directly as wet fish. Nonetheless, it is known that the nature of the fish—i.e. whether they are pelagic or demersal species, from temperate or tropical waters and their size—can affect the duration of storage in ice (Hoffman *et al.*, 1974).

Crushed ice suitable for the storage of shrimp and fish is readily available in the fishing ports of the Gulf of California. Ice is, however, an expensive commodity in Mexico, especially when considering that for proper iced storage a ratio of at least 1 part fish material to 1 part crushed ice must be used. It has been shown that temperate water fish, such as herring and hake, can be maintained in chilled seawater (CSW) (Eddie & Hopper, 1974; Hansen, 1977). This technique allows for the more rapid handling of large catches both at sea and on shore as well as reducing the usage of ice. Further, the use of the CSW system effects more rapid cooling of fish due to their intimate contact with the medium. This can result in the maintenance of fish showing reduced bacterial numbers and lessened occurrence of burst belly walls than for those iced and boxed in the orthodox manner (Eddie & Hopper, 1974).

This paper deals with changes in the quality characteristics, as determined by several physical and chemical parameters, of some of the more common fish species found in Mexican shrimp by-catch. The fish materials studied were stored in CSW or iced in the traditional way. This then allowed for comparisons to be made between storage treatments, fish species and the quality parameters investigated. On the basis of these studies modifications to the existing shrimp vessel design which would permit adequate on-board handling and storage of by-catch fish are proposed. It is hoped that such information will assist the development of appropriate methods for the handling, storage and retrieval of shrimp by-catch fish from the Gulf of California.

Materials and methods

Fish material

Shrimp by-catch fish were collected fresh from the Marsep IV (CET de MAR, Guaymas), a shrimping vessel working in the upper Gulf of California during the 1981/82 shrimping season.

Samples of six fish species (or groups of species) representative of the fish component of the by-catch were separated and washed well with seawater. Fish were

Table 1. Shrimp by-catch fish species studied and their physical characteristics

Scientific name	Common name	Average weight (g)	Average length (cm)
Citharichthys spp.	Lenduados	105.06	21.50
Eucinostomus spp.	Mojarras	20.75	10.06
Paralabrax maculatofasciatus	Cabrilla	76.59	16.87
Orthopristis reddingi	Rayadillo	77.06	16.56
Diplectrum sp.	Cabaicucho	55.31	14.94
Calamus brachysomus	Mojarron	74.60	12.50

then divided into batches and stored as appropriate. The fish included in the investigation can represent up to 60% by weight of all the fish found in shrimp by-catch from this region (Poulter & Poulter, 1984).

The list of species used together with their average weights and lengths are presented in Table 1.

Fish storage

Fish were stored either in crushed ice or in CSW. Fish held in ice were layered into 'isiboxes' (Hansen, 1977), with finely crushed ice (fish/ice—1:1) immediately after capture. Fish stored in CSW were added to 100 litre plastic drums in which 50% of their weight of crushed ice had already been placed. Sea water was then added to cover the fish and the mixture thoroughly agitated by hand to ensure rapid cooling. The containers holding both iced and chilled fish were then held in the insulated ice storage compartment of the shrimp vessel until its return to port where they were transferred to a cold room maintained at +2°C.

Evaluation of fish quality

Samples of each fish specie from each storage treatment were taken at intervals of 2–3 days and evaluated in the following ways.

Using the GR Torrymeter (GR International Electronics Ltd, Camberley, U.K.), readings were taken from sixteen individual fish at a point on the lateral line just posterior to the pectoral fin. The mean value of these readings was then calculated. At this time notes on the appearance of the fish were taken. These included colour and nature of eyes and gills, presence of mucus and skin condition, occurrence of burst belly walls, odour, and texture of flesh.

Total volatile base (TVB) contents of fish flesh were determined by the method described by Pearson (1975) involving the use of the micro-Kjeldhal distillation apparatus and magnesium oxide (MgO) as a catalyst. The samples used for this assay were obtained by pooling one fillet from five individual fish from each fish specie used in the sensory evaluation.

Sensory evaluations were made on fillets of fish that had been boiled in individual plastic bags for 15 min and then allowed to cool. Ten panellists accustomed to eating fish were asked to score each of the fish samples for general acceptability on a scale from 1 to 8, where a score of 1 was unacceptable and a score of 8 was highly acceptable. An average score of 5 or less was taken to indicate that the sample was not acceptable to panellists.

Microbiological evaluations were made with both skin and muscle tissues from each of the fish species. Total viable bacterial numbers were determined using the technique described by Speck (1976). A non-selective nutritive medium was used incorporating 0.5% NaCl. Peptone water (0.1%) was used to obtain dilutions from 10^{-1} to 10^{-7} followed by incubation at 28–30°C for 48 hr.

The results from each of these four evaluatory parameters were tabulated and the respective correlation coefficients (r) calculated.

Results and discussion

The average weights and lengths of the fish species studied are given in Table 1. Average weights range from 20 to 105 g and lengths from 10 to 17 cm. This weight and size variation has to date precluded the use of established mechanical sorting tech-

niques. Furthermore, due to variation in the texture and colour of the flesh of these fish species, pre-selection and sorting according to type has been found to be necessary prior to their processing into certain food products (N. H. Poulter & D. Mejia, unpublished data). Consequently, at the present time the sorting of the shrimp by-catch must be carried out manually (Poulter, 1982b).

Fish taken for the present study were, therefore, separated by hand and washed thoroughly with seawater to remove adhering debris, prior to storage. The changes in the qualities of these fish were then investigated using the techniques described.

The results of GR Torrymeter evaluations are presented in Table 2. The dielectric properties of fish tissue change progressively during storage and the Torrymeter responds to the dielectric loss angle and is claimed to provide an accurate measurement of the freshness of fish. Thus the GR Torrymeter may provide a rapid result from non-destructive sampling. Although its application to tropical fish species has not been proven (Poulter, Nicolaides & Hector, 1978) the Torrymeter has been shown to be a good indicator of the freshness of a number of commercial Mexican fish (R. H. Young, unpublished data).

Table 2. The effects of storage in ice and CSW on GR Torrymeter readings

	Days in storage												
Fish species	1	3	4	5	6	7	8	10	11	12	13	14	17
Lenguados													
Ice	15.4	—	13.0	—	11.5	—	9.6	9.5	—	9.7	—	8.5	8.1
CSW	13.0	11.7	—	11.0	—	9.6	—	—	9.1	—	8.3	—	—
Mo[illegible]as													
Ice	13.5	—	13.4	—	11.1	—	11.0	10.8	—	11.3	—	7.6	9.1
[illegible]W	10.8	9.6	—	9.4	—	9.1	—	—	8.6	—	8.5	—	—
Ice	14.6	—	13.7	—	11.0	—	10.6	13.0	—	12.7	—	11.6	9.6
CSW	10.5	9.3	—	9.0	—	9.0	—	—	7.9	—	7.5	—	—
Rayadillo													
Ice	11.4	—	11.0	—	9.5	—	10.8	9.7	—	8.9	—	8.0	7.0
CSW	9.1	8.3	—	8.3	—	8.0	—	—	7.5	—	7.7	—	—
Cabaicucho													
Ice	14.3	—	12.1	—	10.4	—	—	10.9	—	9.9	—	9.1	7.1
CSW	9.4	6.9	—	6.3	—	6.5	—	—	3.6	—	4.1	—	—
Mojarron													
Ice	12.9	—	12.3	—	11.7	—	—	10.5	—	10.5	—	9.6	8.9
CSW	10.1	10.1	—	9.8	—	9.1	—	—	8.6	—	8.1	—	—

For the six species representative of the majority of shrimp by-catch fish studied here, the mean values from the meter fell progressively with length of storage (Table 2). Thus, the initial mean values for the different fish species held in ice were in the range 12–15. Values then declined slowly so that after 17 days of storage mean values had come to fall in the range 7–9. This progressive decline was also seen for fish held in CSW but it was clear that this decline was more rapid and the mean meter readings for the fish species were found to be in the range of 4–8 after only about 13 days. The rates of decline of meter readings found for the various fish species were generally similar within storage treatments though those for cabrilla, mojarras and mojarron were among the slowest in each trial.

Table 3. The effects of storage in ice and CSW on taste panel scores

	Days in storage											
Fish species	1	3	4	5	6	7	10	11	12	13	14	17
Lenguados												
Ice	7.66	—	7.33	—	5.50	—	5.00	—	5.00	—	3.50	6.00
CSW	8.00	7.00	—	6.00	—	5.66	—	6.00	—	4.00	—	—
Mojarras												
Ice	7.66	—	7.33	—	6.50	—	5.66	—	5.66	—	5.00	4.00
CSW	8.00	7.66	—	7.00	—	6.33	—	6.00	—	4.00	—	—
Cabrilla												
Ice	8.00	—	6.66	—	6.50	—	5.66	—	5.66	—	5.00	5.66
CSW	7.00	7.66	—	7.00	—	6.33	—	6.00	—	4.33	—	—
Rayadillo												
Ice	8.00	—	6.66	—	6.50	—	6.33	—	6.00	—	5.00	5.33
CSW	7.00	7.66	—	7.00	—	6.33	—	5.50	—	4.33	—	—
Cabaicucho												
Ice	8.00	—	7.33	—	6.50	—	6.33	—	5.66	—	6.00	5.33
CSW	8.00	7.00	—	6.00	—	6.66	—	4.75	—	4.66	—	—
Mojarron												
Ice	8.00	—	7.00	—	7.00	—	6.33	—	6.00	—	7.00	6.33
CSW	8.00	7.33	—	6.50	—	6.33	—	5.75	—	4.66	—	—

Fish stored in crushed ice were found to remain acceptable to taste panellists for up to about 16 days and fish held in CSW for up to 11 days (Table 3). Few differences in overall acceptability with length of storage were found between fish species. Thus, the data collected using the GR Torrymeter agreed well with the taste panel results and high positive correlation coefficients were obtained (Table 7). At the times when the fish were considered to be just unacceptable to panels, namely a score of 5 or less, the physical qualities of the fish, as judged by their appearance and condition, were very poor (Table 4). At these time large amounts of surface mucus had appeared on the majority of the fish, particularly those held in ice, and they possessed unpleasant yeasty odours. The eyes had become opaque and sunken and the gills had lost colour. In some species, notably lenguados, the majority of the fish specimens showed burst bellies and this specie, together with mojarras and rayadillos had very soft flesh textures.

The results for the content of TVB in the flesh of the fish species studied are presented in Table 5. The gradual increase in volatile bases during spoilage has been proposed as a means of determining the freshness of fish. Total volatile base values of 30–40 mg/100 g flesh are generally considered to represent the limit of acceptance for temperate and cold water fish (Connell, 1975). From the results presented here it can be seen that these levels were reached within 6–7 days for fish held in both ice and CSW. However, at this time taste panel scores indicated a relatively high degree of acceptability and it was not until levels ranging from 50 to 110 mg/100 g flesh were recorded that the fish had become just unacceptable to the panellists. The increase in TVB levels to these high values were more rapid after about the 6th day with fish stored in CSW than with fish stored in ice. These values were finally attained after approximately 11 and 16 days, respectively.

The results of other studies with tropical fish species have not always indicated a gradual increase in TVB levels with length of storage (Amu & Disney, 1973; Meynell, 1978). Indeed, for the collated results for eleven fish species that have been studied, the

Table 4. (a) Changes in physical appearance of shrimp by-catch fish during storage in ice

Fish species	Characters	Days in storage							
		1	4	6	8	10	12	14	17
Lenguados	Eyes	Flat, clear	Flat, red	Concave, red	Concave, red	Sunken, grey	Sunken, burst	Sunken burst	Sunken, burst
	Gills	Red, fresh	Red, fresh	Red, neutral	Pink, neutral	Mucus, putrid	Brown, putrid	Brown, putrid	Brown, putrid
	Skin/flesh	Bright, firm	Bright, firm	Bright, soft	Dull, soft	Loose, v. soft	Loose pulpy	Mucus, pulpy	Mucus, pulpy
Mojarras	Eyes	Flat, clear	Flat, clear	Concave, red	Sunken, red	Sunken, red	Sunken, red	Sunken, grey	Sunken, grey
	Gills	Red, fresh	Red, fresh	Red, fishy	Red, fishy	Red, fishy	Pink, fishy	Pink, fishy	Pink, putrid
	Skin/flesh	Bright, firm	Bright, firm	Bright, fairly firm	Dull, fairly firm	Dull, fairly firm	Dull, fairly firm	Dull, fairly firm	Mucus, soft
Cabrilla	Eyes	Flat, clear	Flat, clear	Concave, red	Concave, red	Concave, red	Concave, red	Concave, red	Sunken, grey
	Gills	Red, fresh	Red, fresh	Red, fresh	Red, neutral	Red, neutral	Red, fishy	Red, fishy	Pink, fishy
	Skin/flesh	Bright, firm	Bright, firm	Bright, firm	Bright, firm	Bright, firm	Bright, firm	Dull, firm	Dull, firm
Rayadillo	Eyes	Flat, clear	Flat, clear	Concave, red	Concave, red	Concave, red	Sunken, red	Sunken, red	Sunken, grey
	Gills	Red, fresh	Red, fresh	Red fresh	Red, fresh	Pink, neutral	Pink, fishy	Pink, fishy	Pink, putrid
	Skin/flesh	Bright, firm	Bright, firm	Bright, firm	Bright, firm	Bright, firm	Dull, firm	Dull, firm	Dull, firm
Cabaicucho	Eyes	Flat, clear	Flat, clear	Concave, red	Concave, red	Sunken, red	Sunken, red	Sunken, red	Sunken, grey
	Gills	Red, fresh	Red, fresh	Red, neutral	Red, neutral.	Pink, neutral	Pink, fishy	Pink, fishy	Pink, fishy
	Skin/flesh	Bright, firm	Bright, firm	Bright, firm	Bright, fairly firm	Dull, fairly firm	Dull, fairly firm	Dull, fairly firm	Dull, fairly firm
Mojarron	Eyes	Flat, clear	Flat, clear	Concave, red	Concave, red	Sunken, red	Sunken, red	Sunken, grey	Sunken, grey
	Gills	Red, fresh	Red, fresh	Pink, neutral	Pink, neutral	Pink, fishy	Pink, fishy	Mucus, fishy	Mucus, fishy
	Skin/flesh	Bright, firm	Bright, firm	Bright, firm	Bright, firm	Dull, firm	Dull, firm	Mucus, fairly firm	Mucus, soft

Table 4. (b) Changes in physical appearance of shrimp by-catch fish during storage in CSW

Fish species	Characters	Days in storage 1	3	5	7	11	13
Lenguados	Eyes	Flat, red	Concave, grey	Sunken, grey	Sunken, grey	Sunken, grey	Sunken, grey
	Gills	Red, fishy	Pink, mucus	Pink, mucus	White, yeasty	White, H_2S	White, putrid
	Skin/flesh	Dull, fairly firm	Soft, mucus	Loose, v. soft	Belly burst	Pulpy	v. pulpy
Mojarras	Eyes	Flat, red	Concave, grey	Sunken, grey	Sunken, grey	Sunken, grey	Sunken, grey
	Gills	Red, neutral	Red, neutral	Red, fishy	Red, fishy	Red, H_2S	Pink, putrid
	Skin/flesh	Bright, firm	Bright, firm	Dull, firm	Dull, firm	Dull, fairly firm	Dull, fairly firm
Cabrilla	Eyes	Flat, red	Concave, red	Sunken, grey	Sunken, grey	Sunken, grey	Sunken, grey
	Gills	Red, neutral	Red, neutral	Pink, fishy	Pink, fishy	Pink, fishy	Pink, putrid
	Skin/flesh	Bright, firm	Dull, firm	Dull, firm	Dull, firm	Dull, firm	Dull, firm
Rayadillo	Eyes	Flat, red	Concave, red	Sunken, grey	Sunken, grey	Sunken, grey	Sunken, grey
	Gills	Red, neutral	Pink, neutral	Pink, fishy	Pink, mucus	Pink, H_2S	White, putrid
	Skin/flesh	Bright, firm	Dull, firm	Dull, fairly firm	Dull, fairly firm	Dull, fairly firm	Dull, fairly firm
Cabaicuchos	Eyes	Flat, red	Concave, red	Sunken, grey	Sunken, grey	Sunken, grey	Sunken, grey
	Gills	Red, neutral	Pink, neutral	Pink, fishy	Pink, mucus	Pink, mucus	White, putrid
	Skin/flesh	Dull, firm	Dull, fairly firm	Dull, soft	Belly burst	Dull, soft	Pulpy
Mojarron	Eyes	Flat, red	Sunken, grey	Sunken, grey	Sunken, grey	Sunken, grey	Sunken, grey
	Gills	Red, neutral	Pink, fishy	Pinky, fishy	Pink, H_2S	White, H_2S	White, putrid
	Skin/flesh	Bright, firm	Dull, fairly firm	Dull, fairly firm	Dull, fairly firm	Dull, soft	Dull, v. soft.

Table 5. The effects of storage in ice and CSW on TVB contents (mg N/100 g)

	Days in storage										
Fish species	3	4	5	6	7	10	11	12	13	14	17
Lenguados											
Ice	—	28.0	—	28.0	—	32.2	—	37.8	—	47.6	48.3
CSW	30.1	—	30.1	—	28.7	—	47.6	—	68.6	—	—
Mojarras											
Ice	—	30.8	—	35.0	—	46.2	—	42.0	—	58.8	70.0
CSW	21.0	—	25.9	—	30.1	—	66.5	—	74.2	—	—
Cabrilla											
Ice	—	19.6	—	28.0	—	44.8	—	43.4	—	46.2	59.7
CSW	22.4	—	35.7	—	39.9	—	72.8	—	102.9	—	—
Rayadillo											
Ice	—	29.4	—	32.2	—	39.2	—	33.6	—	43.4	53.2
CSW	23.1	—	25.9	—	31.5	—	63.0	—	70.7	—	—
Cabaicucho											
Ice	—	19.6	—	26.6	—	37.8	—	32.2	—	106.4	53.9
CSW	18.2	—	34.3	—	48.3	—	75.6	—	94.5	—	—
Mojarron											
Ice	—	25.9	—	38.5	—	42.7	—	39.2	—	41.3	44.8
CSW	21.0	—	31.5	—	34.3	—	43.4	—	107.8	—	—

measurement of TVB appeared to be a useful index of quality in only four (Poulter *et al.*, 1978).

Those previous studies, which have determined the TVB contents of various tropical fish species, have all employed the micro diffusion method of Conway (Beatty & Gibbons, 1937). This method was initially used in the present investigation but it became clear from the results obtained that the method was not reliable and duplicates could not be obtained. Consequently, the distillation technique described by Pearson (1975) was subsequently used since it proved possible to obtain good duplicates and the procedure also allowed for the more rapid processing of large numbers of samples. Thus, the very high negative correlation coefficients found for GR Torrymeter readings and TVB values in the present study (Table 7), indicating TVB to be a useful index of quality in by-catch fish, may conflict with previous comments on its application to tropical fish. This observation seems to be compounded by the fact that fish were found to remain acceptable to panellists when levels of TVB were greater than those normally considered to represent the limit of their freshness.

The results obtained from the microbiological investigation are presented in Table 6. Values for total viable bacterial numbers were generally similar between fish species within storage treatments. Skin counts were always higher than those for muscle. Counts increased from initial values in the ranges 10^2–10^5 (muscle) and 10^3–10^6 (skin) to 10^5–10^8 (muscle and 10^7–10^{10} (skin) after 14 days. High correlation coefficients were calculated for these total viable bacterial counts with the other physical and chemical analyses conducted with stored fish (Table 7). The high correlations found between the GR Torrymeter readings and these other quality parameters indicate that the GR Torrymeter may be used as a quick and reliable method of determining the quality and freshness of shrimp by-catch fish. Nonetheless, the precise meter readings and ranges in values that may be expected on storage of by-catch fish will be species dependent.

The projected storage life of these common shrimp by-catch fish species does therefore allow for their collection for the majority of the time that vessels are at sea. The average duration of shrimper voyages has been estimated to be about 20 days (Poulter, 1982b). However, where to store by-catch on board is an important question. It has been estabished that there is little free space available on the deck of shrimpers for the addition of any new equipment (Crean & Young, 1979), or for the storage of large quantities of fish material since this could decrease ship stability.

It has been estimated that from 5 to 8 t of by-catch fish could be collected from the last 10–15 days of each voyage. This quantity of fish material might be expected if 65% by weight of the total by-catch consists of fish, if 60 kg/hr of by-catch were caught and if the average trawling time/day were 14 hr (Poulter, 1982c; Schafer *et al.*, 1982).

Data supplied by the Mexican Fisheries Department (Pesca, 1982) indicates that shrimpers working out of Guaymas in the northern Gulf of California yield only an average of 1.6 tonnes of headless shrimp from each voyage. Over the last decade the number of boats included in this shrimping fleet has increased almost 2-fold (1970: 282

Table 6. The effects of storage in ice and CSW on total viable bacterial numbers ($\log_{10}$)

Fish species	Tissue sample*	Days in storage									
		1	2	3	4	7	8	10	11	13	14
Lenguados											
Ice	S	—	5.746	—	5.580	—	7.961	9.114	—	—	9.014
	M	—	2.255	—	4.504	—	3.995	4.539	—	—	6.621
CSW	S	4.251	—	3.927	—	6.315	—	—	8.315	8.334	—
	M	2.609	—	2.255	—	3.171	—	—	4.868	6.661	—
Mojarras											
Ice	S	—	5.556	—	7.797	—	9.129	7.951	—	—	9.276
	M	—	3.115	—	4.291	—	5.888	6.206	—	—	6.343
CSW	S	3.850	—	5.263	—	6.722	—	—	8.421	8.686	—
	M	3.158	—	4.739	—	5.682	—	—	8.165	8.438	—
Cabrilla											
Ice	S	—	5.328	—	6.033	—	9.269	9.410	—	—	10.143
	M	—	3.958	—	3.255	—	4.739	6.796	—	—	4.954
CSW	S	3.454	—	3.799	—	5.431	—	—	8.151	7.842	—
	M	2.767	—	3.409	—	3.947	—	—	8.732	7.327	—
Rayadillo											
Ice	S	—	5.485	—	6.144	—	9.359	9.060	—	—	9.591
	M	—	4.268	—	4.231	—	5.572	6.235	—	—	7.138
CSW	S	4.620	—	5.157	—	6.878	—	—	8.079	7.537	—
	M	5.587	—	4.539	—	6.826	—	—	7.597	8.037	—
Cabaicucho											
Ice	S	—	5.203	—	6.514	—	9.073	9.661	—	—	9.629
	M	—	3.587	—	3.485	—	6.022	4.808	—	—	6.915
CSW	S	4.431	—	4.641	—	6.328	—	—	8.003	7.971	—
	M	3.171	—	3.587	—	4.197	—	—	5.577	8.686	—
Mojarron											
Ice	S	—	5.902	—	5.974	—	8.283	8.948	—	—	8.875
	M	—	2.607	—	5.100	—	2.883	3.607	—	—	6.875
CSW	S	4.165	—	4.653	—	6.308	—	—	8.182	8.346	—
	M	2.352	—	2.908	—	2.431	—	—	4.956	6.510	—

*S, skin; M, muscle.

Table 7. Correlation coefficients (r) between quality parameters for six common by-catch fish held in ice or CSW

Fish species		Tm *versus* Pan		Tm *versus* TVB		Pan *versus* TVB	
		Ice	CSW	Ice	CSW	Ice	CSW
Lenguado		0.95	0.92	0.92	0.85	0.85	0.73
Mojarra		0.88	0.83	0.81	0.97	0.98	0.92
Cabrilla		0.61	0.78	0.61	0.99	0.84	0.90
Rayadillo		0.90	0.69	0.92	0.92	0.83	0.96
Cabaicucho		0.95	0.98	0.47	0.87	0.76	0.80
Mojarron		0.66	0.94	0.74	0.87	0.47	0.99
		Tm *versus* Bac		Pan *versus* Bac		TVB *versus* Bac	
Fish species		Ice	CSW	Ice	CSW	Ice	CSW
Lenguado	S	0.65	0.77	0.92	0.82	0.94	0.94
	M	0.98	0.97	0.81	0.83	0.56	0.89
Mojarro	S	0.88	0.97	0.80	0.84	0.73	0.98
	M	0.92	0.99	0.97	0.83	0.73	0.97
Cabrilla	S	0.99	0.91	0.96	0.82	0.64	0.95
	M	0.83	0.83	0.74	0.76	0.08	0.91
Rayadillo	S	0.76	0.86	0.72	0.80	0.57	0.95
	M	0.85	0.88	0.79	0.91	0.88	0.75
Cabaicucho	S	0.66	0.97	0.97	0.94	0.71	0.94
	M	0.98	0.91	0.97	0.96	0.90	0.77
Mojarron	S	0.99	0.74	0.51	0.96	0.81	0.97
	M	0.03	0.89	0.92	0.85	0.35	0.89

Tm = Torrymeter readings; Pan = taste panel evaluations;TVB = total volatile bases; Bac = total viable bacterial numbers; CSW = chilled sea water; S = skin; M = muscle.

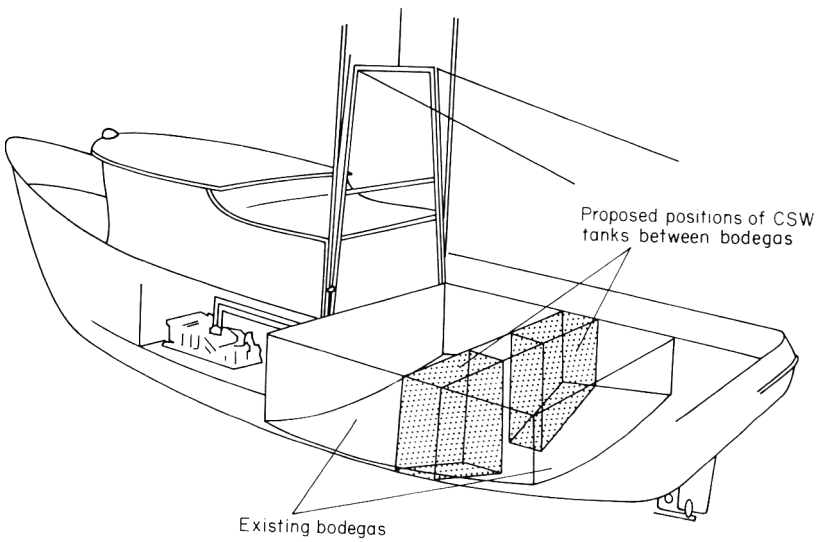

Figure 1. Illustration of shrimp boat tanks and bodegas.

vessels, 1980: 485 vessels), whilst the total yield of headless shrimp has remained stable (about 5000 t/year).

All shrimpers have insulated ice storage capacity in the bodegas found amidships and more recently built boats also have refrigerated capacity. It has been estimated that the space available in these bodegas exceeds 43 m^3, equivalent to approximately 15 t of iced product, but that only about 10% of this volume is utilized over the shrimping season which extends between October and May (J. E. Tamayo, unpublished data). Consequently, it appears that considerable vessel storage capacity remains unutilized.

It would, therefore, appear feasible to store 5–8 t of by-catch fish in ice in shrimper bodegas. However, the icing of large quantities of by-catch fish would undoubtedly be labour intensive for the crew of shrimpers as would be its manual off-loading in port. Furthermore, ice is an expensive commodity in relation to the value of the by-catch fish. Thus, the option of CSW seems more appropriate since reduced quantities of ice would be required for storage and because by-catch fish could simply be stored in tanks to be later discharged in port using vacuum suction methods. Such equipment with capacities for discharging up to 40 t/hr of product are well established and tested in the major ports on the Gulf due to the presence of the large sardine fishery.

It is proposed that tanks for CSW might be easily incorporated into existing shrimping vessels at low cost by modification of the areas found between bodegas (Fig. 1). Two tanks could be incorporated in each boat where each tank would have a capacity of from 6.3 to 9.4 m^3 dependent upon the type of boat design. This would then allow for from 4.5 to 6.7 t/tank of CSW and fish to be stored. Such modification would not affect vessel stability and would not reduce conventional storage capacities.

Incorporation of CSW storage tanks at low cost, therefore, seems feasible and would not greatly increase the work load of shrimper crews at sea. Such storage has been shown to be applicable to sorted shrimp by-catch fish resulting in retention of quality for up to 12 days. A suitable handling technique, compatible with the existing traditional fishing methods, does, therefore, exist allowing for the recovery of high quality by-catch fish suitable for processing into a range of foods for human consumption.

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A preliminary evaluation of the Super-Scan meat analyser for the analysis of poultry meat

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Summary

The Super-Scan meat analyser measures protein, fat and moisture on an homogenate of the sample by infrared absorption in the transmittance rather than the reflectance mode. A preliminary evaluation of the instrument using fifty-two samples of chicken or turkey meat indicated that the instrument gave reliable results for fat but not for protein and moisture. Regression equations were obtained from the combined data and used to correct the protein and moisture results. These equations were tested on a further twenty-eight samples of turkey meat and the results obtained demonstrated that the instrument could be used for the rapid analysis of poultry meat.

Introduction

Proximate analysis is a general requirement in the food industry and it is also needed for some aspects of food research. There is an increasing demand for fast and reliable analyses which implies that they should be instrumentally based and preferably on the same instrument. The conservative nature of the food industry has tended to encourage the automation of existing methods rather than the seeking of new ones. This has led to automatic fat analysers, Kjeldahl nitrogen analysers, etc., but quite often they retain some of the basic limitations of the original methods and it is necessary to have an array of instruments to produce a proximate analysis of a food sample.

There is currently much research interest in instruments using infrared (IR) and near infrared (NIR) measurements because they have the potential of fulfilling the major requirements of being fast and able to analyse all major constituents. While NIR instruments are established in the grain trade and may have the most promising future, IR instruments are well established in the milk industry. The Foss Electric Super-Scan meat analyser is a development of the 'Milkoscan' analyser and it has already been successfully tested on red meat samples (Bjarno, 1981, 1982)

The poultry research programme at the Food Research Institute requires a large amount of analytical work and the Institute is also responsible for assisting the poultry processing industry to maximize its efficiency. Thus all new instruments which fulfil the various requirements are of interest and the opportunity was taken to evaluate an early model of the Super-Scan for the analysis of poultry meat.

The Super-Scan utilizes the Foss-Let reactor (Usher, Green & Smith, 1973) to produce an homogenate of 11 g of the sample in an alkali dispersant medium. Basically it is a single beam IR spectrophotometer utilizing interference filters for wavelength selection (Fig. 1). It is equipped with a pump which automatically passes sample or cleaning solution into a flow cell where it is held while measurements are made.

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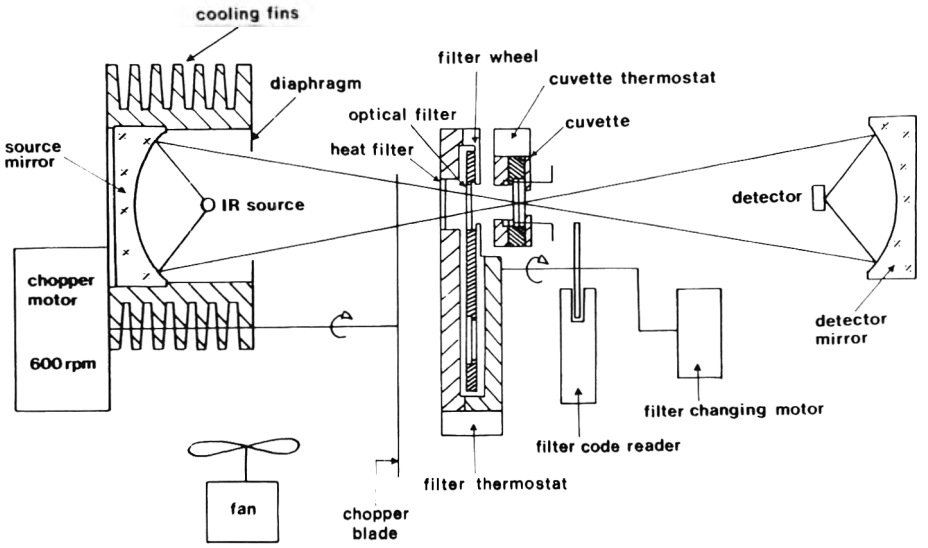

Figure 1. Optical system of the Foss Electric Super-Scan.

Table 1. Analysis of chicken meat (% w/w)

Sample No.	Fat		Protein		Moisture	
	Lab.	IR	Lab.	IR	Lab.	IR
1	2.8	2.7	23.3	25.2	72.4	88.3
2	2.4	2.5	24.1	26.0	72.6	87.5
3	9.9	9.9	19.9	20.7	69.8	78.8
4	2.6	2.8	22.8	25.2	72.9	87.5
5	2.0	2.4	23.4	25.7	73.0	85.0
6	6.2	6.6	21.3	21.9	71.8	83.0
7	2.5	2.5	23.9	25.6	72.3	86.8
8	14.5	15.7	18.5	20.6	65.1	74.3
9	1.0	1.5	23.5	26.1	73.5	86.5
10	11.1	11.4	19.3	20.1	69.2	77.8
11	9.9	10.0	19.9	20.0	69.8	80.0
12	1.0	0.9	24.9	26.2	73.8	87.3
13	5.1	5.1	22.5	22.4	72.3	82.5
14	2.0	2.3	23.4	25.9	73.0	84.5
15	10.9	11.2	18.6	20.5	68.9	81.0
16	4.9	4.3	20.0	21.8	73.8	85.3
17	10.0	10.2	19.5	21.0	69.5	79.0
18	6.2	6.7	21.3	22.2	71.8	81.5
19	5.0	4.6	20.4	21.8	73.5	83.3
20	10.9	11.2	19.3	20.1	69.1	78.8
21	10.9	10.4	19.1	20.4	69.2	77.8
22	11.9	12.4	18.8	20.2	68.7	75.5
23	1.0	1.2	23.5	26.9	73.5	87.5
24	2.6	2.7	22.8	25.7	72.9	86.5
25	1.8	2.1	23.3	26.1	72.9	86.5
26	2.2	2.5	24.1	26.6	71.7	86.8
27	15.8	16.5	17.9	20.0	64.8	72.3
28	14.3	14.6	17.7	19.9	66.3	73.8
29	11.9	12.9	18.8	20.2	68.7	76.0
30	8.6	8.9	20.9	21.4	70.2	77.8
31	9.6	9.9	19.7	21.4	70.5	78.8

Wavelengths are selected by rotation of a filter wheel which contains component and reference filters for each analyte. The signals from the component and reference filters are compared to extract the signal due to the component and the resulting signal is then passed through a logarithmic/linear converter and after amplification it is further subjected to correction prior to being displayed and printed.

Materials and methods

The samples of minced turkey and chicken meat from breast, thigh and drumstick muscles examined had been previously analysed as part of other work in the Institute. Methods of sample preparation were described by Grey *et al.* (1983) and the following methods of analysis used:
Free fat: ISO/1444—1973.
Protein: ISO/937—1978.
Moisture: ISO/1442—1973.

Method of use for the Super-Scan

11.0 g of sample were weighed into the reactor vessel, 100 ml of alkali dispersant (supplied by Foss Electric) at 50°C were added by an automatic diluter (HATI IV). The reactor weight was inserted and the closed container was locked into the reactor. The reactor was set to vibrate for 4 min after which the container was removed and the homogenate de-foamed by addition of de-foaming agent (Foss Electric). It was then filtered through a domestic plastic tea strainer and placed in a stoppered flask in a water bath at 50°C for a minimum period of 5 min before analysis in the Super-Scan. The Super-Scan was zeroed using a solution containing 11.0 g of water and 100 ml of the alkaline dispersant. The time required for the completion of one analysis from the initial weighing was 20 min. After this time, results were obtained continuously at ten per hr, with one operator.

Results

Results for the analysis of chicken meat are given in Table 1, for turkey meat in Table 2, and for analysis of additional turkey meat in Table 3. In these tables IR refers to the averaged result for duplicate uncorrected Super-Scan results from the same homogenate and Lab to the averaged result for duplicate laboratory analyses. The reproducibility of the Super-Scan results was very good, for fat and protein the standard error was less than 0.1% for duplicates from the same homogenate. Some test samples were put through several times and the duplication between different homogenates of the same sample was 0.1% for fat and 0.2% for protein. The reproducibility of the water results was less satisfactory. The result given by the instrument is corrected to the nearest 0.5% and the standard error was 1% for the same homogenate.

Statistical analyses of these results are summarized in Table 4. Regression analysis was carried out on the combined results from Tables 1 and 2. This file was used to produce regression equations for predicting protein and moisture contents which were tested using the data in Table 3 and the results are summarised in Table 5.

Table 2. Analysis of turkey meat (% w/w)

Sample No.	Fat		Protein		Moisture	
	Lab.	IR	Lab.	IR	Lab.	IR
1	1.2	0.8	25.2	27.3	73.4	87.5
2	2.8	2.5	20.4	23.0	74.9	84.3
3	3.8	3.5	20.3	22.0	75.3	83.5
4	3.5	3.2	19.8	21.7	75.7	84.3
5	0.6	0.7	25.1	26.8	73.2	86.8
6	2.9	2.5	20.4	23.0	75.3	85.5
7	5.1	6.5	18.9	22.0	73.4	83.0
8	0.3	0.7	24.4	26.8	73.8	88.8
9	3.4	3.1	20.6	22.7	74.7	87.5
10	3.8	2.9	20.5	24.1	74.2	87.0
11	0.7	0.6	24.7	26.6	73.8	90.0
12	1.2	1.3	23.8	26.7	73.5	88.8
13	0.9	0.9	24.7	26.5	73.2	91.3
14	4.1	4.1	21.0	23.0	73.5	86.3
15	0.6	0.8	23.4	26.7	73.2	89.3
16	3.1	2.5	20.4	23.0	75.5	86.8
17	5.0	4.8	19.4	23.4	74.0	85.5
18	3.1	2.6	20.3	22.4	75.0	86.3
19	4.3	3.5	19.8	23.1	74.6	87.5
20	0.7	0.6	24.0	27.4	72.5	88.8
21	5.6	5.1	19.3	22.5	73.0	84.3

Discussion

The analyser results for fat were in agreement with the conventional method and needed no further correction. There were insufficient data to apply and test regression analysis on different types of poultry, so the regression analysis was carried out on the combined results from Tables 1 and 2. The equations obtained were tested by carrying out the additional analyses on turkey samples when the analyser was available in a local factory, 9 months after the original work. The most important measure of the instrument performance is the standard error of prediction (s.e.p.). The improvement produced by regression analysis in the s.e.p. of the moisture predictions was very marked. The improvement in the s.e.p. of the protein results is most probably due to the introduction of an automatic analyser (Buchi 322/324) and standardization of the digestion procedure. The remaining bias in these results is not thought to be a serious problem. Considering that the instrument had been travelling around the country for 9 months, the regression equations were remarkably stable. These results indicate that the instrument performs to the manufacture's specification, i.e. s.e.p. for fat and protein of 0.50% and for water of 0.55%; thus it is likely that the instrument will be useful in producing results for routine quality control in the poultry processing industry.

The latest production instrument has a new interference filter for measuring water which is reported to give much improved results. It is also being marketed with an option for a desk top computer on which it is possible to carry out regression analysis and the automatic re-calculation of results from the instrument by evaluation of regression equations. We have shown that this would be required for the use of the instrument for the analysis of poultry meat. Although calibrations based on a

Table 3. Additional analyses of turkey meat

Sample No.	Fat		Protein		Moisture	
	Lab.	IR	Lab.	IR	Lab.	IR
1	4.73	4.6	19.65	20.8	74.97	88.0
2	4.46	4.6	20.18	20.7	75.26	88.0
3	0.71	0.9	24.38	26.1	73.66	91.0
4	3.36	6.5	19.62	20.7	73.07	86.0
5	1.17	0.8	22.58	23.9	76.16	91.0
6	7.20	7.5	20.24	21.6	71.91	84.5
7	0.79	0.8	24.21	25.9	74.38	89.5
8	1.14	1.2	23.61	25.9	75.23	90.5
9	4.28	4.6	20.28	21.7	74.76	87.5
10	0.94	0.8	25.32	27.1	73.40	90.0
11	3.68	3.7	20.73	21.8	75.20	87.0
12	6.36	6.1	19.77	21.2	73.07	85.5
13	1.36	1.1	21.72	23.1	76.45	91.5
14	1.41	1.2	24.65	25.8	73.94	90.5
15	4.76	5.1	20.72	21.5	74.13	86.0
16	1.32	1.4	23.16	24.7	75.07	90.5
17	0.92	1.0	24.73	26.6	74.39	89.0
18	4.59	5.6	20.63	21.8	75.18	85.0
19	0.82	1.7	24.81	26.2	74.26	89.5
20	4.80	5.5	20.04	21.7	76.84	86.5
21	6.80	7.3	19.29	20.7	73.81	85.0
22	1.13	1.2	23.68	25.7	74.72	90.5
23	4.03	6.4	19.34	20.8	74.94	86.4
24	4.50	4.7	20.30	21.6	74.71	88.0
25	1.50	1.7	23.95	25.6	73.92	90.5
26	1.01	1.1	24.83	26.4	74.35	90.0
27	5.82	6.4	20.51	21.9	73.61	86.5
28	1.27	1.4	22.85	24.1	75.34	91.5

Table 4. Summary of statistics on raw results

Sample type	Fat				Protein				Moisture			
	n	*r*	Bias	s.e.p.	*n*	*r*	Bias	s.e.p.	*n*	*r*	Bias	s.e.p.
Chicken	31	0.999	−0.10	0.35	31	0.948	+1.87	0.83	31	0.931	+11.1	2.52
Turkey	21	0.981	−0.23	0.36	21	0.951	+2.57	0.68	21	0.429	+12.7	2.75
Turkey	26	0.990	+0.17	0.34	28	0.991	+1.42	0.36	27	0.520	+14.1	1.87

n: number of samples used in test; *r*: correlation coefficient; bias: average of differences between IR and laboratory methods; s.e.p.: standard error of prediction (the s.d. of the differences between methods).

Table 5. Summary of results obtained by regression analysis

Regression equations for the prediction of:

Protein % = 2.27 + 0.816 × IR protein (n = 52)

Moisture % = 95.378 − 0.900 IR fat − 0.791 × IR protein (n = 52)

Results obtained by applying regression equations to Table 3 results:

Constituent	*n*	*r*	Bias	s.e.p.
Protein	28	0.991	−0.61	0.34
Moisture	27	0.854	−0.58	0.54

sufficiently large number of representative samples are likely to be stable it would be essential to carry out regular checks on the instrument with results obtained by conventional methods.

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Texture and mechanical properties of pork backfat

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Summary

A puncture test for measuring the firmness of backfat is described for use in studies of the effects of changes in animal production, and meat processing on the quality of fatty tissue. The operating and recording conditions were chosen from sensory deformation characteristics used in firmness evaluation when pressing fat with the index finger. On average, assessors exerted a maximum force of about 30 N and caused a deformation of 4 mm in 2 sec and the best predictor of firmness was the maximum deformation they applied. Mechanical properties of the fats were determined by stress relaxation and the modulus 2.5 sec after compression was about 80×10^{4} for hard and $< 3\times10^{4}$ N/m^2 for soft fat at 3°C and the logarithm of the modulus was linearly related to the subjective firmness rating.

In the puncture test, the forces required to drive a stainless steel punch (3.5 mm in diameter) 2.5 or 4.0 mm into backfat at 3°C at 2 mm/sec correlated well ($r > 0.8$) with firmness rating and five replicate determinations using the punch proved more discriminating than a panel of four assessors. A combination of the forces required on inner and outer layers of fat accounted for 93% of variation in firmness assessments.

Introduction

Both the quantity and physical properties of fat are important attributes of pig carcass quality. The physical properties of fatty tissue, particularly its softness, affect its appearance, ease of cutting and slicing, and pork carcasses with excessively soft or oily fat are unsuitable for bacon manufacture.

Experienced workers at a pig processing factory and at the Meat Research Institute agree that an initial indication of softness of cold pig carcass fat is seen as transluscency, an oily sheen, or a greyish discolouration of the medial surface exposed by splitting the carcass. The final and more important judment however is obtained by pressing the fat with index finger or thumb and by assessing rigidity in the carcass.

Standard objective methods are available for testing the firmness or refined fats and, in the British Standard (BS 684, 1976), a cone penetrometer is used but most studies of fatty tissue have been concerned with its lipid chemistry rather than its mechanical properties which are determined by the interaction of lipid and supporting cellular and connective tissues (Warwick & Williams, 1973; Hausman, 1978; Stanley and Voisey, 1979). Tschizhikova *et al.* (1971a) determined the breaking stress of mutton backfat and Tschizhikova, Reshetnyak & Mizeretsky (1971b) derived maximum shearing stress for pig backfat. Stanley & Voisey (1979) used sensory assessments and several mechanical tests to measure the texture of cooked Canadian bacon. Bourne (1979) reviewed mechanical puncture testing, and made a distinction between it and penetrometer

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testing; in the former, a cylindrical punch is usually driven into the material to a constant depth and force recorded, whereas in penetrometer testing the depth of penetration at constant force in a given time is recorded. Therefore, the objectives of this work were to examine the criteria by which assessors evaluated the firmness of pig carcass fat, to measure the mechanical properties of fats, and to use this data to devise a simple instrumental method for measuring fat firmness, which could be used to investigate the effects of production, and processing on the quality of fatty tissue.

Materials and methods

Materials

Castrated male pigs at about 80–110 kg live weight were slaughtered in a commercial abattoir. Carcasses were split into sides and cooled to 5°C. 24 hr after slaughter when they were butchered into 'fore-ends', 'middles' and 'legs' by cutting between the fourth and fifth ribs and at the first lumbar vertebra. From the cranial end of the 'middles', blocks composed of skin and all subcutaneous fat dorsal to M. longissimum thoracis were removed. Fat depth at the medial face of the blocks was between 14 and 42 mm. Usually two distinct fat layers (inner and outer) were visible, but, in one case, four layers were seen. Blocks were wrapped in polythene bags to minimize moisture loss, placed in an insulated box with vacuum sealed bags of crushed ice and transported for 1 hr to the Meat Research Institute, where they were stored at 1°C for 2 days.

Sensory evaluation of backfat texture

Assessors were asked to press the fat with the index finger and rate its firmness on a line (marked on 10 mm graph paper) which was labelled near the left extremity 'extremely soft', and 200 mm to the right, 'estremely hard'. Assessments outside these marks were allowed. Assessors were members of the Meat Research Institute's scientific and technical staff, five were male and one female aged between 25 and 40 years. Assessors one, four and five were 'experts', familiar with the commercial and experimental ranges of fat texture. Assessors one and five were licensed meat inspectors. The other three were less experienced, but all had handled pork in the course of their work.

In the first experiment, a block of fat (20–30 mm thick) was removed from a temperature controlled cabinet at 1°C and placed, with its inner subcutaneous fat layer uppermost, on a chilled metal plate above a load sensitive table and was assessed by pressing down with the index finger one or more times on the block. The block was wrapped in polythene and returned to the cabinet. The assessment was repeated with five more blocks, taking about 5 min. The tests were repeated for each assessor in turn. Temperature of the blocks remained below 5°C. The whole series of tests was repeated, presenting the blocks in reverse order, 3 or 4 days later.

In a second experiment, measurements of fat deformation and impulse measurement (see next section) were made simultaneously with assessment of firmness rating. In the first session, done on the 4th and 5th days after slaughter, seven fats were assessed and six of them were reassessed in a second session, 2–3 days later, together with two other fats.

Analysis of variance was used to determine the discrimination by assessors of firmness and the reproducibiity of their judgments.

Forces and deformations applied to backfat during sensory testing

Force was measured on the table of a strain gauge compression load cell (Instron 2511—203) which had a deflection of 0.1 mm at maximum load (50 kgf) and a rated accuracy of 0.1% of full scale output. Output from the load cell amplifier was 0–10 mV and was amplified to 0–10 V d.c. by a signal conditioning amplifier (Microconsultants 2044) to give an output suitable for recording on magnetic tape. To measure fat deformation a linear variable differential transformer (LVDT) with low friction PTFE bearings (RDP Electronics, D5/500A) was mounted vertically above the fat. Identical sand-blasted aluminium keys, attached to each end of the core of the LVDT, were shaped to correspond to the contact area of the index finger area (350 mm^2) when pressed on a firm horizontal surface. The lower key was positioned across the boundary between inner and outer layers of a 20 mm slice of fat and was connected using a ball and swivel joint, allowing it to maintain complete contact with the fat surface while transmitting uniaxial finger force. The frictional force on the LVDT was undetectable using the 5 kg force range used for measuring finger forces. Core and keys of the LVDT weighed 30 gf. Thre range over which the output voltage (amplified to 10 V d.c.) was proportional (±0.4%) to displacement of the LVDT was ± 14 mm. Sensitivity was 8 V (rms)/25 mm with a 5 V, 5kHz energizing supply.

Outputs from the load cell (measuring the normal uniaxial finger force) and the LVDT (measuring fat deformation) were monitored on a dual trace oscilloscope set to the $X-Y$ mode (Scopex, 4D-10B) and recorded synchronously on two channels of an FM instrumentation tape recorder (Racal, Store 7DS) at a tape speed of 191 mm/sec. Force–time and deformation–time plots were obtained by playback at 48 mm/sec to an xy/t chart recorder with 0.25 sec for full scale deflection (Oxford Instruments 3000) and force–deformation records by playback at 24 mm/sec to an x/y recorder (J.J. Lloyd Instruments PL100).

Mechanical properties (stress relaxation) of backfat

The mechanical properties of backfat were investigated by measuring stress relaxation after compression.

The fat blocks used in the first experiment were frozen at −20°C and then tempered for about 1 hr at 1°C to facilitate cutting without distortion. A steel cork borer was used to cut cylinders, 30 mm in diameter, from the periphery of the blocks, at right angles to the skin surface. Cylinders, containing the skin and inner layers of subcutaneous backfat, were trimmed at right angles to the long axis and the cylinders (17–20 mm long and 11 and 14 g) were replaced in the block which was wrapped in polythene and stored at 1°C for 24 hr to complete thawing.

The cylinder of fat, with the inner layer uppermost, was equilibrated for 1 hr in water maintained at 3°C contained on the table of a 50 kgf compression load cell. This temperature was chosen to approximate that of carcass fat in the abattoir chillroom. The cylinder was compressed by up to 10% (approximately 2 mm) or its original height in 0.5 sec. The output of the load cell, amplified to 0–10 V d.c., was fed to a chart recorder with 0.25 sec full scale deflection and recorded continuously for up to 1 hr after the start of compression. The weight of the cylinder before testing was unchanged after testing. An LVDT measured the crosshead movement which, at the compression speed of 250 mm/min, over-ran the set travel by 0.5% (approximately 0.1 mm).

To investigate the viscoelastic behaviour at different strains, cylinders of backfat from a carcass of a 3 year old hybrid (European wild pig × Tamworth) pig were compressed in 0.5 sec by 2–8%. Stress during relaxation was monitored continuously for up to 300 sec.

Puncture test

A flat-ended cylindrical steel punch, 100 mm in length, and 3.5 mm diameter, was narrow enough to test separately the inner and outer layers of subcutaneous backfat at the medial surface where each layer varied in width from 5 to 28 mm, and both layers from 14 to 42 mm. A slice of fat, 20±0.5 mm in thickness was cut from the blocks and stuck (cyanoacrylate glue) to a holder which had side faces to prevent lateral slippage.

After storage at 1°C, the slice was equilibrated and tested in water at 3°C. The chilled punch was screwed into the movable crosshead of an Instron 1102 TM-SM and driven down at 2.1 mm/sec. After touching the surface of the slice the punch travelled a further 10 mm and then reversed automatically. A force *versus* time record was obtained for each test using the Instron chart recorder (Speedomax G, model S 60000). Each layer was proved five times at intervals of 10 mm.

Table 1. Assessors' firmness ratings

(a) Experiment 1

Assessor	A	B	C	D	E	F	Mean	Variance ratio
1	108	180	80	180	70	113	114	31
	75	170	60	170	60	100		
2	70	170	50	160	40	180	88	6
	30	150	30	170	40	60		
3	125	165	78	170	115	90	127	21
	119	184	50	180	130	115		
4	118	190	56	190	36	76	104	11
	26	198	20	190	66	81		
5	49	189	60	190	84	120	109	45
	30	197	47	188	70	80		
6	130	190	50	200	20	110	118	4
	20	200	40	180	120	150		
Means	75	182	52	181	71	98	110	

(b) Experiment 2

Assessor	G	H	I	J	K	L	M	O	P	Mean	Variance ratio
1	125	70	80	30	112	170	100	—	—	105	15
	170	—	54	40	144	130	80	90	180		
3	160	107	110	50	107	187	58	—	—	122	10
	193	—	124	24	175	175	91	72	196		
4	178	146	137	19	90	191	132	—	—	134	11
	197	—	147	29	171	176	109	92	196		
6	190	90	105	40	150	175	65	—	—	108	6
	180	—	100	40	140	195	—	120	175		
	75	—	30	0	120	160	20	15	180		
Means	163	103	99	30	134	173	82	78	185		

Values are replicate firmness rating (0 = extremely soft; 200 = extremely hard) for fifteen fats (A to P) and the variance ratios (excluding H, O and P which were not always assessed twice) between the fats for each assessor in (a) experiment 1 (in which impulse during sensory testing was also measured) and (b) experiment 2 (in which stress and strain were also measured).

Results

Sensory discrimination

Firmness of fats in the two experiments varied from 30 to 185 (Table 1), i.e. 78% of the scale from extremely soft (0) to extremely hard (200). As a panel, the least significant difference between samples in experiment 1 was 17 and the grand mean score was 110.

It was clear however that variations between assessors was as high as 40 and some assessors were more discriminating than others. The most experienced assessors, one and five, had the highest variance ratios; the other 'expert' assessor 4, had a lower ratio but it was higher than those of the less experienced assessors two and six (Table 1).

In the second experiment (in which the LVDT was placed between finger and fat thereby removing the finger contact with the fat) the variance ratios of firmness for two out of four assessors were lower than in the first experiment but this was probably due to the smaller range of firmness in the second experiment rather than to the use of the LVDT.

Finger forces and fat deformation

In the first experiment assessors pressed the fat directly producing a force–time record. The number of times the fat was pressed differed between fats and between assessors; assessor one always used one push, assessor six used up to seven. Forces ranged from 6 to 61 N overall, with a grand mean of 33 N which, using a representative

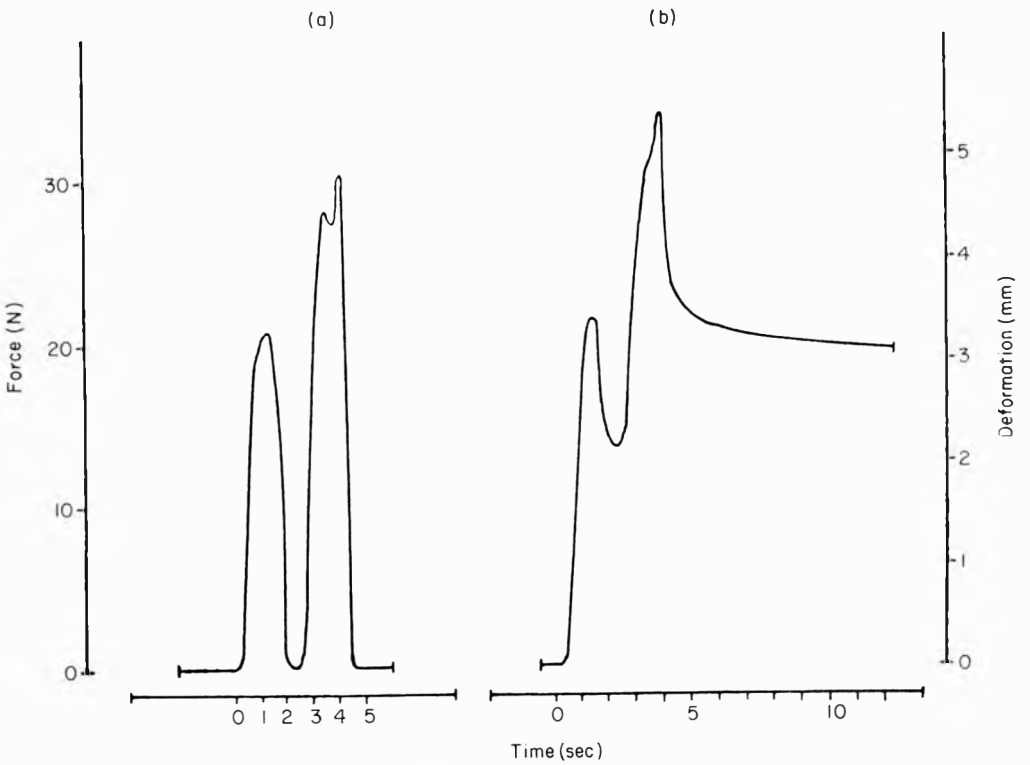

Figure 1. Typical time records of (a) finger force and (b) fat deformation. Partial recovery of deformation after applications of 30 N in a second finger push is shown.

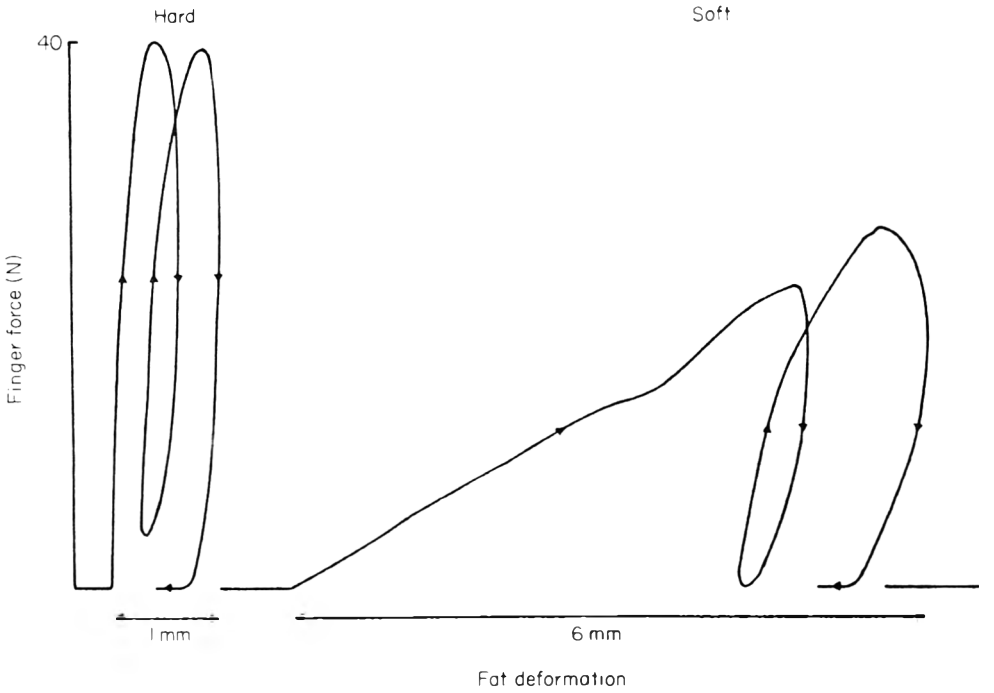

Figure 2. Synchronous finger force and fat deformation during evaluation of firmness of hard and soft pork backfat.

finger contact area of 350 mm^2, is equivalent to an average pressure of about 0.1 N/mm^2. The time taken to apply the first peak force varied between 0.2 and 2.4 sec, with a grand mean of 1 sec. Although the first peak force applied by the assessors was the only force which differed significantly (from 20 to 30 N) between fats it varied much more (17–48 N) between assessors, with assessor one applying an almost invariable 48 N to every fat.

In experiment 2, both force and deformation were recorded. In the example shown in Fig. 1 (a and b), partial recovery of the fat occurred between the two pushes and, in the 10 sec following assessment, the fat recovered 2 of the 5 mm maximum deformation.

The relationship between force and deformation for a hard and a soft fat, tested by assessor three, who was of average discrimination (Table 1), is shown in Fig. 2. For the hard fat the maximum force applied was about 40 N and the maximum deformation was 1 mm, for soft fat the values were 26 N and 6 mm respectively. Both fats recovered some of the deformation between the pushes. Data taken from the records of force and deformation with time of all fats and assessors are summarized in Table 2. The mean and ranges of the force and related variables were similar to those from experiment 1 except that the average time taken to apply the first peak force was 1.7 sec compared to 1 sec in experiment 1. With increasing softness of the fat, the average rate of deformation increased from 0.2 mm/sec to over 6 mm/sec and the total deformation increased from 0.8 to 6.6 mm. The maximum deformation rate was 24 mm/sec, given by assessor three in fat I.

The variation of force and related variables between assessors was generally larger than that of deformation and its related variables. Analysis of variance of the data for each assessor showed that the deformation, the work done up to the first peak force and

Table 2. Deformation characteristics induced in fat by assessors and their relationship firmness

Variate	Grand mean	Minimum–maximum			Linear correlation coefficients				
						Assessor (df = 13)			
		Overall	Assessor	Fat	Overall (df = 58)	1	3	4	6
Firmness, scored on 200 mm line (0 = extremely soft, 200 = extemely hard)	114	0 –197	94 –134	29 –188	—	—	—	—	—
Number of finger pushes	2.2	1 –7	1.1 –3.9	1.5 –2.8	ns	ns	ns	ns	ns
Analysis of first finger push									
Maximum force—MF (N)	29	8 – 51	22 –39	22 –39	ns	0.6*	0.5*	0.6*	ns
Time to MF (sec)	1.7	0.7– 4.1	1.3– 2.5	1.5– 2.2	ns	ns	ns	ns	ns
Impulse (N sec)	31	3 –101	12 –58	16 –48	ns	ns	ns	ns	0.5*
Deformation at MF (mm)	3	0.4– 8.8	2.2– 4.2	0.8– 5.8	−0.8***	−0.9***	−0.9***	−0.7***	−0.9***
Force at 0.4 mm, deformation (N)	9	0.6– 48	8 – 9	1.5–23	0.6*	ns	0.6*	0.7**	0.9***
Force at 0.6 mm, deformation (N)	11	0.7– 38	10 –13	2 –30	0.8***	0.8***	0.8**	0.7**	0.9***
Initial slope of force–deformation plot (N/mm)	18	1 –164	16 –22	4 –65	0.4***	ns	ns	0.6*	0.9***
Work to MF (J 10^{-2})	4.4	0.3– 14.8	2.5– 7.3	1.3– 7.8	−0.6***	−0.8***	−0.7**	ns	−0.7**
Power (W 10^{-2})	2.6	0.2– 10.2	1.9– 3.2	0.6– 4	−0.7***	−0.9***	−0.7**	ns	−0.7**
Average deformation rate (mm/sec)	1.9	0.2– 6.3	1.9– 2	0.3– 4.3	−0.8***	−0.9***	−0.8**	−0.8***	−0.8***
Overall									
Maximum force (N)	32	12 – 51	29 –39	25.0–41.0	0.3*	0.6*	0.6*	0.6*	ns
Maximum deformation (mm)	3.8	0.6– 8.8	3.4– 4.4	0.8– 6.6	−0.8***	−0.9***	−0.9***	−0.8**	−0.9***

Data are from two sessions where four assssors each tested a total of fifteen backfats.
ns = not significant; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

the maximum deformation were good ($P < 0.001$) discriminants and there were no significant session differences, indicating that they were reproducible.

Prediction of firmness from finger forces and fat deformation

In experiment 1, firmness judgements were significantly related ($r = 0.9$) to first maximum finger force, time to this maximum, impulse and maximum force applied for assessors two and five but not for the other four assessors. Mean panel ratings were poorly related to the maximum force ($r = 0.28$) and this was not significantly improved by stepwise multiple linear regression using all force measurements.

In experiment 2, force values were again poorly related to firmness but deformation of fat was highly correlated with firmness (Table 2). Maximum deformation was the best single predictor and accounted for 85% of the variation in sensory firmness. The relationship between firmness and deformation (Fig. 3) was linear but the slopes of the regression lines differed between the four assessors with assessor six deforming the fat by 9 mm whilst assessor one deformed the fat by only 7 mm.

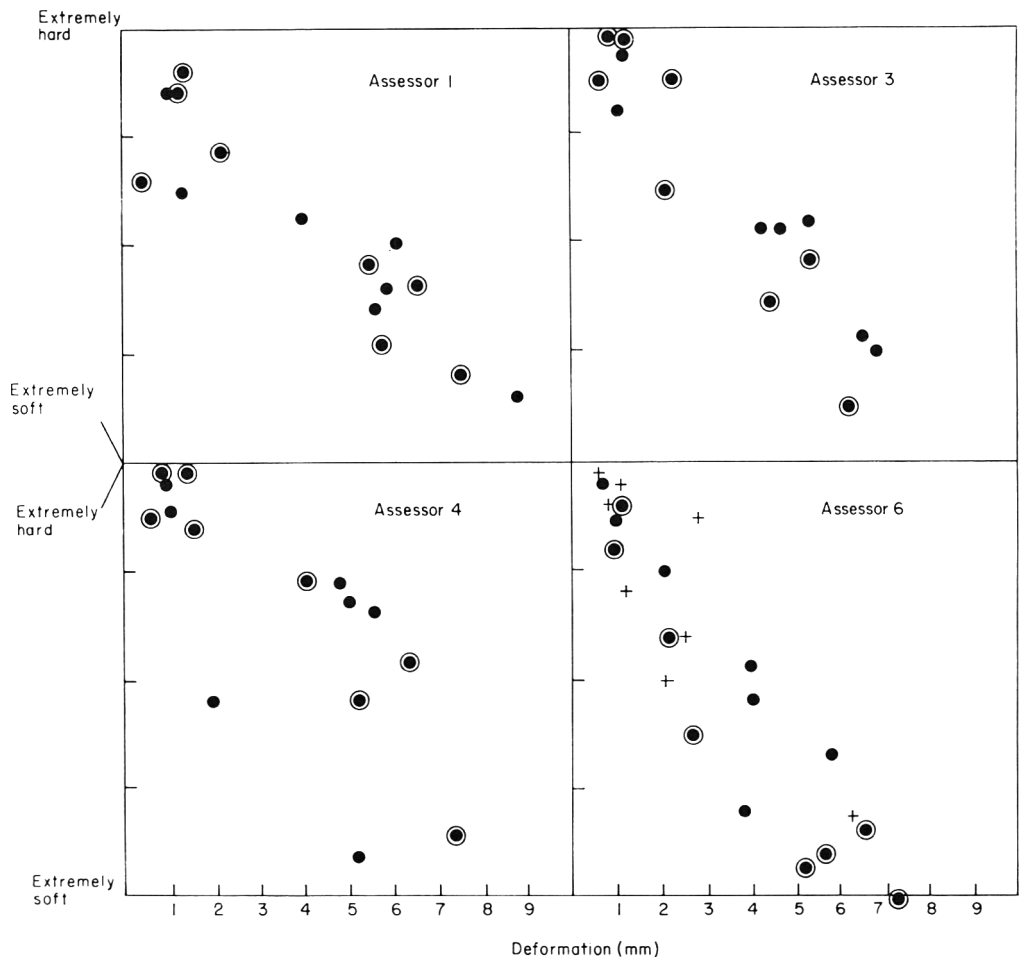

Figure 3. Relationships between firmness, assessed by pressing pork backfat with the index finger, and the maximum deformation applied to the fat. Data for assessors 1, 3, 4 and 6. The codes are for session 0, (+), session 1, (•), and session 2 (◉).

Although the firmness ratings given by assessor six were related to his maximum deformation of fats ($r = -0.9$, Table 2), his discrimination was lower than that of the other three assessors (Table 1, low variance ratio). Therefore his firmness ratings were strongly related to the deformation he applied but he had relatively large variations of ratings between replicates and this warranted further study. For fat G which he assessed three times he applied different deformations. Removing that variation using the overall regression, the ratings he would have given if he had used the average deformation was calculated on all three occasions. This was repeated for fats F, J, K and L giving fifteen calculated ratings in all. Analysis of variance of those ratings gave a variance ratio between 'fats' of 12.2—i.e. double that using the original ratings and similar to that of the other assessors (Table 1).

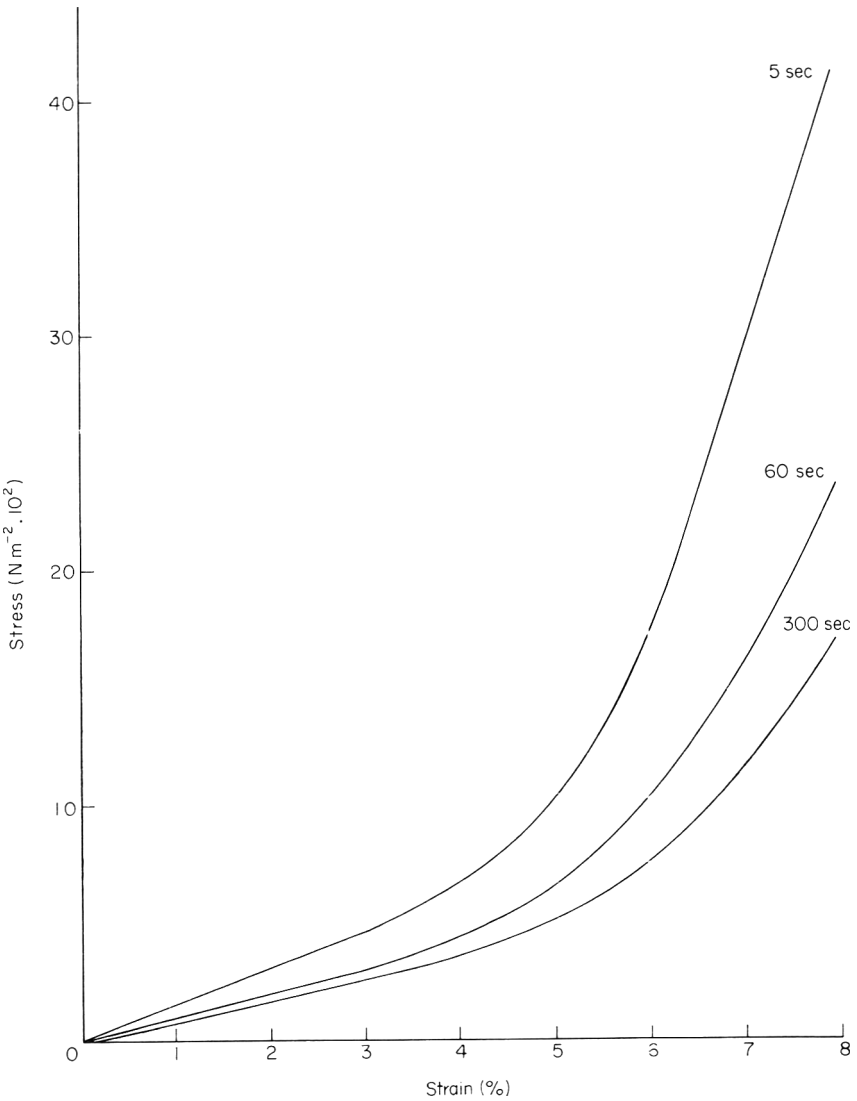

Figure 4. Isochronal stress–strain curves following uniaxial compression of pork backfat at 3°C for 0.5 sec at various speeds. Stress was measured at 5, 60 and 300 sec.

Mechanical properties

Stress relaxation data were plotted as the stress against strain at given times, after compression. These isochronals (Fig. 4) were not linear but stress increased progressively with the strain. With strain up to 8% therefore fatty tissue was not linearly viscoelastatic although there appeared to be linearity at strains less than 3% which is worthy of further study.

After 10% compressive strain applied in 0.5 sec at 3°C the peak forces varied between 4 and 111 N for the soft and hard samples in experiment 1. With increase in firmness the peak force increased progressively. The time dependent modulus at 0.5 sec after the start of compression was about 150×10^4 N/m^2 for hard fat and about 5×10^4 N/m^2 for soft. After 1 hr the value for soft fat was close to equilibrium at about 1×10^4 N/m^2, but for hard fat the value was 30×10^4 N/m^2 and still decreasing. Values of modulus, E_t, together with the average of the six assessors firmness ratings are shown in Table 3. There was a linear relationship between the logarithm of E_t and firmness (for E_t at 1 hr, $r = 0.99$, $P < 0.001$).

Table 3. Firmness, peak force and relaxation modulus

Fat	Firmness*	Peak force (N)	Relaxation modulus (N/m$^2 \times 10^4$) 2.5 sec	1 hr
C	52	4.8	3.4	1.6
E	71	9.0	9.2	3.4
A	75	9.0	6.3	3.0
F	98	19.6	14.9	5.4
D	181	103.0	77.5	27.0
B	182	106.4	79.0	28.1

Cylinders of pork backfat were compressed by 10% of height in 0.5 sec at 3°C. The moduli are means from measurements on two cylinders from each fat. Firmness was judged after pressing fat with the index finger.

*0 = extremely soft; 200 = extremely hard.

Table 4. Puncture test data related to firmness grade

	Mean	Soft	Medium	Medium/hard	Hard
Inner backfat					
First peak force	20.7	5–25	10–42	20–32	22–28
Maximum force	21.6	5–25	10–42	21–34	22–30
Force at 2.5 mm punch travel	12.3	< 2	3– 7	11–22	17–25
Force at 4 mm punch travel	16.1	< 5	5–15	15–28	21–28
Outer backfat					
First peak force	36.8	11–47	15–54	32–51	29–49
Maximum force	36.9	11–47	15–54	32–51	29–50
Force at 2.5 mm punch travel	13.9	< 5	5–12	8–24	14–28
Force at 4 mm punch travel	24.6	< 10	11–27	16–38	26–44

Values are the forces (N) obtained using the puncture test on inner and outer layers of backfat from thirty-eight carcasses categorized from soft to hard by an experienced factory worker.

Puncture testing of fats

Force–time records for the puncture of hard and soft fat are shown in Fig. 5. In hard fats a rapid increase in force occurred immediately after the punch touched the surface. The force increased with increasing deformation (time) and a change in slope was observed at about 0.8 mm travel. Further travel produced a lower rise to a peak force when the punch pierced the fat. This was succeeded by a variable number of peaks (at similar forces) as the surface area of punch in contact with the fat increased. Much lower forces are recorded for soft fat. Initial slopes were lower, and yield points were less distinct. Most of the increase in force was associated with indentation, and when puncture occurred it was only towards the end of the 10 mm travel.

To characterize the puncture test, force at 2.5 and 4.0 mm deformation, first peak

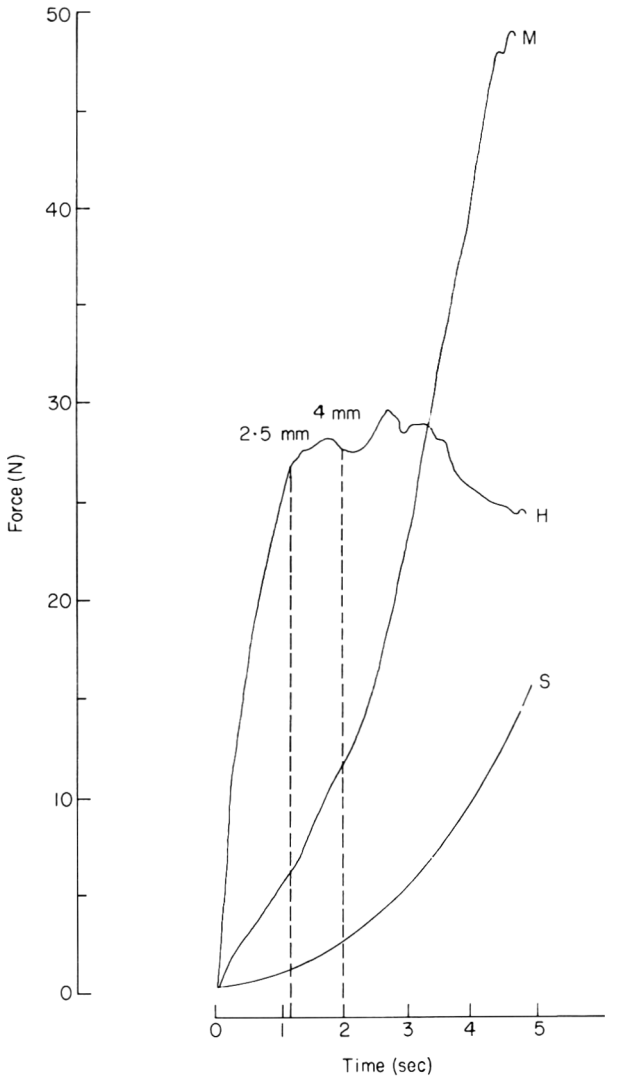

Figure 5. Typical force–time records for puncture tesing the inner subcutaneous layer of hard (H), medium (M) and of soft (S) pork backfat. The forces at 2.5 and 4 mm punch travel were used as measures of firmness.

force and maximum force were measured. There was apparently, no relationship between firmness graded by an experienced factory worker and first peak, or maximum force since some samples judged 'soft' had higher peak and maximum forces than samples judged 'hard' (Table 4). Forces in both layers at 2.5 and 4 mm punch travel increased from soft to hard and had average coefficients of variation at 2.5 and 4.0 mm of 12% for the inner and 8% for the outer layers. With the exception of force at 2.5 mm punch travel, forces in the outer layer were about 1.5 times higher than those in the inner subcutaneous fat layer. In the first experiment the variance between fats relative to that within fats for the force at 4 mm deformation was 159 for the inner and 62 for the outer subcutaneous fat layers and in the second experiment was 124 and 143 respectively. These values are much higher than those of any of the laboratory assessors (Table 1) Showing the greater discrimination of the probe used in this way.

In the second experiment, linear correlation coefficients between assessors' judgment of firmness and first peak and maximum forces in the puncture test were low and indicated that these forces were unreliable predictors of firmness. Correlation coefficients for the forces from the inner fat layer varied from 0.72 to 0.90 ($P \leq 0.05$) between individual assessors, and was 0.83 ($P \leq 0.01$) when assessors' judgments were averaged. But for the outer layer comparisons, correlation coefficients were lower and varied between 0.56 and 0.78 ($P \leq 0.10$) for assessors and 0.71 ($P < 0.05$) for the panel. Correlation coefficients between firmness and forces at 2.5 mm and 4.0 mm

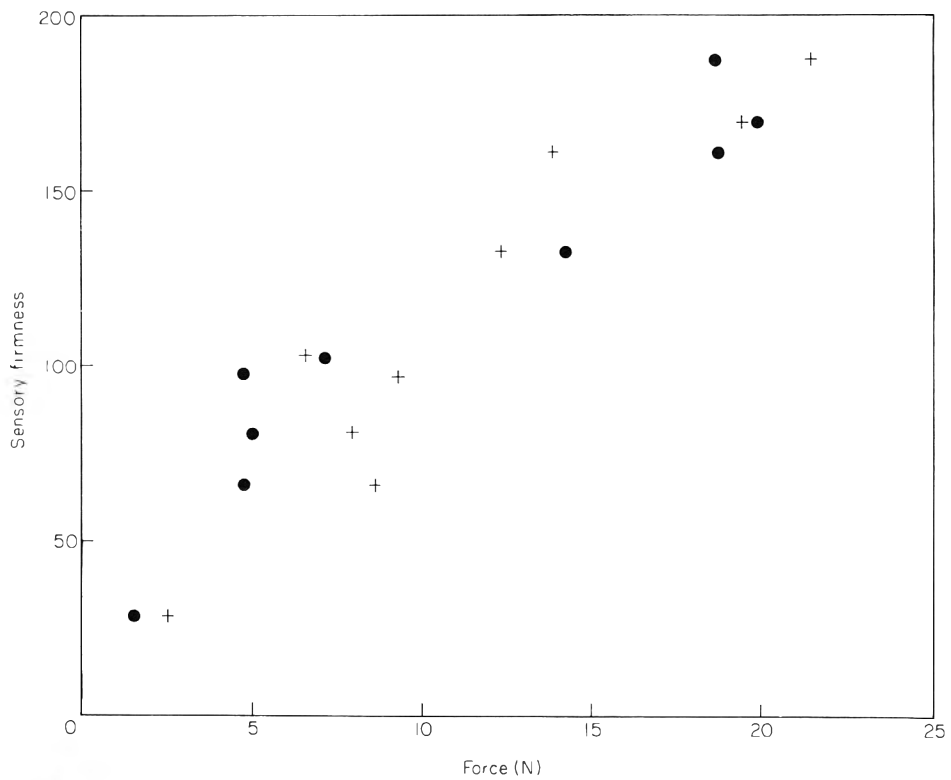

Figure 6. Relationship between sensory firmness and 2.5 mm punch travel in puncture testing pork backfat. Zero on the firmness scale corresponds to extremely soft and 200 to extremely hard. The scores are the average for four assessors judging nine fats. Puncture measurements were on inner (•), and outer (+) subcutaneous layers of nine fats.

travel were above 0.8 ($P \leq 0.01$) for each of four assessors, and above 0.9 for assessors one, three and six. Coefficients were highest using puncture measurements on the inner layer of fat and were 0.95 ($P < 0.001$) for the inner fat layer and 0.93 ($p < 0.001$) for the outer. The relationship between averaged firmness for the four assessors and 2.5 mm force for inner and outer subcutaneous layers is shown in Fig. 6. The best predictor was the force at 2.5 mm for the inner layer combined linearly with the force at 4.0 mm for the outer layer and accounted for 93% of the variation in panel firmness judgments.

Discussion

Sensory evaluation

Finger testing (or 'thumbing') of foods has been practiced for many years and much is known of the sensory scoring and sensing (mainly tactile and kinaesthetic; Harper, 1972) by experts but little is known of the mechanics of sensory assessment. Lack of knowledge of the mechanics of finger testing and the mechanics of mastication has limited the modelling of texture evaluation in the mouth (Kokini, Kadane & Cussler, 1977) and between finger and thumb (DeMartine & Cussler, 1975) since they assume certain operating conditions (geometry, forces, deformation rates). Although sensory testing can be studied by asking assessors to perform in a particular way, e.g. by applying a constant pressure (Scott Blair, 1966), when allowed free choice, as we did, the operative rate depends on the rheological properties of the food and mode of evaluation (Shama, Parkinson & Sherman, 1973). When testing fats most people used two to three pushes with the finger although it was clear that most of the information could be derived from the first push and one assessor used one only. The forces used were similar to those used in testing onions (40 N) and tomatoes (16 N) but we do not know the deformations given to those foods (Voisey & Crête, 1973). One assessor used a constant force irrespective of the fat firmness and also, in general, deformation was related to firmness better than the forces used.

Because we were unable to account completely for the rheological properties of the fats, we cannot be sure that these correlations represent the mechanism by which people assess firmness or whether the same mechanism is used for soft and hard fats. The complexity of feedback mechanism during measurement (Taylor, Lederman & Gibson, 1973) has restricted speculation about the parameters used for judging which may not follow Newtonian dimensions (Scott Blair, 1966). Measuring the operations of one assessor enabled his performance to be improved by allowing for inconsistencies in his testing methodology. It appeared therefore that his poor performance was due to inconsistencies in his method of assessment rather than his ability to perceive firmness. Although we did not study his performance over many trials, it is likely that it would improve with training or experience (Cardello *et al.*, 1982) and we can speculate that this would occur by improving the consistency of his method or by learning how to compensate for those variations.

Even after training, however, it is clear that some assessors would be better than others. One factor in this would be the mechanical characteristics of the fingers since it can be argued (Peleg, 1980) that discrimination is related to the ratio of hardness of the finger to the test material. For example, when two materials are very hard, say 10 to 100 times that of the finger, discrimination is poor whilst at 1 to 10 times discrimination would be higher. To study this, mechanical properties of the fingers of these subjects were assessed by compression at 2 mm/sec for 0.5 sec. Unfortunately, deformation at the maximum force used by an assessor (taken as the measure of softness) was not

significantly less in the more discriminating (higher F ratio) assessors indicating that their fingers were no harder than those of the poorer assessors. Although only the average testing condition was chosen it is unlikely that a relationship would have been found using different conditions.

Structure, deformation and mechanical properties

Subcutaneous adipose tissue is an irregular connective tissue (Warwick & Williams, 1973) in which fat cells are the principal component and vary with age and sex of the animals. The cells contain storage lipids—mainly triglycerides—in a single droplet, and have a characteristic signet ring shape after release from the tissue. Cell size varies with animal growth and nutritional status and, for the pigs in this study, the diameter would be expected to be between 50 and 100 μm. In transverse section the cells have a polyhedral appearance with four to six faces and are surrounded by connective tissue fibres (Stanley & Voisey, 1979). Pork subcutaneous backfat has two principal layers. The outer layer, in which the supporting connective tissue appears to be a continuous three dimensional network with ramifications to the dermis, is separated from the inner layer by a thin layer of connective tissue. In immature animals that part of the inner layer adjacent to the underlying muscle consists of sub-layers, each bounded by a flat criss-cross lattice of connective tissue and, as the animal matures, the sub-layers fill with lipid and become less distinct (Hausman, 1978). These complex structures will impart anisotropy in mechanical properties.

At chill temperature, probing the high stiffness of hard fat allows relatively high shearing forces to be applied to the network of supporting tissue which can result in penetration and sequential rupture of the network. In softer fat, the probing is accompanied by flow which is eventually retarded by the supporting tissue producing higher compressive forces than in hard fat. In soft fat indentation rather than puncture occurs, the supporting tissue being concentrated under the probe.

Further evidence for the importance of components other than lipid in texture of backfat is shown by the difference between inner and outer layers. By the probe method, the outer layer was about 1.5 times harder than the inner. The outer layer, however, contains a greater proportion of unsaturated fatty acids (mainly 18 : 1 but also 18 : 2 and 16 : 1), a smaller proportion of saturated fatty acids (mainly 16 : 0 but also 18 : 0 and 14 : 0) and has a lower melting point (44°C compared with 47°C for the inner layer) which indicate that it ought to be softer (Wood *et al.*, 1978). Connective tissue is also a major contributor to breaking stress under tension which is 3.3×10^5 N/m^2 in mutton backfat at 4°C (Tschizhikova *et al.*, 1971a) and is related to firmness in pig backfat (Wood *et al.*, 1984).

The mechanical properties of pork adipose tissue were assessed from the time dependent stress relaxation modulus (E_t) measured at 3°C. This was clearly related to the sensory assessment of firmness, but in a limited series of stress relaxation experiments the isochronal relaxation moduli increased with strain above 3% showing that the tissue became progressively stiffer with increasing compression. Such changes are consistent with compression of lipid and the generation of tension within or the reorientation of connective tissue surrounding the fat cells. This non-linear viscoelasticity is a common property of biological materials, including collagen rich tendons (Cohen, Hooley & McCrum, 1976). Below 3%, stress was proportional to strain after 300 sec relaxation with a modulus of $8.1 \cdot 10^5$ N/m^2 but a full interpretation of the stress relaxation data awaits an acceptable model for non-linear viscoelasticity.

Puncture testing of hard fats produced a characteristic yield force but caused only

indentation of soft fat. Therefore we could not calculate coefficients of compression and shear using the cross-sectional area and the perimeter of the punch respectively (Bourne, 1975). However Tschizhikova, *et al.* (1971b) used a conical plastometer and derived maximum shearing stress for backfat from Large White pigs. With a ribbed cone (angle $2\alpha = 30°$) at 4°C this value was 1.2×10^5 N/m^2 similar to our values for fats A and F (Table 3).

Firmness by probe

The probe travel was selected at 2 mm/sec, because it was the average rate of finger compression. Combining the average of five probe measurements of initial resistance to deformation of inner and outer subcutaneous layers gave a better discrimination than experienced assessors and accounted for 93% of the variation in firmness judged by the panel. The probe therefore gives a reliable and accurate firmness measurement for chilled backfat and is more valuable than extracted fatty acid concentrations and melting or slip temperatures which are less well related to sensory firmness (Enser *et al.*, 1984).

The orientation of the fat blocks in the laboratory was standardized so that the medial surfaces of the inner and outer layers of subcutaneous backfat, exposed by splitting the carcase, were probed. This orientation would be readily accessible in the abattoir chillroom. A factory instrument relying on either the force measurement at a given precise punch travel or penetration at a given force would have to be made independent of operator influence and be temperature compensated. Within layers, backfat thickness at the sampling site of the fifth to seventh rib was greater than 14 mm but if the probe was to be used on fat from leaner pigs, some reorientation of the fat block might be necessary.

Acknowledgments

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Hydrocyanic acid levels in fermented cassava

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Summary

The extent of loss of hydrocyanic acid during the fermentation of cassava tubers selected from both sweet and bitter varieties in the traditional method (whole unpeeled tubers) compared with the fermentation of peeled tubers and crushed pulps with or without the addition of water. Although the traditional fermentation is terminated after 3–4 days, in this study the fermentation was allowed to proceed for 8 days. Loss of cyanide from the whole tubers was 80–87% after 8 days and was only 51–53% after 4 days. Loss of cyanide from the whole sweet tuber was not significant after 5 days. The loss of cyanide from the peeled tubers was comparable to the whole tubers after 8 days of fermentation. However, there was a marked decrease in free cyanide in the 1st day of fermentation of the peeled tubers compared to whole tubers. The loss in cyanide in the crushed pulp, which occurred primarily in the 1st day, appears to be due to the action of endogenous linamarase rather than hydrolysis by fermentation. When water is added to the crushed pulp the reduction in cyanide was 83–91% with marked decrease in bound cyanide in the 1st day of fermentation. It seems that autohydrolysis is enhanced by addition of water to the crushed pulp.

Introduction

Cassava (*Manihot esculenta* Cranz) is a dominant staple of primary or secondary importance for about 300 million inhabitants of the tropics (Nestel, 1973). Cassava contains cyanogenic glycosides in the form of linamarin and to much less extent, lotaustralin. Two main types of cassava are culivated in Sudan namely: the 'sweet' variety which is associated with low cyanogenic glycosides and the 'bitter' variety which is usually reported to have high cyanide content. The cyanogenic glycosides produce hydrocyanic acid (HCN) when the action of an endogenous enzyme linamarase is initiated by crushing or otherwise damaging the cellular structure of the plant (Conn, 1973). The utilization of cassava roots for both human and animal nutrition appears to be limited by the presence of these cyanogenic glycosides. As a result, the roots have to be processed by a wide variety of traditional methods (Coursey, 1973) in order to reduce their toxicity and improve their palatibility. The fermentation of cassava is a very important step during the preparation of cassava foods. According to Ngaba & Lee (1979) cassava fermentation was mainy due to lactic acid bacteria, *Lactobacillus* sp. and to a lesser degree *Straptococcus* sp. Its significance has been reported (Ketiku *et al.*, 1978) to bring about detoxification of cassava by the liberation of hydrocyanic acid at low pH and the development of a characteristic flavour.

In southern Sudan, tubers are fermented by immersing them in pools of stagnant water for 3–4 days. No information is available on the hydrocyanic acid levels during cassava fermentation. In this study the hydrocyanic acid levels in the traditional

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fermentation (whole unpeeled tubers), in the peeled tubers and in the crushed pulp with or without added water are determined.

Materials and methods

Sweet and bitter cassava varieties were cultivated in the Food Research Centre Experimental Farm near Khartoum. At the age of 1 year, the tubers were harvested and 30 kg of each variety were collected. The tubers were washed free from any adhering soil. Six tubers were peeled by making an incision with a knife followed by the removal of the peel by hand. The peeled tubers were grated into small pieces, mixed thoroughly and divided into six portions. Each portion was blended in a kitchen mixer for 1 min and samples placed into screw cap jars for chemical analysis. Total and free cyanide were determined by the methods of Cooke (1978). Titratable acidity was determined as described in AOAC (1975). The remaining tubers were divided into three equal lots of 7 kg. In the first treatment whole tubers were immersed in 10 litres of tap water in a plastic bucket. The bucket was covered and kept at room temperature (25–28°C) for 8 days. In the second treatment the tubers were peeled and treated in the same manner as for whole tubers. In the third treatment the peeled tubers were crushed in a meat mincer for 2–3 min at room temperature and the crushed pulp divided into two portions. One portion was placed in a plastic bucket with cover and left to ferment for 8 days. To the second portion water was added in the ratio of 4 : 1 (crushed pulp : water) and was mixed thoroughly and left to ferment. Samples were drawn at 24 hr intervals from the three treatments for chemical analysis. Portion of 50–100 g from each piece of fermenting cassava tubers were cut out from not less than half of the total soaked number (eight to nine tubers).

Results and discussion

Table 1 shows changes in pH, titratable acidity and the cyanide content during fermentation of the whole tuber from the bitter variety. The pH of the pulp dropped from 6.0 to 3.95 and acidity increased from 0.111 to 0.712% as lactic acid. Total cyanide content in the pulp of the whole tuber decreased by 80% during the 8 day fermentation period. In the traditional village fermentation of cassava the fermentation process usually takes 3–4 days (Akinrele, 1964). On the 4th day of fermentation the hydrocyanic acid content was reduced by 51.8%. The free and bound cyanide contents in the whole tuber pulp dropped to 9.5 ± 0.50 and 5.2 ± 0.45 mg/kg fresh basis, respectively, in 8 days. The concentration of the free cyanide in the fresh cassava tubers was found to be higher than the known quantities of 10–20% (Table 1). The breakdown of bound cyanide could be due to the disintegration process prior to immersion in phosphoric acid.

Fermentation of the whole tuber from the sweet variety resulted in a pH drop from 6.2 to 3.9 in the pulp and acidity increased from 0.152 to 0.790 as lactic acid. The total cyanide content in the pulp of the whole tuber from the sweet variety decreased by 87.2% during the 8 day period. The decrease on the 4th day of fermentation represents a drop of 53.8% in total cyanide content. The kinetics of loss in cyanide from the pulp of the sweet whole tuber are similar to those shown in Table 1. The reduction of cyanide in the pulp of the fermented whole tuber of the sweet variety was not significant ($P < 0.01$) after the 5th day of fermentation. The pulp contained lower quantities of total, free and bound cyanide (6.5 ± 0.71, 4.4 ± 0.28 and 2.1 ± 0.23, respectively) compared to the pulp from the bitter variety at the end of fermentation (Table 1).

Table 1. Mean changes ± s.d. for total, free and bound cyanide during cassava fermentation

Treatment	Time (days)	pH	Titratable acidity (%)	Mg/kg fresh basis		
				Total cyanide	Free cyanide	Bound cyanide
Bitter variety						
Whole tuber:	0	6.0	0.111	69.9 ± 0.99[d]*	35.0 ± 0.30	34.9 ± 0.57
Pulp	1	5.9	0.142	65.9 ± 0.57[d]	31.9 ± 0.48	34.0 ± 0.28
	2	5.85	0.132	49.3 ± 0.60[c]	26.1 ± 0.60	23.2 ± 0.12
	3	5.8	0.171	40.8 ± 0.67[c]	23.7 ± 0.40	17.1 ± 0.28
	4	5.35	0.250	33.7 ± 0.60[b]	21.4 ± 0.57	12.3 ± 0.31
	5	4.4	0.413	30.0 ± 0.60[b]	18.1 ± 0.50	11.9 ± 0.12
	6	4.2	0.560	27.0 ± 1.30[b]	14.7 ± 1.07	12.3 ± 0.35
	7	4.1	0.600	19.8 ± 1.30[a]	12.1 ± 0.50	7.6 ± 0.83
	8	3.95	0.712	14.7 ± 0.80[a]	9.5 ± 0.50	5.2 ± 0.45
Peel	0	6.1	0.101	208.6 ± 0.67[f]	03.3 ± 0.40	105.3 ± 0.28
	1	5.7	0.113	186.3 ± 0.60[e]	94.2 ± 0.57	92.1 ± 0.31
	2	5.6	0.242	156.0 ± 0.50[d]	84.0 ± 0.35	72.0 ± 0.17
	3	4.8	0.261	139.0 ± 0.66[c]	76.0 ± 0.40	63.0 ± 0.26
	4	4.7	0.350	117.0 ± 0.44[b]	63.0 ± 0.25	54.0 ± 0.28
	5	4.4	0.452	106.6 ± 0.53[b]	56.8 ± 0.47	49.8 ± 0.05
	6	4.1	0.612	92.9 ± 0.51[a]	53.5 ± 0.50	39.4 ± 0.11
	7	3.9	0.740	81.3 ± 0.60[a]	43.9 ± 0.36	37.8 ± 0.26
	8	3.85	0.801	72.0 ± 0.76[a]	36.2 ± 0.67	35.8 ± 0.28
Soaking water	1	4.8	0.102	10.6† ± 0.40	5.8 ± 0.45	4.8 ± 0.06
	2	4.35	0.191	15.7 ± 0.45	7.66 ± 0.36	8.1 ± 0.15
	3	4.1	0.262	25.7 ± 0.41	15.7 ± 0.36	10.0 ± 0.05
	4	3.8	0.500	35.8 ± 0.53	20.5 ± 0.51	15.3 ± 0.17
	5	3.8	0.693	40.9 ± 0.67	22.4 ± 0.45	18.5 ± 0.23
	6	3.8	0.760	52.7 ± 0.55	25.5 ± 0.62	27.2 ± 0.32
	7	3.8	0.792	60.7 ± 0.55	29.6 ± 0.35	31.1 ± 0.42
	8	3.8	0.890	66.2 ± 0.50	32.8 ± 0.59	33.4 ± 0.20

*Means in a column sharing the same letter are not significantly different ($P < 0.01$) as determined by Duncan's multiple range test.

†As mg/l.

The peels from bitter variety contained considerably higher amounts of cyanide compared to the pulp (Table 1). The soaking water from both varieties contained both free and bound cyanide. The total cyanide content in the soaking water from the bitter variety increased from 10.6 ± 0.40 in the 1st day of fermentation to 66.2 ± 0.50 mg/l in the last day. Similarly in the sweet variety the increase was from 17.3 ± 0.43 to 56.0 ± 0.41 mg/l. This high cyanide content in the soaking water necessitates dewatering of the pulp before consumption. Maduagwe & Oben (1981) used a screw press for dewatering fermented cassava and reported a 63.8% reduction in cyanide content as a result of the pressing step.

In Table 2 is shown the changes in pH, acidity and cyanide levels during fermentation of the peeled tuber from the bitter variety. The pH of the fermented pulp dropped to 3.9 and acidity increased to 0.781% as lactic acid. The total hydrocyanic acid content decreased by 81.8% during the 8 day period. This drop is comparable with that of the whole tuber from the same variety. On the 4th day of fermentation the decrease was up to 61.7%. Loss of cyanide in the peeled tuber from the bitter variety was not significant ($P < 0.001$) after the 5th day of fermentation. It is observed that there is a

Table 2. Mean changes ± s.d. for total, free and bound cyanide during cassava fermentation

Treatment	Time (days)	pH	Titratable acidity (%)	Mg/kg fresh basis		
				Total cyanide	Free cyanide	Bound cyanide
Bitter variety	0	6.0	0.111	69.99 ± 0.85^{f}	35.0 ± 0.30	34.9 ± 0.57
Peeled tuber:	1	5.8	0.121	55.6 ± 0.40^{e}	24.7 ± 0.30	30.9 ± 0.11
Pulp	2	5.7	0.222	42.2 ± 0.55^{d}	21.9 ± 0.50	20.3 ± 0.05
	3	5.3	0.226	34.1 ± 0.61^{c}	17.3 ± 0.55	16.8 ± 0.10
	4	4.8	0.320	26.8 ± 0.39^{b}	14.2 ± 0.35	12.6 ± 0.06
	5	4.2	0.451	21.8 ± 0.42^{a}	11.8 ± 0.35	10.0 ± 0.15
	6	4.1	0.582	19.9 ± 0.35^{a}	10.6 ± 0.35	9.3 ± 0.06
	7	4.0	0.690	16.5 ± 0.40^{a}	9.0 ± 0.35	7.5 ± 0.06
	8	3.9	0.781	12.7 ± 0.37^{a}	7.4 ± 0.21	5.3 ± 0.26
Soaking water	1	4.5	0.131	4.3 ± 0.57	2.3 ± 0.20	2.0 ± 0.36
	2	4.1	0.262	6.3 ± 0.65	3.8 ± 0.26	2.5 ± 0.40
	3	4.0	0.440	7.3 ± 0.42	5.2 ± 0.36	2.1 ± 0.21
	4	3.95	0.521	9.8 ± 0.25	6.7 ± 0.32	3.1 ± 0.15
	5	3.95	0.643	11.8 ± 0.55	6.9 ± 0.40	4.9 ± 0.15
	6	3.6	0.832	14.1 ± 0.66	9.1 ± 0.40	5.0 ± 0.26
	7	3.8	0.790	16.7 ± 0.30	11.7 ± 0.35	5.0 ± 0.05
	8	3.85	0.792	19.7 ± 0.45	12.5 ± 0.35	7.4 ± 0.10

higher drop of free cyanide during the 1st day of fermentation in the peeled tuber compared to the whole tuber of the same variety (Table 2).

Total cyanide content of the soaking water of the peeled tubers is considerably lower than that of the whole tubers (Table 2). The increase in total cyanide content was from 4.3 ± 0.57 to 19.7 ± 0.45. Also, free and bound cyanide content of the soaking water were considerably lower in the peeled tuber compared to the whole tuber.

Fermentation of the peeled and crushed tuber from the bitter variety resulted in a pH drop to 3.8 and an increase in acidity to 0.802% (Table 3). The total cyanide content of the crushed pulp decreased by 69.8% during the 8 day period. Loss of cyanide was not significant ($P < 0.01$) after 4 days of fermentation which represented a reduction of 60.8%. For the sweet peeled and crushed tuber the pH dropped to 4.6 in the 3rd day and then increased to 6.7 in the last day. This fluctuation in the pH of the crushed pulp in air could be attributed to termination of lactic acid fermentation and the participation of other microorganisms that do not produce acid. Acidity increased to 0.420% in the third day and diminished to 0.101% in the last day. Total cyanide content of the crushed pulp decreased by 64.6% as a result of fermentation for 8 days. Loss of cyanide was not significant ($P < 0.01$) during the first 6 days of fermentation. The initial loss in cyanide in the crushed pulp could be attributed to the action of endogenous linamarase which is reflected in the rapid loss of bound cyanide (Conn, 1973).

Fermentation of the crushed pulp with added water for the bitter variety resulted in a pH drop to 3.8 and increase in acidity to 0.82% (Table 3). Total cyanide content decreased by 91.7% during the 8 day period. There was a marked decrease in bound cyanide in the 1st day of fermentation (77.9%). In the 4th day the cyanide content decreased by 80.3%.

The pH of the crushed pulp with added water from the sweet variety dropped to 3.8 and acidity increased to 0.86% (Table 3). Total cyanide content decreased by 83.0% during the 8 day period. Loss of cyanide is not significant ($P < 0.01$) after 4 days of fermentation which amounted to 76.5%.

Table 3. Mean changes ± s.d. for total, free and bound cyanide during cassava fermentation

Treatment	Time (days)	pH	Titratable acidity (%)	Mg/kg fresh basis		
				Total cyanide	Free cyanide	Bound cyanide
Bitter variety	0	6.0	0.111	69.9 ± 0.99^{d}	35.0 ± 0.30	34.9 ± 0.57
Crushed pulp	1	5.6	0.220	39.4 ± 0.65^{c}	23.0 ± 0.36	16.4 ± 0.32
	2	5.25	0.321	33.1 ± 0.75^{b}	21.6 ± 0.20	11.5 ± 0.55
	3	5.0	0.361	29.4 ± 0.70^{b}	20.2 ± 0.45	9.2 ± 0.25
	4	4.75	0.422	27.4 ± 0.50^{a}	19.7 ± 0.46	7.7 ± 0.10
	5	4.3	0.522	25.9 ± 0.57^{a}	18.6 ± 0.35	7.3 ± 0.21
	6	4.2	0.671	24.9 ± 0.89^{a}	15.8 ± 0.40	9.1 ± 0.49
	7	4.0	0.718	24.1 ± 0.78^{a}	13.4 ± 0.61	10.7 ± 0.17
	8	3.8	0.802	21.1 ± 0.55^{a}	12.4 ± 0.41	8.7 ± 0.15
Crushed pulp + water (4 : 1)	0	6.0	0.111	69.9 ± 0.99^{d}	35.0 ± 0.30	34.9 ± 0.57
	1	5.0	0.311	22.6 ± 0.36^{c}	14.9 ± 0.26	7.7 ± 0.21
	2	4.8	0.390	16.9 ± 0.25^{b}	12.7 ± 0.21	4.2 ± 0.10
	3	4.6	0.402	15.4 ± 0.26^{b}	11.2 ± 0.24	4.2 ± 0.10
	4	4.3	0.542	13.8 ± 0.36^{b}	9.9 ± 0.30	3.9 ± 0.10
	5	4.0	0.660	11.9 ± 0.25^{b}	8.3 ± 0.10	3.6 ± 0.15
	6	3.9	0.741	9.4 ± 0.36^{a}	6.3 ± 0.20	3.1 ± 0.17
	7	3.8	0.750	7.5 ± 0.41^{a}	5.5 ± 0.21	2.0 ± 0.21
	8	3.8	0.820	5.8 ± 0.40^{a}	3.8 ± 0.30	2.0 ± 0.12
Sweet variety	0	6.2	0.152	50.6 ± 0.96^{d}	31.2 ± 0.42	19.4 ± 0.43
Crushed pulp	1	4.9	0.352	37.6 ± 0.41^{c}	29.4 ± 0.28	8.2 ± 0.13
	2	4.6	0.420	36.5 ± 0.36^{c}	29.4 ± 0.28	7.1 ± 0.08
	3	5.2	0.303	34.1 ± 0.56^{c}	27.2 ± 0.28	6.9 ± 0.28
	4	6.0	0.222	33.6 ± 1.30^{c}	26.8 ± 0.57	6.8 ± 0.73
	5	6.2	0.160	32.3 ± 0.41^{c}	26.0 ± 0.27	6.3 ± 0.14
	6	6.4	0.122	29.7 ± 0.49^{c}	23.6 ± 0.21	6.1 ± 0.28
	7	6.6	0.111	24.0 ± 0.41^{b}	18.6 ± 0.14	5.4 ± 0.27
	8	6.7	0.101	17.9 ± 0.57^{a}	12.9 ± 0.42	5.0 ± 0.15
Crushed pulp + water (4 : 1)	0	6.2	0.152	50.6 ± 0.96^{d}	31.2 ± 0.42	19.4 ± 0.43
	1	4.6	0.442	22.4 ± 0.42^{c}	14.9 ± 0.28	7.5 ± 0.14
	2	4.3	0.682	17.1 ± 0.28^{b}	13.2 ± 0.14	3.9 ± 0.14
	3	4.3	0.680	15.1 ± 0.23^{b}	12.0 ± 0.14	3.1 ± 0.09
	4	4.25	0.781	11.9 ± 0.42^{a}	9.2 ± 0.28	2.7 ± 0.14
	5	4.2	0.811	10.4 ± 0.28^{a}	7.9 ± 0.14	2.5 ± 0.14
	6	4.1	0.830	9.4 ± 0.21^{a}	7.0 ± 0.14	2.4 ± 0.07
	7	4.1	0.860	8.9 ± 0.14^{a}	6.4 ± 0.07	2.4 ± 0.07
	8	3.8	0.860	8.6 ± 0.28^{a}	6.3 ± 0.21	2.3 ± 0.08

Crushing and addition of water resulted in rapid initial loss in bound cyanide (77.9% for the bitter variety and 61.3% for the sweet variety). Also, it seems that autohydrolysis is enhanced by addition of water to the crushed pulp.

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Ripening and spoilage of sugar salted herring with and without nitrate. I. Microbiological and related chemical changes

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Summary

Microbial and chemical changes during ripening with and without nitrate and spoilage of sugar salted (barrel salted) herring were studied using fish from different fishing grounds and different initial quality and fat content. The fish were stored for ripening at 4–6°C for 18 months.

The microbial activity in the barrels during ripening was generally low. The flora, which was predominantly aerobic, did not change much during storage and consisted mainly of moderately halophilic gram negative rods (~ 70%), gram positive cocci (~ 20%) and yeast (~ 3%). Some of the microorganisms present were able to reduce trimethylamine-*N*-oxide (TMAO), but none attacked sucrose, which is the sugar added to the curing salt in the barrels. There was no correlation between TMAO reduction and ripening.

As both the viable counts and, sometimes, the composition of the microflora could vary considerably in barrels classified as normal, the microorganisms are considered to play a minor role in the ripening process.

Two types of microbial spoilage have been identified. The most common type, characterized by development of sour, sour/sweet or putrid off odours and flavours, was caused by growth of a gram negative obligate anaerobic halophilic organism. A second type of spoilage, characterized by development of fruity off odour, was caused by growth of osmotolerant yeast species.

Introduction

A great variety of semi-preserved fish products are marketed in Scandinavia. Some of these, such as Kryddersild and Tidbits, are produced from sugar salted fish (mainly herring), which have gone through a long ripening process (up to 1.5 years), in wooden barrels during storage at low temperature. The fish, either ungutted or headed and nobbed, are mixed with salt (15–17%), sugar (6–7%) and spices in the barrels. During ripening the flesh becomes tender and acquires an aromatic flavour and taste which is characteristic of the product. When ripening is complete, the fish are filleted and packed in a retail container (can or glass jar) with a sauce containing much less salt (approx. 4%), more sugar (35%) and some acetic acid (10–12%). In some cases a chemical preservative is added to the final product.

In spite of the long historical tradition for production of salted and sugar salted fish in barrels, there is a remarkable lack of information on the precise nature of the

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ripening process. There are some reports dealing with the sensory and chemical changes taking place (Luijpen, 1959; Alm, 1964; Constantinides & Dymoza, 1972; Kiesvaara, 1975; Filsinger *et al.*, 1982), and it is generally accepted that the texture changes are due to the action of proteolytic enzymes which leach out from the pyloric caecum. Little or no information is available on the microbiological changes and the possible role of microbes in the ripening and spoilage of this type of product. However, it has been stated that the microflora—partly by fermenting the added carbohydrates—is responsible for the development of the characteristic aroma and flavour (Zaitsev *et al.*, 1969).

The present work deals with the normal microbiological and chemical changes during ripening of sugar salted herring and with the microbiological causes of certain types of spoilage. The work is part of a large scale experiment, where the main objective has been to study the effect of added nitrate in the curing salt (Knøchel & Huss, 1984).

Materials and methods

Raw material

Herring from different fishing grounds, of different initial quality and fat content were used in this experiment. Three hundred barrels of herring were produced in Iceland using fish caught in Icelandic coastal waters during the months of October and November. Another 300 barrels were prepared in Denmark, using fish caught in the North Atlantic, Danish coastal waters and the Baltic Sea, respectively, during the period September–February. At each locality the material was prepared in ten series of thirty barrels, each series consisting of fish from the same catch, treated exactly the same way until landing.

Salting and storage

On landing, the fish were washed, headed and nobbed. Between 90–100 kg of fish were mixed with 16 kg NaCl, 6 kg sucrose and spices, and the mixture was filled into wooden barrels (120 l). Of the thirty barrels within one series, ten had 50 g KNO_3, ten had 25 g KNO_3 and ten had no KNO_3 included in the mixtures. The barrels were left for 24 h for the fish to settle and draw brine. Before being closed the barrels were filled completely with saturated brine to exclude all air. They were then stored in a horizontal position at 4–6°C for 18 months for ripening at two commercial plants. During this period, the factory quality control routinely checked all barrels for degree of ripening and signs of spoilage.

Sampling

Based on differences in fat content, six series (three from each locality) were selected for laboratory analyses in order to monitor the normal changes taking place. Samples were always taken from two barrels with 50 g KNO_3 and two without KNO_3 from each series at times shown in Table 1. Samples from all barrels with signs of spoilage were likewise taken to the laboratory for further examinations.

Organoleptic assessment

Based on appearance and texture of the fish, eight to ten experienced panellists were asked to place the samples in one of the following three grades: normal, slightly off, reject. The term normal does not imply that the herring were of the same quality, but simply that the fish were perfectly fit for further processing and had no off odour or

Table 1. Sampling of sugar salted herring during ripening

Location of production	Series No.	Fat content of fish (%)	Storage time (weeks) when sampled			
Iceland	C	20.4	17	35	53	69
	E	17.1	6	34	52	68
	G	17.9	15	33	51	67
Denmark	I	25.9	22	45	59	77
	IV	18.9	15	37	51	69
	IX	14.9	3	25	39	57

flavour. In cases where off odours and flavours were found, the panellists were asked to describe these.

Chemical examination

This was carried out on fish and brine. The fish samples consisted of eight to ten fillets without skin taken from the same number of fish and homogenized in a Waring blender. The samples were analysed for protein (Kjeldahl's method), oil (Bligh & Dyer, 1959), dry matter (dried at 105°C for 24 h) and NaCl (Volhard's method). pH and oxidation reduction potential (Eh) were measured using a pH-meter 28 (Radiometer), in the latter case mounted with platinum electrode and calomel reference electrode (Huss & Larsen, 1979). Trimethylamine-*N*-oxide (TMAO) and total volatile bases (TVB) were estimated using the method of Conway & Byrne (1933).

Enumeration of microorganisms

Viable counts were carried out on fish and brine. Material from belly flaps of four fish were pooled into one sample of 10 g and homogenized (Stomacher) in 90 ml sterile 15% (w/v) NaCl solution containing 0.1% peptone. The same solution was used for serial dilutions.

All plating was done as surface plating.

General viable aerobic and anaerobic counts were made on Brain Hearth Infusion Agar (BHI) (Difco) supplement with 15% (w/v) NaCl and $MgSO_4$ (0.2% w/v) to meet the specific requirement of halophilic organisms for Mg^{2+} (Gibbons, 1969). Plates for aerobic counts were incubated in polyethylene pouches to avoid evaporation. For anaerobic incubation Oxoid Anaerobic System (H_2/CO_2 generators) was used. Incubation time was 2 weeks.

Yeast was counted on APT (Difco), supplemented with 100 ppm oxytetracycline and 15% (w/v) NaCl after incubation for 4 weeks.

The general viable aerobic counts at 7% NaCl plus the number of gelatin liquefiers were counted on Veal Infusion Broth (Difco) supplemented with (g/l): NaCl 70: peptone 10: yeast extract 2: gelatine 10, and solidified with agar (15 g/l).

The number of microogranisms able to produce slime from sucrose was counted on a medium containing (g/l): tryptone 10; yeast extract 5: K_2HPO_4 2; NaCl 150; sucrose 50, and solidified with agar (15 g/l).

Incubation temperature was always 22°C (± 1°C).

Direct microscopy was performed on the brine at × 1000 magnification.

Selection and examination of isolates

The composition of the microflora was examined at each sampling occasion in the Danish produced series with 50 g KNO_3 and without KNO_3 (twenty-four barrels). Around thirty colonies were randomly selected from BHI agar with 15% NaCl and subcultured in BHI broth containing 15% NaCl. The same procedure was used when examining barrels with off-odours (six barrels) and barrels with fully ripe Icelandic Herring (five barrels). Gram staining properties were tested using material from newly formed colonies on BHI agar and using both Hucker's modification and the KOH method (Gregersen, 1978). Cytochrome oxidase was tested by the method of Kovacs (1956) and catalase by gas formation when exposed to 3% H_2O_2. Carbohydrate utilization (sucrose, glucose and fructose) was tested by the method of Hugh & Leifson (1953). Nitrate reduction was tested according to Cowan (1974) and N_2 production was detected in Durham tubes.

Reduction of TMAO and H_2S production was tested in a medium containing TMAO, cysteine, Na-thiosulphate, ferric citrate and a redox indicator, resazurin (Jensen, Marjun & Schulz, 1980). Proteolytic activity was tested on gelatin agar but supplemented with 15% (w/v) NaCl.

Lipolytic activity was tested in tributyrin agar (Oxoid).

Production of H_2S by anaerobic strains was tested in deep stabs in Triple Sugar Iron agar (Difco).

Production of indole by anaerobic strains was tested using the medium of Spray (1936). The same medium supplemented with carbohydrates was used for testing acid production from sucrose and glucose. Indicators were added to the fully grown cultures.

Carbohydrate fermentation by yeasts was tested as described by Beech *et al.* (1968).

All diagnostic media were adjusted to contain 15% NaCl (w/v) and 0.2% $MgSO_4$ (w/v). Growth in 0.5 and 20% NaCl was tested in BHI broth adjusted accordingly.

All anaerobic isolates were examined for spores after prolonged incubation (at least 3 weeks) in Robertson's cooked meat medium by phase contrast microscopy. Although no sporulation was observed the cultures were heat treated for 10 min at 80° and subsequently streaked on BHI agar.

Results

Raw material

The quality and origin of herring used in the experiments are shown in Table 2. The fish used in Iceland were always very fresh, being caught close to the shore and having spent a maximum of 2 days in ice before salting. The fat content of the fish varied little (Table 3) due to the short catching period. In contrast, the initial organoleptic quality of the raw material used in Denmark varied greatly due to widely scattered and often very distant fishing grounds. Also the fat content of this material showed greater differences (Table 3) as the ten series were collected over a period of nearly 5 months.

Organoleptic changes

In the great majority of the barrels, the ripening process proceeded normally and the fish were considered to be normally matured on the last day of sampling.

After approximately 1 year of storage, off odours and flavours were found in

twenty-five barrels. Two barrels were rejected due to rancidity and failure to ripen normally, the fish being hard, pale and dry. In the rest (twenty-three barrels), two distinct types of spoilage could be identified as shown in Table 2. The table also shows that spoilage, particularly type 1 spoilage, occured most frequently in fish produced from the poorest raw material (most days in ice). Indeed, in the series made from the oldest raw material 30% of the barrels were rejected due to type 1 spoilage.

Table 2. Procesing and spoilage of sugar salted herring

Origin of raw material	Production place	Quality of raw material (days in ice)	No. of barrels produced	No. of spoiled barrels*	
				Type 1	Type 2
Icelandic coastal waters	Iceland	< 2	300	0	0
North Atlantic	Denmark	4–13	180	17	0
Kattegat	Denmark	1–6	60	2	1
Baltic	Denmark	3–5	60	0	3

*Type 1 spoilage was characterized by the presence of sour, sour/sweet or putrid odour and flavour.

Type 2 spoilage was characterized by the presence of fruity odours and flavours.

Chemical change

Table 3 shows chemical changes during ripening. Herring from Danish coastal waters and the Baltic Sea tended to retain the fat in the fillet during ripening. This was not the case with herring from Iceland. After maturation the fish from the latter area had 12–14% fat in the fillet, irrespective of the fat content of the raw material.

The content of TMAO in herring from the North Atlantic was higher than in herring from Danish coastal waters and the Baltic Sea. TMAO was partly reduced to TMA during ripening. The TMAO was not completely reduced in the normally matured fish, whereas in the type 1 spoiled fish all TMAO had disappeared.

The content of total volatile bases (TVB) in the fish flesh increased during ripening, but never exceeded 75 mg N/100 g fish in a normally matured fish. In type 1 spoiled fish the TVB content was always higher.

The pH of the fish flesh decreased during ripening to about 5.7–5.8, but when type 1 spoilage occurred, pH again increased above 5.9.

The chemical changes of type 2 spoiled fish were not different from those observed in normally matured herring.

Microbiological changes

In organoleptically normal fish the range of the general viable aerobic counts recorded was, with a few exceptions, between 10^3 and 3×10^5/g fish, using media with 15% NaCl, and no specific development was noted during the storage period. The viable counts were always higher in the brine (Table 3), while aseptic sampling of fillet

Table 3. Chemical and microbiological change during maturation and spoilage of sugar salted herring

		Raw fish	Normally matured fish	Spoiled fish: Type I (sweet-sour, putrid)	Spoiled fish: Type II (fruity odour)
No. of samples		6	24	19	4
Protein (%)		15–17	15–17	14–16	14–15
Fat (%)	Iceland	17–21	12–14		
	Denmark	15–26	15–22		
NaCl (%) (aqueous phase)		0.9	20–22	20–22	20–22
pH		6.3–6.4	5.7–5.8	> 5.9	5.7
TMAO (mg N/100 g fish)	Iceland	35–40	> 2	0	5–16
	Denmark	20–25			
TVB		10–25	< 75	> 75	47–72
Viable counts in 15% NaCl					
Aerobic	($\log_{10}$/g fish)		$\bar{x}$ = 4.69 (s = 1.10)	$\bar{x}$ = 5.04 (s = 0.76)	$\bar{x}$ = 4.64 (s = 0.49)
Aerobic	($\log_{10}$/g brine)		$\bar{x}$ = 5.30 (s = 1.06)	$\bar{x}$ = 5.68 (s = 0.92)	$\bar{x}$ = 5.54 (s = 0.58)
Anaerobic	($\log_{10}$/g brine)		< 5.00*	$\bar{x}$ = 6.33÷ (0.87)	$\bar{x}$ = 3.87* (s = 0.15)
Yeasts ($\log_{10}$/g fish)			$\bar{x}$ = 3.32 (s = 0.69)	< 3.00	$\bar{x}$ = 4.17 (s = 0.12)
Yeasts ($\log_{10}$/g brine)			N.T.	N.T.	$\bar{x}$ = 5.29 (s = 0.38)

*Mainly cocci.
÷Mainly gram negative rods.

tissue showed that the flesh itself was almost sterile even after 18 months of storage. With 7% NaCl in the media, counts were slightly higher but the proportion of cocci to rods in some randomly selected samples did not always correspond to the proportions observed by direct microscopy.

The general viable anaerobic counts were low, generally $< 10^3$/g, and never exceeding 10^5/g in brine from barrels with normally ripe fish. Colonies from two normal samples with high anaerobic counts proved upon microscopical examination to consist of cocci.

No slime forming colonies on sucrose containing agar were recorded in this study.

The presence of yeasts varied greatly between the individual experimental series and no general pattern was observed. Counts from less than 10^2/g up to 7×10^4/g were recorded in organoleptically normal fish.

Type 1 spoiled fish differed from normal fish in having high anaerobic counts (range: 6×10^5–10^8/g brine) partly or mainly consisting of obligate anaerobic rods. Increasing anaerobic counts correlated with an increasing degree of spoilage as shown in Table 4.

Type 2 spoiled fish were characterized by the presence of yeasts in numbers from 10^4 to 10^5/g fish, while the aerobic and anaerobic counts were low.

The studies of flora composition were sometimes complicated by the loss of many isolates. In a few cases up to 50% of isolates from one primary plate did not survive transfer to secondary broth culture.

A total of 666 aerobic isolates from normal barrels were examined according to the criteria shown in Table 5.

Table 4. Characteristic changes measured in three barrels representing three different stages in type I spoilage

Organoleptic assessment	Sour	Sour/sweet	Putrid
TMAO (mg N/100 g fish)	0	0	0
TVB (mg N/100 g fish)	120	132	178
Eh (mv) in brine	+107	+11	−70
Total anaerobic count	10^6	1.3×10^6	1.5×10^8
Composition of anaerobic flora			
Microaerophilic cocci	20%	0%	0%
Anaerobic rods	80%	100%	100%
No of isolates examin.	20	20	30

Table 5. Types of microorganisms found in the aerobic flora on sugar salted herring

	Gram negative rods		Gram negative or positive rods	Gram positive cocci			Yeasts
Biochemical activity	1	2	3	4	5	6	7
Ability to reduce TMAO	+	−	−	−	−	−	−
Ability to reduce NO_3^-	+	+	−	−	+	−	+*
Catalase	+	+	+	−	+	+	
Oxidase	+	+	+	−	−		
Sucrose	−	−	−	−	−		−
Glucose	−	−	−	+	+†		+
Growth in 20% NaCl	+	+	+	+	+		+
Growth in 0.5% NaCl	−	−	−	+	+		+
Gelatinase	−	−	(+)	−	+		−
Lipase	−	−	−	−	−		(−)
No. of isolates	220 (33%)	226 (34%)	55 (8%)	58 (9%)	30 (5%)	25 (4%)	19 (3%)

*A few strains were able to assimilate nitrate very slowly.
†Oxidatively.

Seven types of microorganisms were found to be dominating. By far the most numerous were two types of gram negative halophilic rods separated only on their ability to reduce TMAO, while both were able to reduce nitrate. A third small group of rods containing both gram negative and positive strains were unable to reduce any of these two compounds.

Three types of cocci were isolated. The main group (group 4 in Table 5) was unable to reduce TMAO and nitrate, while a second type (group 5) was catalase positive and able to reduce nitrate. The last ype of coccus, which was only found in a few barrels, was catalase positive but unable to reduce nitrate and TMAO.

A number of yeasts were isolated. They all had in common that they did not reduce TMAO, while a few strains assimilated nitrate very slowly. None of them produced acid from sucrose but did from glucose and fructose.

Table 6. Composition in % (all series pooled within each period) of the aerobic microbial flora during ripening

	Weeks of storage					
	1–12	13–26	27–39	40–52	53–65	66–78
Rods	73	77	80	89	72	75
Cocci	23	18	20	8	27	24
Yeast	5	5		4	1	1
No. of isolates examined in period	62	133	60	200	114	97

The composition of the flora varied somewhat from barrel to barrel and could not be correlated to storage time. The general pattern is presented in Table 6.

A total of 176 microorganisms isolated from the aerobic flora in barrels with off odours were likewise examined. All of them belonged to group 1, 2 and 4 and no correlation could be made to the type of spoilage found in the barrels.

The obligate anaerobic rods isolated from the type 1 spoiling fish were characterized as follows: 70/70 isolates examined all showed violent gas production when grown anaerobically in BHI with 15% NaCl. Size and shape of the organisms varied, but the colonies on BHI+15% NaCl were characteristically round, semi-transparent and yellowish. All strains were gram-, catalase- and oxidase-negative, they did not reduce nitrate and none liquefied gelatin. They produced acid from glucose and, in some cases, also from sucrose. All isolates produced indole and large amounts of H_2S. All grew in 20% NaCl and none in 0.5% NaCl. No spores were observed microscopically.

Five strains of yeast isolated from barrels with fruity odour all produced acid and gas under anaerobic conditions from glucose and fructose, but not from galactose, sucrose, maltose and lactose. When inoculated into a naturally contaminated brine containing TMAO and nitrate, these strains were able to produce the same fruity odours as noticed in the barrels with type 2 spoiled fish.

Discussion

The microbial activity is generally low during ripening of barrel salted herring stored at low temperatures. The flora is mainly aerobic and dominated by moderately halophilic gram negative rods. These organisms are not included in the genus *Halobacteriaceae* as defined in Bergey's Manual (Buchanan & Gibbons, 1974), but organisms having similar characteristics were isolated by Hornsey & Mallows (1954) and Ingram (1958) in beef and bacon curing brines.

The main group of cocci found in our study has many features in common with *Pediococcus halophilus* as described in Bergey's Manual (Buchanan & Gibbons, 1974), and similar organisms have been isolated from curing brines (Deibel & Niven, 1958;

Hornsey and Mallows, 1954, soy sauce (Sakaguchi, 1958; Ueno & Omota, 1961) and from fermented fish sauce (Crissan & Sands, 1975). The second type of coccus found in our study was able to reduce nitrate and could belong to the *Micrococcaceae*. Organisms from this genus are often isolated from very salty environments.

The existence of osmotolerant or osmophilic yeasts is well known (Tilbury, 1980). The latter have been isolated from barrel salted herring (Erichsen & Molin, 1964) and fermented fish sauce (Crisan & Sands, 1975).

The environment in the barrels is free of oxygen, but a large part of the microbial flora is able to reduce nitrate and TMAO and it is likely that they use these compounds as alternative electron acceptors in their anaerobic respiration. 'Nitrate respiration' is well known, and respiration by means of TMAO has recently been shown to several types of bacteria (Sakaguchi & Kawai, 1975; Strøm, Olafsen & Larsen, 1979; Easter, Gibson & Ward, 1982) and could well be possible also for these partly unknown halophiles. Not all the available TMAO is reduced during ripening, and there is no indication in our work of any correlation between TMAO and/or nitrate reduction and degree of ripening. In some barrels the fish were fully mature while practically all of the TMAO remained in the flesh.

The aerobic and microaerophilic flora was biochemically rather inactive. Only few organisms possess proteolytic enzymes (ability to liquefy gelatine) and none are able to attack the carbohydrate (sucrose) added to the barrels.

The majority of the herrings were able to ripen normally regardless of the number of microorganisms present and the composition of the microbial flora. It seems therefore likely that the ripening process is predominantly autolytic and that the microbial flora plays a minor role in the development of the characteristic odour.

The examination of spoiling or completely spoiled fish showed that at least two types of spoilage are caused by microbial action. The gram negative, obligate anaerobic halophilic rods isolated from type 1 spoiled fish are biochemically very active and can be classified as true 'spoilers'. Bergey's Manual (Buchanan & Gibbons, 1974) does not mention any organisms of a similar nature, but in an early work by Baumgartner (1937) an obligate, anaerobic, halophile organism (*Bacterioides halosmophilus*) was identified. However, this organism did not produce indole and H_2S with the methods applied in the work cited.

The gram negative spoiler isolated is an obligate anaerobe, and it does not seem to grow in a natural substrate (fish+brine) until this is depleted of compounds such as TMAO and nitrate (if added) by the growth of harmless aerobes. Consequently type 1 spoilage is first noted in fish being naturally low in TMAO, such as poor raw material or fish from areas with low salinity—such as seen in this study.

A second type of spoilage, characterized by development of fruity off odours, is caused by growth of osmotolerant yeast species. These yeasts are not affected by the presence of nitrate and TMAO, and it is possible, therefore, for type 2 spoilage to develop much earlier than type 1 spoilage.

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Ripening and spoilage of sugar salted herring with and without nitrate
II. Effect of nitrate

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Summary

The technological effect of nitrate and the possible role of this compound in the formation of volatile nitrosamines during ripening and spoilage of sugar salted herring have been examined. In total, 600 barrels of fish with various amounts of nitrate added to the curing salt were prepared and stored under commercial conditions. During storage (18 months) the organoleptic, microbiological and chemical changes were studied. Additionally, a number of model experiments were carried out to evaluate the effect of nitrate on microbial growth and chemical changes. It was found that addition of nitrate significantly influences the colour of the fish while texture and flavour are not affected. Based on both the practical experiments and the model studies it is concluded that nitrate *per se* does not inhibit any microbial growth. Growth of strict anaerobes does not occur until the substrate is depleted of TMAO, and nitrate is effective in delaying the most common type of spoilage by delaying the TMAO reduction.

Small amounts of nitrosodimethylamine—up to 2.9 ppb has been detected—was formed in the sugar salted herring, but the addition of nitrate to the curing salt had no influence.

Introduction

Traditionally nitrate has been included in the mixture of curing salt used in the production of sugar salted (barrel salted) fish in Scandinavia. The amount of nitrate used has been reduced over the years, and now the maximum amount allowed in Denmark is 500 mg KNO_3/kg fish corresponding to approx. 300 ppm NO_3^-.

Recently it has been shown that sugar-salted and pickled fish occasionally contain traces of carcinogenic volatile nitrosamines (Pedersen & Meyland, 1981). As it was believed that the use of nitrate was partly responsible, it was suggested that nitrate might be deleted from the list of permitted additives. Nitrate has been used for many years by the fish industry, and it is a practical experience that addition of nitrate is necessary in order to avoid spoilage and to obtain a correct (red) colour of the product. However, the specific technological effect has never been demonstrated in controlled experiments.

The present work was carried out to determine the effect of nitrate on the organoleptic properties (colour, texture and flavour) in the normal ripening process of sugar salted herring. Further, the ability of nitrate to prevent spoilage has been studied and the influence of nitrate on the microbial development both in the natural environment

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(the barrels) and in model experiments is demonstrated. Finally this paper also describes the possible role of nitrate in the formation of volatile nitrosamines during ripening and spoilage of sugar salted herring.

Materials and methods

Fish

Six hundred barrels of sugar salted herring were produced, using raw material of various origin and quality as previously described (Knøchel & Huss, 1984). The barrels contained 90–100 kg fish and a mixture of curing salt consisting of 16 kg salt, 6 kg sugar (sucrose) and spices. Of this material, 200 barrels had 50 g KNO_3 and 200 barrels had 25 g KNO_3 added to the curing salt while 200 barrels were prepared without addition of nitrate.

The experimental material was stored during ripening at 4–6°C for 18 months at two commercial plants. During this time the ripening was checked routinely by the factory quality control personnel. All barrels with off odours and flavours were sampled for laboratory analysis, while the normal material was sampled as indicated previously (Knøchel & Huss, 1984).

Organoleptic assessment

All samples of normal material were subjected to triangle tests, where nitrate fish were tested against nitrate-free fish. A number of fillets without skin from each group were presented to a panel of six experienced persons. Assessment of colour was carried out in daylight, while odour/flavour and texture were evaluated in red light.

The organoleptic assessment of samples with off odours and flavours was carried out as previously described (Knøchel & Huss, 1984).

Chemical analysis

Determination of nitrate and nitrite was carried out according to the ISO standard method (ISO 4099-1978 E). Volatile nitrosamines were determined essentially as described by Eisenbrand *et al.* (1972). All other chemical analyses were carried out as described by Knøchel and Huss (1984).

Eh measurements

Oxidation reduction potential (Eh) was measured using a pH-meter 28 (Radiometer) with a platinum electrode and a calomel reference electrode (Huss & Larsen, 1979). The electrodes were changed before each measurement and the Eh values read after approx. 1 hr, when they had become fairly stable.

Effect of nitrate on microbial growth

Twenty aerobically isolated strains from sugar salted herring were examined. Thirteen nitrate reducing microorganisms (twelve rods, one coccus), five non-reducing (four cocci, one rod) and two yeasts were inoculated into test tubes with Brain Heart Infusion (BHI) broth (Difco) with 15% NaCl (w/v) and supplemented with 0, 250, 500 and 750 ppm KNO_3, respectively. The tubes were incubated microaerobically (standing cultures) and anaerobically (in N_2 atmosphere) at 22°C.

Three obligate anaerobic rods isolated from a barrel with spoiling fish were likewise inoculated with 0, 250, 500, 750 and 1000 ppm KNO_3, respectively, into test tubes sealed with a paraffin layer.

Growth was measured as OD at 400 nm.

Effect of nitrate on the reduction of TMAO

Ten ml brine from a barrel with early signs of spoilage were inoculated into fermenting jars with BHI broth, 15% NaCl and 0 and 500 ppm KNO_3, respectively. pH was adjusted to 6.0 with a potassium phosphate buffer. Approximately 450 ppm TMAO (of own synthesis) was added after autoclaving. The jars were bubbled through with pure N_2.

Effect of nitrate and TMAO on physico-chemical conditions and corresponding microbial development

Ten ml brine from a barrel with fully ripe herrings were inoculated into a fermenting jar as described above with the addition of 500 ppm KNO_3. Aerobic and anaerobic viable counts were determined as described previously (Krøchel & Huss, 1984). On plates with between ten and 100 colonies all were examined for shape and catalase reaction.

Results

Organoleptic properties

A significant difference (95% level) in colour was seen in ten out of twenty-four triangle tests carried out (42%), and in all cases the panel agreed to the colour being more red in herring cured with addition of nitrate (Fig. 1). When evaluating flavour, the panellists were unable to distinguish between herring cured with or without addition of nitrate. A significant difference (95% level) in texture was noted on two occasions but results in these two tests were not in agreement with each other.

Off odours and flavours or failure to ripen normally were noted in twenty-five barrels. The majority of spoiled barrels (nineteen) had a sour, sour/sweet or putrid

Where produced	Experimental series	When produced	Criteria	Time for organoleptic examination (months after salting)																	
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Iceland	C	October	Colour					X					X			▨			▨		
			Odour/flavour					X					X			X			X		
			Texture					X					X			X			X		
	E	November	Colour			X							X			X			▨		
			Odour/flavour			X							X			X			X		
			Texture			X							X			X			X		
	G	November	Colour				X					X			X				▨		
			Odour/flavour				X					X			X				X		
			Texture				X					X			X				X		
Denmark	I	September	Colour					▨							X		■			▨	X
			Odour/flavour					X							X		X				
			Texture					X							X		X				▨
	IV	November	Colour				X						X		■				▨		
			Odour/flavour				X						X		X				X		
			Texture				X						X		X				X		
	IX	February	Colour	X						X		X					▨				
			Odour/flavour	X						X		X					X				
			Texture	X						X		▨					X				

Figure 1. Organoleptic examination (triangle test) of sugar salted herring prepared with and without addition of nitrate to the curing salt. All examinations are carried out in duplicate. X indicates that in both tests there was no significant difference between fish prepared with or without nitrate. Hatched area means that SD was seen in one test. Black area means that both tests showed a SD between the samples.

odour and flavour (type 1 spoilage), while four barrels were rejected due to characteristic fruity off odours and flavours (type 2 spoilage). The number of type 1 spoiled barrels were significantly lower when nitrate had been added to the curing salt as shown in Table 1, while all type 2 spoiled fish had 25 g nitrate/100 kg fish added to the curing salt.

Table 1. The significance of nitrate in preventing spoilage of sugar salted herring

	No. of barrels	Addition of nitrite in curing salt (g)		
		0	25	50
Type 1 spoilage	19	13	4	2
Type 2 spoilage	4	0	4	0
No maturation	2	0	0	2

Fate of nitrate and formation of volatile nitrosamines

Small amounts of nitrate were found in raw fish and in fish salted without nitrate (Table 2). In ripening and normally ripe fish, salted with 50 g of nitrate/100 kg, between 100 and 325 ppm of nitrate was measured in the fillet, and 200–600 ppm of nitrate was found in the brine.

Table 2. Nitrate (ppm) and nitrite (ppm) in sugar salted herring produced with and without addition of nitrate in the curing salt

	Raw fish	Ripening and fully ripe fish			Type 1 spoiled fish			Type 2 spoiled fish		
KNO_3 in curing salt (g/100 kg fish)		0	25	50	0	25	50	0	25	50
Nitrate in						11, 82	13, 15			
fish	0–5	0–20	NT	100–325	0–12	0, 16			120–150	
brine		0	NT	200–600	NT	NT	NT		NT	
Nitrite										
fish	0	0	NT	0–10	0–3	0	3		3	
brine		0	NT	0	NT	NT	NT		NT	
No. of samples	6	48	0	48	13	4	2	0	4	0

NT: not tested.

In type 1 spoiled fish only small amounts of nitrate were found, even if the fish had originally been salted with addition of 25 g or 50 g nitrate/100 kg fish. In contrast, 120–150 ppm of nitrate could be found in type 2 spoiled fish originally prepared with 25 g nitrate/100 kg fish.

Only small amounts of nitrite (< 10 ppm) were found in a few samples of fish.

Analyses for nitrosodimethylamine (NDMA) were done regularly during the ripening period. However, statistically the influence of the time factor was not significant, and all results have therefore been pooled in Table 3. NDMA could not be detected in raw fish but it was found in eighty-seven out of 106 samples of ripening and

ripe fish. Mean value of apparent NDMA of nitrate fish was 0.8 ppb, while that of nitrate-free fish was 0.7 ppb. In five barrels of spoiled fish prepared with nitrate the mean value of apparent NDMA was 1.3 ppb, while NDMA could not be detected in two samples of nitrate-free spoiled fish.

Table 3. Volatile nitrosamines (nitrosodimethylamine, NDMA (μg/kg) in sugar salted herring prepared with and without addition of nitrate to the curing salt

	Raw fish	Barrels with ripening fish				Barrels with spoiled fish (type 1+2)	
Nitrate in curing salt		+	+	−	−	+	−
		Fish	Brine	Fish	Brine		
No. of samples	3	24	24	24	24	5	2
Apparent NDMA (mean)÷	BD*	0.8	0.7	0.7	0.4	1.3	BD

*Below detection level (0.2 μg/kg).
÷Standard deviation on all calculations is 0.5 μg/kg.

The effect of nitrate on microbial growth

A total of 633 strains, isolated from ripening and ripe sugar salted herring were tested for their ability to reduce nitrate. After a few weeks of storage the great majority of strains isolated from the fish were able to reduce nitrate as shown in Table 4. This applied whether or not the fish were cured with addition of nitrate.

Table 4. Number of nitrate reducing microorganisms in barrel salted herring prepared with and without addition of nitrate to the curing salt (No. of NO_3 reducers/No. of organisms examined)

	Weeks of storage					
Curing salt	1–12	13–26	27–39	40–52	53–65	66–78
With nitrate	18/34 (53%)	63/68 (93%)	19/27 (70%)	88/96 (92%)	34/53 (64%)	25/53 (46%)
Without nitrate	7/28 (25%)	55/65 (85%)		98/104 (94%)	48/61 (79%)	32/42 (76%)

Twenty isolates, representing the most commonly occurring aerobic microorganisms, were examined for microaerobic and anaerobic growth in the presence of varying levels of nitrate. None of the isolates was inhibited by nitrate in the concentrations examined (up to 750 ppm). In contrast, all microorganisms able to reduce nitrate (twelve gram negative rods and one micrococcus) were greatly stimulated and unable to grow anaerobically without the addition of nitrate (Fig. 2).

Ten obligate anaerobic gram negative rods isolated from spoiling sugar salted herring were examined for anaerobic growth in the presence of 0–1000 ppm of nitrate. No inhibition due to nitrate addition was recorded.

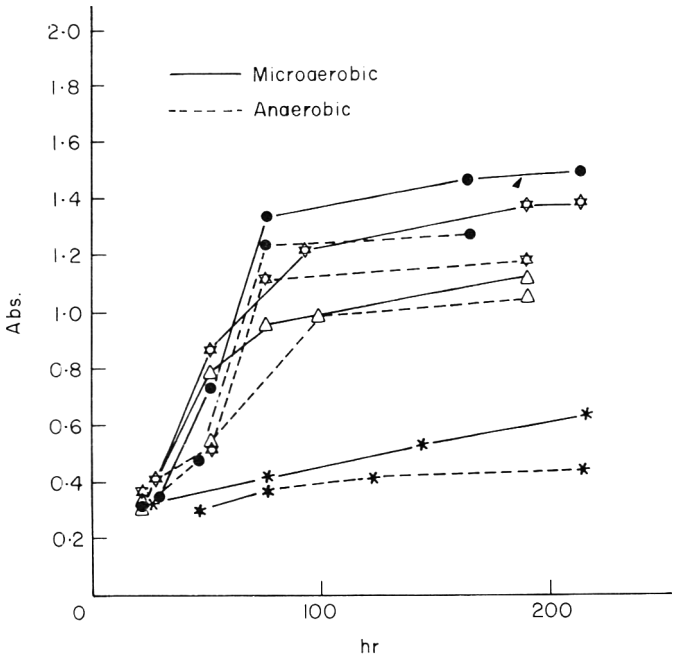

Figure 2. Microaerobic and anaerobic growth in the presence of various amounts of nitrate, of an aerobic organism isolated from sugar salted herring. *, 0 ppm; △, 250 ppm; ✡, 500 ppm and ●, 750 ppm KNO_3.

The effect of nitrate on the reduction of TMAO

The chemical changes taking place in two fermenting jars prepared with and without nitrate and inoculated with 10 ml brine containing a mixed flora from barrel salted herring are shown in Fig. 3. It shows that reduction of TMAO occurred much more rapidly in the jar without any nitrate in the growth medium. A great increase in TVB was seen when the medium was depleted of TMAO.

The effect of nitrate and TMAO on the physico-chemical conditions and the corresponding microbial developments

In this experiment the fermenting jar was prepared both with nitrate and TMAO and thereafter inoculated with a mixed culture (10 ml brine) from barrel salted herring. Results in Fig 4 show that during the first 4 days the microflora in the chemostat was completely dominated by aerobic rods. After approximately 4 days the nitrate started to disappear and shortly afterwards the TMAO was also decreasing. During this period (4–7 days) the aerobic and anaerobic counts were of the same order, and the microflora consisted mainly of microaerophilic cocci. When the medium was completely depleted of nitrate and TMAO (day 7) the Eh decreased to near −350 mV and the microflora changed to be mainly anaerobic and consisted of gram negative rods only.

Discussion

It is well documented that nitrate, serving as a reservoir for nitrite and nitric oxide, is able to stabilize the red colour of meat by formation of nitrosylmyoglobin (and nitrosyl-

haemoglobin) (Dryden & Birdsall, 1980). Accepting that similar reactions are taking place in nitrate-cured fish the added nitrate must be reduced to nitrite and nitric oxide before any reactions are taking place. A large part of the microflora in barrel salted fish is able to reduce nitrate, and decreasing levels of nitrate are recorded in the fish. However, due to our sampling procedures and the unknown proportion between amounts of fish and brine in the barrels, it is not possible to produce a mass balance for nitrate. No accumulation of nitrite was seen in fish or brine which indicates that any formed nitrate is rapidly further reduced. This is confirmed in our model experiments (Fig. 3). In our work a significant difference in colour was not seen regularly until after

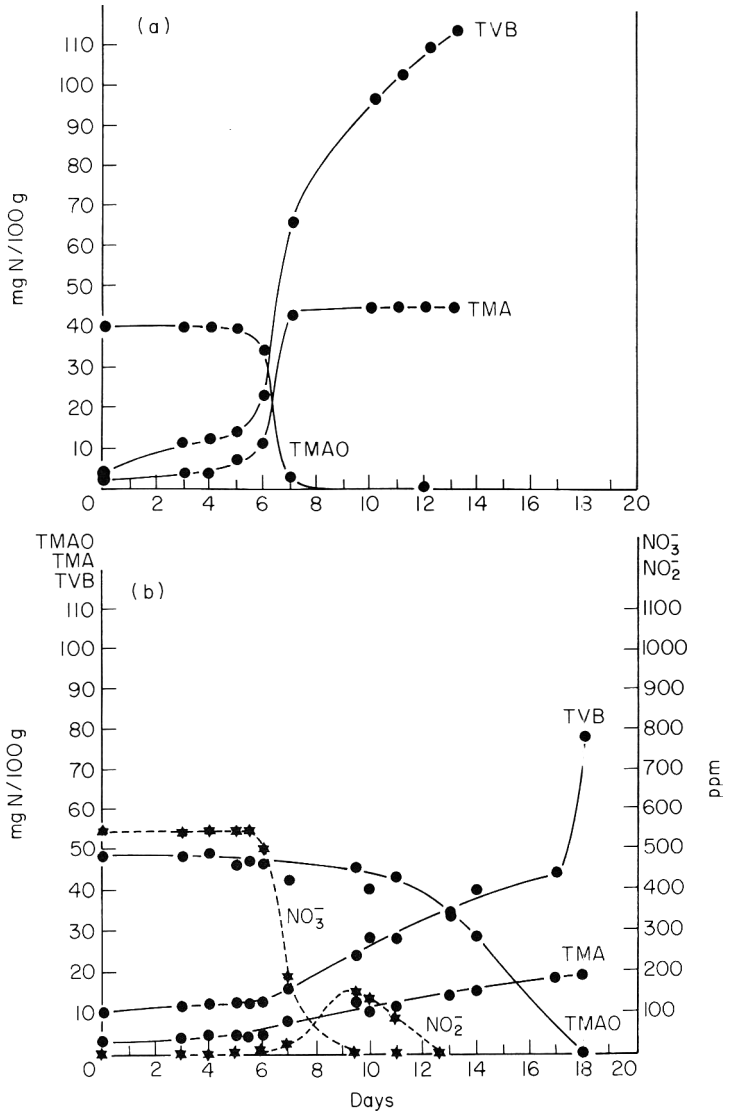

Figure 3. The reduction of trimethylamine oxide (TMAO) by a mixed microbial population (10 ml of brine from barrels with sugar salted herring) (a) without and (b) with nitrate added to the growth medium.

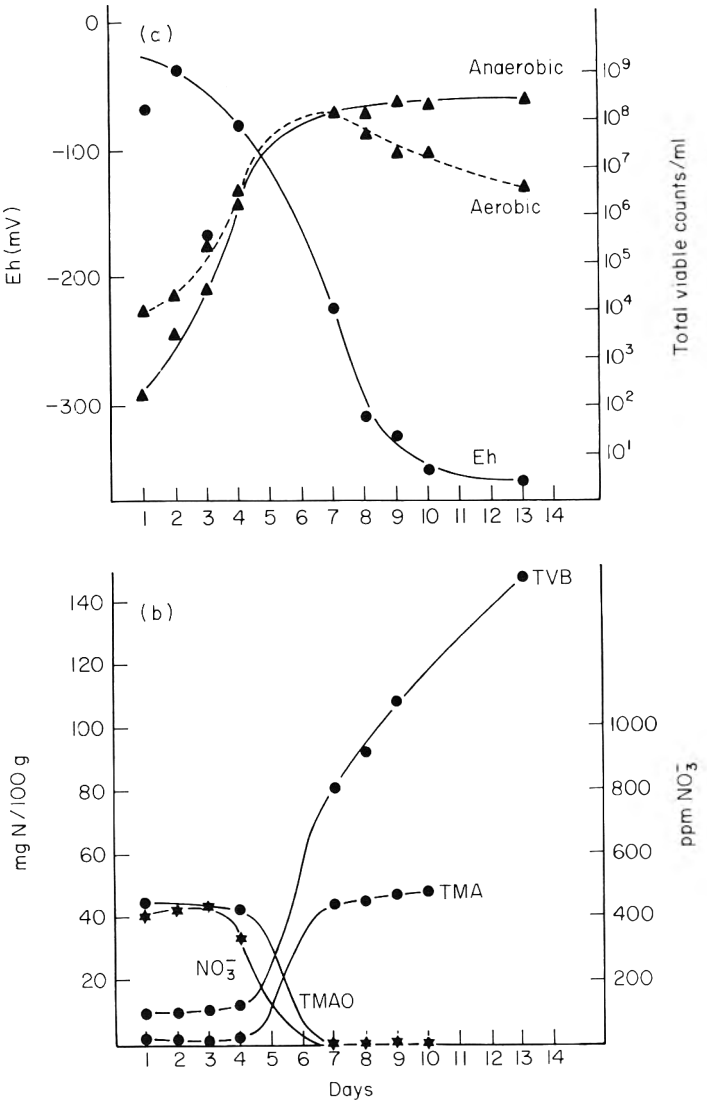

Figure 4. Relationship between microbial development, reduction of nitrate and TMAO and changes in the oxidation reduction potential (Eh) in a chemostat inoculated with a mixed flora (10 ml brine from barrels with sugar salted herring).

approximately 1 year of storage. The reason for this could be that it takes a long time before sufficient nitric oxide is formed or accumulated.

At the same time, the pH of the fish flesh has decreased from originally 6.3–6.4 to 5.7–5.8 (Knøchel & Huss, 1984). If some pH-dependent factor is involved in the colour fixing process this would be another reason for the late reaction.

In our work there is no indication of nitrate or breakdown products of nitrate playing any role in the normal ripening process or in imparting a specific flavour to the fish in the same way as is known for meat products (Gray *et al.*, 1981).

The effect of nitrate *per se* on the microbial development seems to be one of stimulation rather than inhibition. A large part of the microflora in the barrel salted

herring is able to reduce nitrate and our results (Fig. 2) strongly indicate that the organisms tested use nitrate in an anaerobic respiration as is known for a number of bacteria. In spite of this stimulating effect, the addition of nitrate to the curing salt does not influence the composition of the microflora in the barrels. The reason for this may be that fish flesh contains TMAO which is also a strong oxidizing compound and able to stimulate growth of aerobic or facultative anaerobic microorganisms under anaerobic conditions (Strøm, Olafsen & Larsen, 1979; Easter, Gibson & Ward, 1981).

Both in the natural environment (barrel salted herring) and in the model studies it is demonstrated that the presence of nitrate delays the reduction of TMAO. Not until the medium is also depleted of TMAO are conditions favourable for growth of obligate anaerobic organisms capable of causing (type 1) spoilage of the fish (Knøchel & Huss, 1984). In this way, addition of nitrate to the curing salt has a technological effect in preventing or delaying the most common type of spoilage. This effect is most pronounced if the fish is low in naturally occurring TMAO such as poor quality raw material or fish from areas with low salinity such as seen in this study.

The level of nitrosodimethylamine (NDMA) in sugar salted herring found in this work is low, and there is no significant effect of nitrate added to the curing salt. Our results are comparable to those of Pederson & Meyland (1981), who found up to 2.2 μg NDMA/kg pickled fish. Key *et al.* (1982) examined nine types of fish, including three smoked products, and found that the NDMA concentrations in the raw fish ranged from < 0.1 to 1.0 μg. After cooking by gas the levels were 0.2–2.0 μg/kg. Much higher amounts (up to 55.8 μg/kg) were found in hot smoked fish (Röper, Heyns & Gunther, 1981).

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Influence of gamma radiation on the rheological and functional properties of bread wheats

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Summary

The effects of gamma irradiation on some biochemical, rheological and functional properties of bread wheats were studied. Two wheat cultivars were selected to represent medium-strong and weak dough mixing strengths. Falling number values were severely depressed at doses of 500 and 1000 krad. Rheological dough properties, as assessed with the mixograph and farinograph, were also investigated. Radiation at medium doses produced an increase in the farinograph water absorption for both wheats. Radiation decreased the amount of bound water as compared to the control sample. For the medium-strong wheat low levels of radiation produced bread with volumes and overall bread quality equal to or slightly better than those of the control flour, whereas for the weak wheat an improvement of the baking performance was obtained at all the low doses of radiation. However, the overall bread quality of both wheats was highly reduced at medium doses of radiation.

Introduction

Research carried out in several countries over the last four decades has shown the technological feasibility of using various types of radiation to preserve cereal grains and reduce post-harvest losses caused by insect pests and by microbial decomposition (Lorenz, 1975; Pape, 1973). Radiation is also used for producing biochemical, physicochemical and functional changes to enhance the quality of grain products (Giddings & Welt, 1982; Rao *et al.*, 1978; Srinivas *et al.*, 1972). The need for irradiation of grain for insect disinfestation could be increased in the near future in view of the pressure to curb or ban the use of chemicals that leave residues of various levels of toxicity. Moreover, with recent international recognition of the safety of radiation, the commercialization of this technology might be greatly increased in the near future (Giddings & Welt, 1982).

At present there is controversy in the literature about the irradiation effects at low and medium doses (up to 100 and between 100 and 1000 krad, respectively), on the quality of grain products and especially on the physicochemical, technological and functional properties of bread wheats. MacArthur & D'Appolonia (1982) have found that γ radiation reduces the power consumption for wheat milling. Pape (1973) sustains that no objection can be found to the bread quality made from irradiated wheat. Rao *et al.* (1978) report that at dose levels up to 1000 krad there is an increase of reducing

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sugars and up to 200 krad the loaf volume is significantly improved. Similar results were found by Lai, Finney & Milner (1959). On the other hand, other workers (MacArthur & D'Appolonia, 1982; Miller *et al.*, 1965) have reported that in bread from wheat and flour disinfested with low and medium doses the organoleptic quality deteriorates. In view of this situation, we decided to conduct research work to observe the effects of γ radiation on the rheological and functional properties of two important wheat varieties in the Mexican market of medium–high and weak dough mixing strength. In this investigation low to medium doses of γ radiation were applied.

Materials and methods

Wheat samples and irradiation

The two wheat cultivars Tesia and Salamanca were selected on the basis of their importance in the local wheat market. Wheat samples (1000 g) were packed in polyethylene bags, heat sealed and irradiated in a ^{60}Co γ cell 222 (Atomic Energy of Canada, Ltd) having flux of 5.8 krad/min at 0.5–1000 krad levels.

Tesia and Salamanca were milled on the Brabender Quadrumat Junior mill. Flour extraction was 70.2 and 67.9% for Tesia and Salamanca, respectively. Tesia flour had a protein content of 8.9% (14% mb) and had medium–strong dough mixing characteristics as assessed with the farinograph. Salmanca flour presented a protein content of 10.8% (14% mb) and weak strength mixing characteristics. For some determinations whole wheat flour was obtained; wheat was ground on a cyclone mill (Udy Analyzer Co.) to pass through an 80-mesh screen. All ground samples were kept in desiccators at 4°C and removed as required for analyses.

Rheological tests and bound water determination

The falling number was determined on 7 g of ground whole wheat with a type Faling Number apparatus, using AACC method 56–81B (AACC, 1976). The equation of Lorenz & Wolt (1981) was used to calculate falling numbers at sea level.

The 35 g mixogram procedure was used according to AACC method 54–40 (AACC, 1976). Farinograms were produced in a Brabender farinograph (method 54–20: AACC, 1976). The amount of bound water was estimated using the monolayer BET method (Caurie, 1982; Karel, 1976).

Baking test

Breads were prepared by a sponge dough procedure of the Instituto Nacional de Investigaciones Agrícolas (Castilla-Chacón, 1982). Baking was done at 220°C for 25 min. The resulting bread was scored subjectively in a similar way as reported by Paredes-López & Bushuk (1983). Crumb colour indicates visual brightness; the higher the number, the brighter the crumb. Letters are added to indicate other features such as yellow (y), cream (c) and brown (b). For crumb texture, the numerical value relates to the thickness of the cell walls; the thinner the wall, the higher the score. General appearance of breads was graded as not satisfactory (ns), satisfactory (s) and very satisfactory (vs). General appearance was classified as: ns if crumb colour and texture was graded with ≤ 8; s if graded > 8 to > 10; and vs if graded with 10.

Results and discussion

Table 1 shows the results obtained from the falling number determination. Falling number values ranged from 384 to 62 sec for Tesia wheat and from 395 to 68 sec for Salamanca wheat. Radiation treatments presented remarkable decreases in falling number at doses of 500 to 1000 krad. Alpha and β amylases are reported to be resistant to γ radiation, either in free or bound form (Deschreider, 1963). Rao *et al.* (1978) found remarkable increases of reducing sugars in wheat flour at radiation doses higher than 200 krad. Hence, the observed differences in falling number values may arise from an increased susceptibility of starch to enzyme activity. These findings are in disagreement with the results of MacArthur & D'Appolonia (1981). These workers did not obtain significant differences of reducing sugars with radiation. In order to explain the dramatic changes of falling numbers, it would be interesting in a future work to measure α amylase activity and levels of reducing sugars at the various doses of radiation. Table 1 also presents falling number values corrected at sea level with the equation of Lorenz & Wolt (1981). As observed, there is a general decrease for the falling numbers at sea level, and there is also a deviation from this equation at the minimum values of falling numbers. Lorenz & Wolt (1981) found that falling numbers increased with increasing elevation because water has a lower boiling point under reduced atmospheric pressures. They postulated that lower water bath temperatures and, therefore, lower flour suspension temperatures, were outside the optimal activity range of wheat α amylase and therefore produced higher falling numbers.

Table 1. Changes of falling number values at different levels of radiation

Dose level (krad)	Falling number (sec)			
	Tesia		Salamanca	
	A	B	A	B
0	384 ± 5.5	283	395 ± 2.0	288
0.5	390 ± 18.5	285	382 ± 0.5	282
5	370 ± 8.5	276	377 ± 3.0	280
10	376 ± 17.5	280	360 ± 12.0	272
25	360 ± 15.5	272	371 ± 16.9	277
50	341 ± 6.0	263	330 ± 5.9	257
70	336 ± 12.0	261	327 ± 5.0	256
100	327 ± 6.0	256	326 ± 4.0	256
200	284 ± 5.0	233	282 ± 2.0	232
500	120 ± 18.6	90	90 ± 0.5	40
1000	62 ± 0.0	−21	68 ± 0.0	−5

A = Falling numbers at 1760 m of altitude.
B = Falling numbers reported at sea level using the equation of Lorenz & Wolt (1981).

The influence of radiation on the rheological behaviour of some of the doughs under study, as assessed with the mixograph, can be seen in Table 2 for Tesia and for Salamanca wheats. Data presented here revealed that peak time for both wheats, as compared to the control sample, reached the lowest value at 1000 krad. The only defined trend presented by the mixograms was a reduction of the graph width,

Table 2. Peak time from the mixograph experiments

Dose level (krad)	Peak time (min)	
	Tesia	Salamanca
0	2.5	2.5
0.5	3.0	2.0
5	2.5	2.5
10	3.5	2.5
25	3.5	2.25
50	2.5	2.5
70	2.5	2.25
100	2.25	2.25
200	2.25	2.25
500	2.75	2.5
1000	2.0	1.75

especially at higher doses of radiation (not shown here). This effect was more evident in the case of the stronger wheat (Tesia). These results seem to agree with earlier investigations (Ananthanswamy, Vakil & Sreenivasan, 1971; Deschreider, 1968) that significant breakdown of gluten proteins appears to take place at doses of radiation of 1000 krad or higher.

Mixing properties of wheat flour, water absorption and dough development time were measured in a farinograph (Table 3). For Tesia, the water absorption of the control sample was 62% (14% mb). There were only very slight changes in water absorption at radiation levels up to 200 krad (62–62.8%); however, at 1000 krad absorption reached the value of 66.6%. Again, for Salamanca the absorption reached the highest level (57%) at 1000 krad. Pape (1973) and Srinivas *et al.* (1972) have also found that irradiation treatment increased the farinograph water absorption. At the radiation doses used, there were some slight to medium changes for both wheats in

Table 3. Farinograph data of irradiated wheat

Dose level (krad)	Water absorption (%)*		DDT (min)		Mixing stability (min)	
	T	S	T	S	T	S
0	62.0	55.2	2.0	1.0	3.5	1.0
0.5	62.6	53.8	1.5	1.0	4.5	2.0
5	62.8	55.0	1.5	1.5	4.5	2.0
10	62.6	54.6	1.5	1.0	1.5	2.0
25	62.4	55.2	2.0	2.0	3.0	1.0
50	62.5	55.3	2.5	1.0	3.0	2.0
70	62.6	56.6	1.5	1.5	3.0	1.0
100	62.2	56.2	1.5	1.5	4.5	1.0
200	62.6	56.8	1.5	1.0	4.5	1.0
500	63.6	57.0	1.5	1.0	2.5	1.0
1000	66.6	57.0	1.5	1.0	2.5	0.5

*At 14% mb.

DDT = Dough development time; T = Tesia; S = Salamanca.

Table 4. Effect of irradiation on baking properties of wheat*

Dose level (krad)	Loaf volume (cc)		Crumb colour		Crumb texture		General appearance	
	T	S	T	S	T	S	T	S
0	670	675	9 y–c	8 y	8	6	s	ns
0.5	675	850	9 y–c	9 c	8	8	s	s
5	670	800	9 y–c	10 c	8	9	s	s
10	695	800	9 y–c	10 c	8	9	s	s
25	685	800	9 y–c	10 c	8	9	s	s
50	690	850	9 y–c	10 c	8	10	s	vs
70	685	800	9 y–c	10 c	8	9	s	s
100	685	805	8 y	10 c	8	9	s	s
200	680	805	8 y	10 c	8	9	s	s
500	650	710	7 y	7 y	7	7	ns	ns
1000	610	670	6 b	6 b	6	6	ns	ns

*Key for sensory evaluations: y = yellow; c = cream; b = brown; ns = not satisfactory; s = satisfactory; vs = very satisfactory.

T = Tesia; S = Salamanca.

terms of dough development time and mixing stability. It is interesting to note that a change in the farinograph curve pattern was observed for all levels of radiation; the farinograph curve presented a second peak of development. MacArthur & D'Appolonia (1981, 1982) have also observed this same effect in microwave irradiated wheat samples.

In view of the changes in water holding capacity of irradiated wheat found in this study, and results reported earlier by Pape (1973) and Srinivas *et al.* (1972), we decided to determine the influence of radiation on the level of bound water for some of the samples under study. It was found that γ radiation decreased the monolayer value for water (or bound water) in both wheats; this parameter was reduced from 9.6×10^{-2} in the control sample of Tesia to 8.1×10^{-2} and 8.0×10^{-2} g H_2O/g solids in the irradiated sample of 50 and 500 krad, respectively. Same samples from Salamanca wheat flour exhibited a pattern closely related to that of Tesia. It might well be that these changes in water distribution were highly responsible for the rheological and functional behaviour of irradiated wheat samples; this aspect requires further studies.

The radiation treatments produced some important changes in the baking properties of the Tesia and Salamanca wheats (Table 4). With radiation doses used up to 200 krad, loaf volume of Tesia remained about the same as, or improved slightly over, that of the control; for all these doses of radiation, general appearance of breads was graded as satisfactory. On the other hand, with low levels of irradiation Salamanca showed some improvement in its baking performance; the overall bread quality of the control graded as not satisfactory was changed to either satisfactory or very satisfactory. For both wheats, radiation doses of 500 and 1000 krad reduced in general the loaf quality (Figs 1 and 2).

Ionizing radiation processing of wheat could provide an alternative to current disinfestation and fumigation practices which use potentially hazardous and toxic chemicals. It seems to be safe, versatile, low energy method which can reduce post-harvest losses and improve wheat preservation by reducing or eliminating pathogenes and other undesirable contaminants (IFT, 1983; Willis, 1982). The cost of radiation

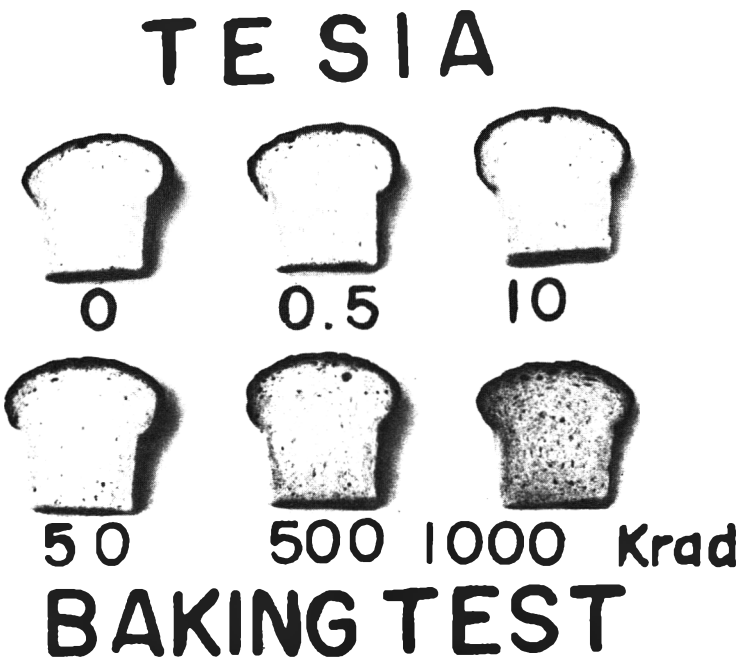

Figure 1. Effect of various levels of radiation on the breadmaking properties of Tesia wheat.

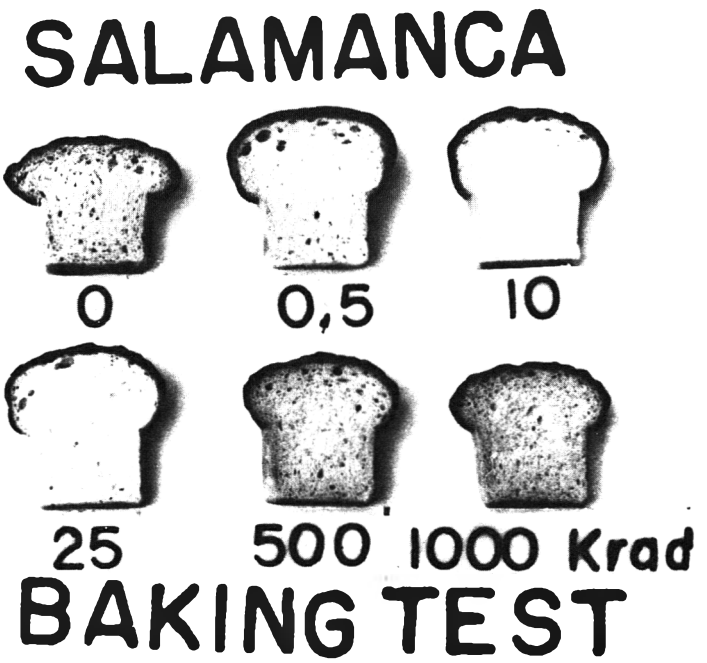

Figure 2. Effect of various levels of radiation on the breadmaking properties of Salamanca wheat.

processing must be competitive with the cost of alternate technologies if it is to be accepted in the marketplace. As it was observed in this study, radiation treatment of wheat brings about some rheological and functional changes which depend, to a certain extent, on the mixing strength of the wheat flour. At low levels of radiation the baking properties of the weak wheat were always improved as compared to the stronger variety. It needs to be pointed out that radiation doses require to be adjusted according to each wheat cultivar and intended food uses of each particular material. It is most likely that the rheological and functional modifications observed in irradiated wheat are produced by the combined effects of changes in water distribution, as it was found here, in conjunction with hydrolysis and changes in interactions of proteins, starch and lipids.

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Protein isolates rich in methionine from the edible dry bean (*Phaseolus vulgaris* L.)

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Summary

Several extraction and fractionation schemes were investigated for their ability to fractionate edible dry bean seed proteins into classes of variable methionine content. Isoelectric precipitation or dialysis from a protein solution of low ionic strength resulted in protein isolates having methionine content equal to or lower than the average seed methionine. A protein isolate rich in methionine was obtained by the adjustment of a high ionic strength protein solution at pH 4.1. This methodology excluded the major storage seed proteins, which are known for their low methionine content.

Introduction

Edible dry beans are a common food staple in many parts of the world. Their nutritional limitations are mainly due to their deficiency in methionine, their reduced digestibility and the presence of anti-nutritional factors (Jaffe, 1973; Liener, 1979). The methionine content of edible dry beans estimated as methionine sulphone has been found to range from 1.1 to 1.7% (Tobin & Carpenter, 1978) and 1.6 to 2.2% (Herrick, Lawrence & Coahran, 1972). An average of 1.4% methionine has been found by this investigator, by gas liquid chromatography (glc), for eighty-two improved edible dry bean cultivars and breeding lines (Apostolatos, 1980).

These values may be contrasted with the methionine content of the main storage proteins of edible dry beans, vicilin and legumin, accounting approximately for 70% of the total seed protein, which have a calculated methionine content of approximately 0.8% (Derbyshire, Wright & Boulter, 1976). It is clear that other proteins must contribute significantly to the observed methionine content and must be appreciably higher in that compartment than that of the major storage proteins.

This study examines the possibility that one or more protein fraction rich in methionine exists in beans. The presence of such protein fraction(s) could then account for the observed methionine difference between the total seed methionine and that accounted for the major storage proteins.

Materials and methods

Plant material

Two cultivars of edible dry bean 'Cuva 168-N' and 'Star' selected for their significant protein and methionine content were used in this study. Freshly harvested dried seeds were ground to pass through an 80-mesh sieve. The meal was defatted by 5 vol. of petroleum ether (3x), subsequently dried under vacuum and stored in stoppered glass vials.

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Protein extraction

Proteins were extracted by stirring 5 g of defatted seed meal in 50 ml extractant for 30 min at room temperature. The suspension was centrifuged (2000 × *g*, 5 min) and the residue reextracted with 25 ml extractant and centrifuged. The combined supernatant liquids were further centrifuged at 25000 × *g* for 30 min at 4°C. Clarified protein extract obtained from 0.1 M phosphate buffer pH 7.5, containing 0.5 M NaCl, was adjusted to pH 4.1 by 3 N HCl. Similar protein extracts were exhaustively dialysed at 4°C against acidic buffers. Protein isolates recovered either as supernatant liquids or precipitates, were further purified by ammonium sulphate precipitation, desalted by passing through a Sephadex G-25 column, freeze dried and stored at − 18°C.

Chemical analyses

Nitrogen content in the crude seed meal and in the protein isolates was determined by a modified Berthelot assay following sulphuric acid digestion of the samples (Apostolatos, 1980). Methionine was determined after cyanogen bromide digestion of the samples and quantitation of released methylthiocyanate by g.l.c. (Apostolatos & Hoff, 1981). Acid hydrolysates of the crude protein extract and methionine-rich isolates were analysed on an amino acid analyzer equipped with an automatic integrator. Methionine and cysteine content in the samples were determined as methionine sulphone and cysteic acid respectively, after performic acid oxidation of the samples and subsequent acid hydrolysis.

Effects of pH on nitrogen solubility

Nitrogen solubility curves were determined by extracting 5 g of defatted seed meal with either sodium phosphate buffer or distilled water. In each case the clarified extract was adjusted to pH values from 2.0 to 11.0 by addition of either 0.5 N HCl or 0.5 M NaOH. Nitrogen content in the supernatant and precipitated fractions were determined.

Results and discussion

A serious problem that is encountered in quantitative analyses is that extraction is usually incomplete and that preparative losses are usually significant. Of various extractants tested (Table 1), nearly quantitative extraction of seed nitrogen was

Table 1. Protein extractability from edible dry bean seed meal

Extractant	Extracted protein % of the total (N × 6.25)
Distilled water	49.5
Distilled water (0.5 M NaCl)	60.5
Phosphate buffer (0.1 M, pH 7.5)	69.0
Phosphate buffer (0.5 M, pH 7.5)	72.5
Phosphate buffer (0.1 M, pH 7.5, 0.5 M NaCl)	88.0
NaOH solution (10 mM)	95.0
0.1 M acetic acid	20.0
0.25 M ascorbic acid (0.5% HCl (v/v))	21.0
0.8 M trichloroacetic acid	8.0
70% EtOH (v/v) (0.5% HCl (v/v))	6.0

Average values of triplicated extractions.

obtained only by sodium hydroxide solution. However, partial protein denaturation was evident as protein dialysates were insoluble in neutral buffers of 0.5 μ ionic strength and produced excessive streaking when analysed by polyacrylamide gel electrophoresis. Neutral to slightly alkaline buffer solutions gave variable results, depending upon the ionic strength of the solution. Acidic buffer solutions extracted only a small portion of seed nitrogen. Finally, acidified ethanolic solution and solution of 0.8 M tricholoracetic acid were used for estimation of non-protein nitrogen.

There is a broad pH range (pH 3.0–6.0) in which protein precipitation occurs, but the relative amounts of precipitation protein varied with the ionic strength of the extract as well as with the protein concentration in the extract. Maximal protein precipitation occurred in the pH range 3.0–3.5; at this range the relative percentages of protein remaining in solution were 21, 30 and 67% for extracts obtained with distilled water and phosphate buffers of 0.1 and 0.5 M respectively. Polyphenolic substances present in the hulls of bean were responsible for coloured protein extracts, but did not affect the protein precipitation profiles; the colour disappeared at acidic pH values, but it came back when protein solutions were adjusted to neutral and alkaline pH values (see Fig. 1).

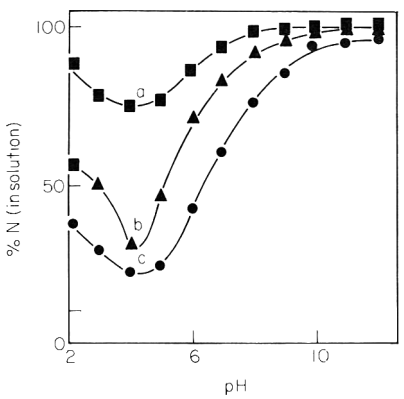

Figure 1. Nitrogen precipitation curves of edible dry bean protein extracted with: (a) sodium phosphate buffer 0.1 M, pH 7.5 containing 0.5 M NaCl; (b) the same buffer system without NaCl; (c) distilled water. Protein concentration in the extract ranged from 7 to 14 mg/ml.

Several fractionation schemes were investigated for their ability to fractionate proteins into classes of variable methionine content. The phosphate buffer (0.1 M, containing 0.5 M NaCl) protein extract was adjusted to pH 4.1; the precipitated protein was found to be rich in methionine (Table 2). The protein fraction remaining in solution at this pH contained the major globulin-type storage proteins, as well as, the acid-soluble albumins. Variable results were obtained for both protein and methionine content, when this phosphate extract was dialysed against acetate buffers pH 5.6 and 4.1, as well as against 0.1 M acetic acid pH 3.0 and distilled water. Above the average seed methionine content was found in the supernant fractions of these dialysates.

Amino acid analysis of the methionine-rich protein isolates is given in Table 3. These isolates are rich in methionine, lysine and histidine, while the considerable amount of aspartic and glutamic acid present, are characteristic of seed storage proteins. However, the rather low cysteine content is responsible for the lower than the proposed by FAO sulphur amino acid content in these isolates.

Table 2. Protein and methionine content of various isolates from edible dry bean seed

Phosphate buffer protein extract (0.1 M, pH 7.5, 0.5 M NaCl)		'Cuva' % protein	'Cuva' % met.	'Star' % protein	'Star' % met.
Acidification at pH 4.1	*S*	68	1.2	65	1.1
	P	17	2.4	20	2.3
Dialysate against buffer (0.1 M, pH 5.6)	*S*	45	1.4	48	1.3
	P	40	1.6	37	1.5
Dialysate against buffer (0.1 M, pH 4.1)	*S*	20	1.8	23	1.7
	P	65	1.4	62	1.3
Dialysate against acetic acid (0.1 M, pH 3.0)	*S*	15	1.8	17	1.8
	P	70	1.4	68	1.3
Dialysate against distilled water	*S*	20	1.8	20	1.7
	P	65	1.4	65	1.3

S: supernatant; *P*: precipitate.
Percentages are given on dry basis of total extracted protein.

Table 3. Amino acid composition of total bean protein extract (TBPE) as well as, of methionine-rich protein fraction (MRF)

	'Star'		'Cuva'	
	TBPE	MRF	TBPE	MRF
Asp	11.7	12.5	12.2	12.0
Thr	4.2	3.8	4.5	4.0
Ser	5.6	4.6	5.4	4.8
Glu	16.0	14.8	15.5	15.0
Pro	5.2	5.0	4.9	5.4
Gly	4.6	4.0	4.5	4.2
Ala	6.0	5.8	6.2	5.9
1/2 Cys	0.9	1.0	1.2	1.0
Val	6.1	6.3	6.4	6.5
Met	1.5	2.2	1.6	2.3
Ile	4.2	4.0	6.0	4.5
Leu	6.1	6.5	5.5	6.4
Tyr	3.5	3.2	3.9	3.8
Phe	6.4	5.8	5.4	5.2
His	2.7	2.9	2.5	2.6
Lys	7.6	7.6	7.4	6.8
Arg	7.5	7.0	7.4	6.8
NH_3	2.0	1.8	1.8	1.5

It is significant that the fractionation methods described (Table 2) resulted in segregation of methionine-rich proteins. Such segregation does not occur when employing common extraction and fractionation methods, involving isoelectric precipitation or precipitation by dialysis from a salt protein solution. Under the latter circumstances, the methionine-rich proteins evidently coprecipitate or aggregate with the proteins low in methionine.

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Lipids in French fries: a retail and laboratory study

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Summary

The lipid content of prepared restaurant and takeaway French fries varied from 6.2 to 20.8 g/100 g, and of frozen retail fries from 2.1 to 5.3 g/100 g, as purchased, and from 12.0 to 20.0 g/100 g after finish frying. The lipid composition (fatty acid profile and cholesterol content) of all retail samples reflected the predominant use of animal fat in the Australian industry. Laboratory studies showed that the lipid content of French fries was increased by decreased chip size, and by the common commercial practice of split frying which caused a doubling of fat uptake when compared with single frying. Commercial practices appear to increase the potential of fries as a fat carrier.

Introduction

In Australia, where food habits are largely based on the British pattern, potato consumption presently stands at 55.2 kg/capita/year (Australian Bureau of Statistics, 1982) and increased consumption is being encouraged (Commonwealth Department of Health, 1981). However, the most popular form of potatoes is French fries, deep fat fried potato strips, which are sold ubiquitously as a snack or takeaway food, and these are recognized as a significant source of fat in the Australian diet. As in many other industrialized countries, concern has been expressed about the relationship of excess fat consumption to degenerative disease, and the national dietary goals (Commonwealth Department Health, 1981) contain a recommendation to reduce total fat consumption by, among other things, avoiding fried foods, including French fries.

The common commercial practice of French fries preparation is to split the frying process into two phases. Par frying partially cooks the potato and prevents undesirable metabolic changes from occurring, while finish frying, which takes place at the point of consumption many hours or even days later completes the cooking process. This split frying process has been used partly for sensory reasons since it produces fries of good quality, but also for reasons of convenience since frying time at the point of purchase is greatly reduced (Nonaka, Sayre & Weaver, 1977).

This study reports on the levels and types of lipid used in the preparation of French fries sold from a range of commercial outlets in Sydney, Australia. Laboratory trials were also carried out to investigate whether lipid levels in French fries could be reduced by varying preparation practices.

Materials and methods

Retail studies

French fries were obtained frozen from supermarkets and ready cooked from

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restaurants and takeaway food bars in Sydney, Australia. Samples of fifteen brands of frozen fries were measured for size and analysed for moisture, total fat and cholesterol content, and fatty acid composition; samples of eleven of the brands (i.e. the plain strip types only) were fried according to the manufacturers' instructions (2.0–3.5 min at 190°C) and then reanalysed for moisture and fat content.

Restaurant samples were obtained from four inexpensive restaurants ($2–4 per main course), four medium-priced restaurants ($3–6 per main course) and four expensive restaurants ($6–12 per main course). Takeaway samples of French fries were purchased from twelve independent takeaway food bars, each in a different Sydney suburb. Suburbs were situated right across the socioeconomic spectrum, three suburbs in each of the rankings A (highest), B, C and D (lowest) (Congalton, 1961, modified to include subsequent urban changes). All restaurant and takeaway samples were measured for approximate size, then weighed and analysed for moisture, fat and cholesterol content, and fatty acid profile.

Laboratory studies

Kennebec potatoes (the variety most used commercially) were used in the laboratory studies to test the effects of frying temperature, fries size, single and split frying and draining time on fat content of French fries. Strips of 5.0 × 1.1 × 0.9 cm (medium) were cut by hand for all tests except for the study of fry size, in which strips were also hand cut in the following sizes: 5.0 × 0.5 × 0.5 cm (very small), 5.5 × 0.8 × 0.6 cm (small) and 5.0 × 1.5 × 1.3 cm (large). Frying was carried out in a domestic deep fryer (4.5 litre capacity) using an animal/plant blend cooking fat. A batch size of 50 g was used in all cases. A frying temperature of 175°C (± 5°C) was used except in the study of frying temperature where various temperatures in the range 160–200°C were used. Recovery time to reach the desired temperature after fries immersion was 30 sec. Cooking times in the range of 0–30 min were used to assess fat uptake in single frying. In split frying, various combinations of par and finish frying times totalling 8 min (cooked) and 15 min (overcooked, but edible) were used. Frying times in the temperature and size studies varied according to the point at which fries were assessed as cooked. Except where otherwise stated fries were cooked to the same degree of 'doneness' as assessed by appearance and texture. After (and between) fryings, fries samples were agitated in air in the fryer basket for 10 sec to shake off excess fat, then drained in a single layer on absorbent paper for 30 min, being turned once mid-draining. In the draining study, the period on absorbent paper varied over the range 0–30 min. All laboratory studies were replicated three times, except for the draining study which was replicated five times.

Analytical methods

Moisture content was determined by vacuum drying, fat content by acid hydrolysis, and fatty acids and cholesterol by gas chromatography of methyl esters and trimethylsilyl ethers, respectively. All methods were described by Wills, Balmer & Greenfield (1980) and Wills & Greenfield (1982).

Results

Retail studies

The results of the retail study are shown in Table 1. The composition of the takeaway French fries was essentially similar for purchases from differently ranked suburbs, with a mean fat content of 13.1 g/100 g. Restaurant French fries had a similar

composition to takeaway fries overall but within the restaurant group there were significant differences between restaurant types ($P < 0.05$), the fat content of fries from expensive restaurants (17.8 g/100 g) being highest, and fries from inexpensive restaurants (9.8 g/100 g) being the lowest.

Table 1. Moisture, fat and cholesterol content and fatty acids ratio of retail French fries

Source	*n*	Moisture (g/100 g)	Fat (g/100 g)	Cholesterol (mg/100 g)	*P*:*S* ratio (*n*:1)
Takeaway food bars	12	50.1 ± 6.1* (40.2–60.1)	13.1 ± 2.8 (9.5–17.5)	19 ± 4.6 (11–24)	0.16 ± 0.12 (0.04–0.50)
Restaurants	12	49.8 ± 10.7 (31.9–65.3)	13.6 ± 4.9 (6.2–20.8)	13 ± 7.4 (1–21)	0.54 ± 1.09 (0.04–3.8)
Inexpensive	4	57.6 ± 4.8[a] (50.5–61.0)	9.8 ± 3.2[a] (6.2–14.0)	18 ± 2.2[a] (15–20)	0.12 ± 0.1[a] (0.04–0.24)
Average price	4	49.6 ± 13.2[a] (37.6–65.3)	13.3 ± 5.4[ab] (7.2–18.7)	8 ± 7.1[a] (2–16)	0.46 ± 0.6[a] (0.05–1.35)
Expensive	4	42.2 ± 8.1[a] (31.9–50.3)	17.8 ± 2.1[b] (16.1–20.8)	13 ± 8.8[a] (1–21)	1.04 ± 1.84[a] (0.05–3.8)
Total prepared fries	24	49.9 ± 8.5	13.4 ± 3.9	16 ± 6.7	0.34 ± 0.78
Supermarket, frozen					
As purchased	15	70.5 ± 2.2 (66.8–74.6)	3.7 ± 1.0 (2.1–5.3)	3 ± 0.8 (2–4)	0.15 ± 0.02 (0.13–0.16)
Finish fried	11	48.6 ± 7.2 (31.0–55.9)	15.0 ± 2.7 (12.0–20.0)	†	†

*Values are means ± s.d., range in parentheses.

†Not determined.

Values with the same superscript letters are not significantly different at the 5% level.

For the frozen French fries, as purchased, fat was present in all samples at a mean level of 3.7 g/100g, showing that they had been par fried during manufacture. The upper level of the range (5.3 g/100 g) approached the lowest level for prepared fries from restaurants (6.2 g/100 g). There was no significant correlation between fat content and the surface area : volume ratio of the frozen fries as purchased ($r = 0.256$). Frozen French fries, when finish fried, contained a mean fat content of 15.0 g/100 g, similar to the levels present in the prepared fries purchased from restaurants and takeaway food bars. Fat content of the finish fried fries (y g) was significantly correlated with the surface area : volume (x) of the fries ($y = 2.4x + 1.2$; $r = 0.909$, $P = 0.001$).

In all except two of the retail French fry types examined the fat had a low *P*:*S* ratio, i.e. contained high levels of saturated fatty acids. Only two samples had a *P*:*S* ratio of > 1.1 (one medium-price restaurant, 1.35 : 1; one expensive restaurant, 3.8 : 1). The levels of cholesterol present in the retail fries ranged from 1 to 24 mg/100 g, equvalent to 60–183 mg cholesterol/100 g of cooking fat. These data for *P*:*S* ratio and cholesterol content approximate closely to the data obtained in a study of Australian commercial cooking fats (Wills, Myers & Greenfield, 1982) which found a *P*:*S* ratio range of 0.1–0.3 : 1 and cholesterol contents in the range 87–157 mg/100 g. The findings thus suggest the predominant use of saturated cooking fats of animal or part-animal origin in the Australian French fries' industry, and mirror our findings for fries produced by U.S.-based fast-food chains in Australia (Wills & Greenfield, 1980; Wills, Wimalasiri & Greenfield, 1981).

Laboratory studies

Single and split frying. Medium-size fries were single fried for 30 min or par and finish fried for various combinations of time totalling 8 or 15 min. Fries were assessed as cooked after 8 min by either method of frying but were also edible at 15 min. The results are shown in Fig. 1. In single frying the greatest increases in fat uptake were in the first 5 min and then after 20 min, although at the latter stage the French fries were obviously overcooked. The moisture content also decreased during frying (80.0, 64.4, 61.3, 52.5, 49.2, 44.2, 34.9 and 27.8 g/100 g after 0, 2.5, 5, 10, 15, 20, 25 and 30 min) but the same relative changes in fat content was present if the data were considered on a dry weight basis.

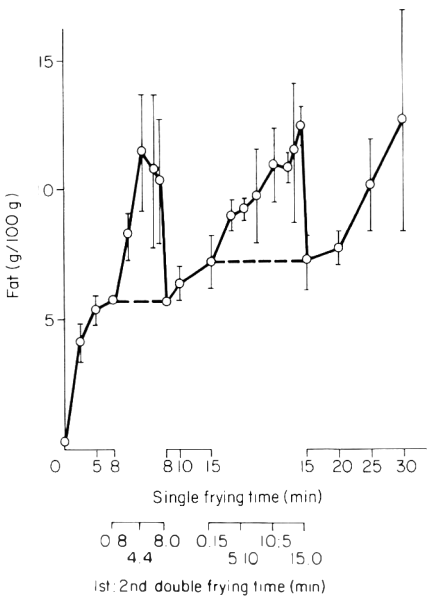

Figure 1. Fat content (wet weight basis) of single and par and finish fried French fries (5.0×1.1×0.9 cm), showing two par and finishing frying time combinations of 8 and 15 min. Values are means of three replicates with s.d. Frying temperature = 175±5°C.

In split frying, all frying time combinations produced significantly higher ($P < 0.05$) fat levels than the use of a single frying of the same total time duration. Some split frying combinations produced a doubling of fat uptake compared with a single frying for the same period. The procedure of split frying, or as it should be more accurately termed, double frying, seems to be the important factor in increasing the fat content (not total cooking time) as the fat levels were not very different in fries fried for a total of 8 or 15 min.

Size. The results of frying four different sizes of potato strips till cooked (i.e. 4, 7, 8 or 10 min) are shown in Table 2. Decreased fries size (x) significantly increased fat content of fries (y g) in a linear fashion for both single frying ($y = 1.5x - 0.6$, $r = 0.93$, $P < 0.001$) and split frying ($y = 2.3x + 0.4$, $r = 0.945$, $P < 0.001$). The slopes of the two regression lines (shown in Fig. 2) were significantly different ($P < 0.001$) confirming the finding that split frying increases the fat content of fries.

Table 2. Effect of size on fat and moisture content of French fries

Size	Surface area: volume		Cooking time (min)	Moisture (g/100 g)	Fat (g/100 g)
Very small	8.4	Single fried	4	47.2 ± 6.5*	11.8 ± 2.4
		Double fried	4	26.8 ± 3.6	19.2 ± 2.0
Small	6.2		7	46.7 ± 6.2	9.6 ± 1.0
			7	35.3 ± 1.4	14.9 ± 2.0
Medium	4.4		8	60.6 ± 1.4	5.9 ± 0.6
			8	50.3 ± 2.7	9.9 ± 1.6
Large	3.3		10	65.4 ± 2.3	4.3 ± 0.5
			10	56.2 ± 2.9	8.0 ± 0.9

*Values are means of three replicates ± s.d.

Frying temperature. Commercial operators often employ different frying temperatures for French fries, and in small businesses temperatures may fluctuate considerably. However, when the effects of temperature over 10° intervals between 160 and 200°C were tested, no significant differences in the fat and moisture content of fries were found. (Fat content 6.0 ± 0.7 g/100 g at 160°, 6.6 ± 1.4 g/100 g at 200°.)

Draining. The efficiency of draining of cooked fries might be expected to influence the fat content, however the fat contents of cooked fries after air agitation in the frying basket followed by periods of draining on absorbent paper at intervals between 0 and 30 min showed no significant changes (fat content 7.3 ± 0.5 g/100 g at 0 min, 6.3 ± 0.8 g/100 g at 30 min). It would appear that excess fat is mainly removed during the initial agitation in the fryer basket.

Discussion

The data obtained from the laboratory studies suggest that some of the variation in fat content of retail French fries may be due to differences in fry size. The values for the retail fries were positioned according to size (Fig. 2) which in the case of the fries purchased hot was, by rough measure, either 'medium' or 'small'. Size *per se* accounted for part of the difference in fat content, for example, between samples from different priced restaurants, where small fries were more common in the more expensive restaurants (small, $n = 7$, fat content = 17.3 g/100 g ± 2.1; medium, $n = 5$, fat content = 8.6 g/100 g ± 1.8; $p < 0.001$).

The regression lines obtained for the laboratory studies for single and split fried samples are also given in Fig. 2. It seems that overall few of the retail samples were single fried and more than 50% of the samples contained more fat than could be accounted for by split frying and size alone. Other factors which may influence fat content are cultivar differences and procedures such as steam blanching and freezing which take place in the manufacture of French fries. Overall, commercial practices tended to increase fat content considerably over laboratory practice. The potential nutritional impact of the practice of split or double frying appears to be counter-productive to dietary recommendations to reduce fat consumption. Techniques to reduce fat used in par frying have been explored from the economic angle (Nonaka, Weaver & Sayre, 1974), but work could be done to develop means of reducing the final

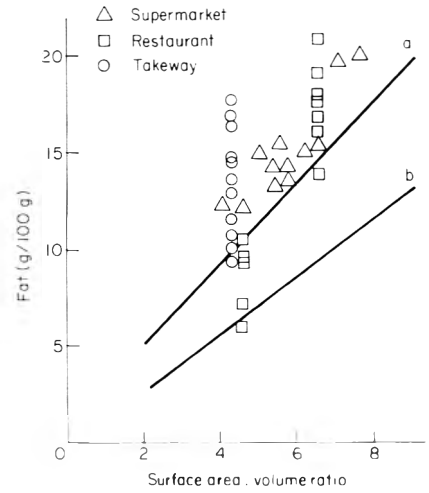

Figure 2. Fat content (wet weight basis) (y) of French fries with increasing surface area to volume ratio (x), showing individual data for French fries from three classes of commercial outlets, and the regression lines determined in the laboratory for (a) par and finish fried French fries ($y = 2.3x + 0.4$), and (b) single fried French fries ($y = 1.5x - 0.6$). Frying temperature for laboratory studies = 175 ± 5°C.

fat content of finish fried French fries, since this would also be beneficial from the nutritional point of view.

In the case of fatty acids and cholesterol in French fries, our data agree with those of others (Slover, Lanza & Thompson, 1980; Hubbard *et al.*, 1977) who have reported data for French fries from several fast-food chain restaurants in the U.S. showing that the majority of fries samples contained cholesterol and predominantly saturated fat. This evidence of animal fat use in Australia and the U.S. casts doubts on general reports which state that unsaturated vegetable oils are used in the main for commercial frying (FAO, 1977).

Conclusion

The common industrial and food service practices of cutting small size French fries and of par and finish frying them in animal fat significantly increases their potential as a saturated fat carrier. In view of official health recommendations to increase the consumption of starchy foods and of vegetables (Commonwealth Department of Health, 1981), means of reducing the lipid content of French fries should be investigated. Meanwhile advice about fat intake should stress the need to avoid commercial French fries and to prepare frozen fries by oven heating instead of frying, rather than focussing on elimination of fries altogether since potatoes are an important source of vitamin C and dietary fibre in the Australian diet.

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Effect of salting and roasting on the carbohydrates and proteins of Iranian pistachio kernels

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Summary

The free sugars which make up 2.7% of Iranian pistachio cv Badami were identified as fructose, glucose, sucrose, maltose, isomaltose, cellobiose, raffinose and stachyose. Both protein and free amino acids contained the same seventeen amino acids but lacked tryptophan, asparagine and glutamine. After salting and roasting, total available carbohydrates, total starches and dextrins, and total free sugars all decreased compared to controls, as did a number of individual sugars, especially the reducing sugars. While free amino acids had severely decreased, total protein amino acids and individual protein amino acid were not affected at all. The decreases in reducing sugars and in free amino acids may be due in part to their taking part in Maillard reactions.

Introduction

Roasting alters and substantially improves the flavour, texture, colour and appearance, and is one of the most common forms of processing for pistachio nuts which contain approximately 50–60% oil (Clarke, Brar & Procopiou, 1976). Kashani & Valadon (in press) have shown that after salting and roasting of six varieties of Iranian pistachio kernels, there was an increase in free fatty acids, in phosphatidic acid and in peroxide value, thus suggesting some degradation of the oil. There are other nutritive components of pistachio kernels, e.g. carbohydrates and proteins which make up 10 and 20% respectively of the fresh weight of the kernels (Kashani, 1982). Compared with labile nutrients such as vitamins, carbohydrates are generally regarded as less vulnerable to heat processing (Bender, 1978) although proteins are often affected through decreases in the nutritive value of the food by the degradation of certain amino acids.

During roasting, caramelization may take place due to severe heating of the sugar molecules. One of the most important reactions of carbohydrates with other food components is undoubtedly the so-called non-enzyming browning or Maillard reaction (Hurrell, 1977), which occurs between reducing sugars and nitrogenous compounds, in particular amino acids and proteins. A great number of studies with model and food systems have been carried out, but still Maillard reactions are not fully understood.

Although the composition of pistachio kernels are known to a certain extent (Clarke *et al.*, 1976; Kamangar, Farohi & Mehran, 1975), no thorough investigations of carbohydrates and proteins have been carried out to date. The purpose of this study was therefore to investigate the effect of roasting on the carbohydrates and proteins of Iranian pistachio kernels, and to possibly obtain some understanding of the Maillard

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reaction. Very little is known concerning the fate of small amounts of carbohydrates and proteins in pistachio nuts where lipids are in such large amounts. It would therefore be most informative to ascertain what happens to these food components during salting and roasting.

Materials and methods

The same six varieties of Iranian postachio nuts (Kashani & Valadon, 1983) kindly provided by the Institute of Modification and Preparation of Seeds, Karadj, Iran were used. The cultivars were from different regions of Iran and varied somewhat in their sizes from 90.5 to 135.5 g/100 nuts (Kashani & Valadon, 1983). Kashani (1982) has studied these six cultivars in detail but did not observe very wide differences in their total carbohydrates and proteins. The effect of roasting was studied on all six, but the differences between them were so small that the only one discussed in detail in this paper is the variety Badami from Rafsanjan: the same one whose lipid components were fully discussed by Kashani & Valadon (1983).

Carbohydrate extraction

Ten g of pistachio kernels were homogenized twice with 150 ml petroleum ether (40–60°C) to remove the lipids and the ethereal layer discarded.

Total available carbohydrates

The defatted and dried kernels were digested with perchloric acid and total available carbohydrates which comprise starches and dextrins together with soluble sugars were determined colorimetrically by the anthrone method (Osborne & Voogt, 1978).

Soluble sugars

Five g of defatted, dried kernels were homogenized twice with a mixture of methanol:chloroform:water (1:1:4 v/v). This was allowed to stand overnight at 4°C and 5 ml of the suspension centrifuged at 20000 rev/min for 5 min (Clarke *et al.*, 1976). To 1 ml of the supernatant liquid was added 2 ml of 1.8% barium hydroxide solution, followed by 2 ml of a 2% zinc sulphate solution, and then centrifuged at 20000 rev/min for 5 min. This supernatant liquid contained the free soluble sugars (Delente & Ladenburg, 1972).

The soluble sugars were estimated by tlc (Farhangi & Valadon, 1983) and by glc using peak identification and quantitation after silylation (Delente & Ladenburg, 1972).

Gas–liquid chromatography (glc)

Gas–liquid chromatography was carried out with a PYE series 104 gas chromatograph fitted with a flame ionization detector and glass columns (180×2.0 mm). Two columns and programmes were used: (i) 3% OV-1 on Chromosorb W (HP) 80–100 mesh; temperature was programmed from 150 to 350°C, 16°C/min (Delente & Ladenburg, 1972); (ii) 3% OV-17 on Gaschrom Q, 80–100 mesh; 100°C for 15 min, then to 320°C, 4°C/min (Aman, 1979).

Starches and dextrins

This estimation was obtained by subtraction of soluble sugars from total available carbohydrates (Farhangi & Valadon, 1983).

Protein extraction and determination

Ground pistachio kernels (0.5 g) were incubated with 10 ml N NaOH for 12 hr at 20°C. The solution was centrifuged at 20000 rev/min for 2 min at −5°C and 3 M trichloroacetic acid (TCA) was added to the supernatant liquid to precipitate the proteins which were then estimated by the Folin–phenol reagent of Lowry *et al.* (1957). The results were expressed as mg/g fresh weight.

Determination of amino acids

Free amino acids and total amino acid composition were extracted with modifications of methods used by Russell (1944) and Naguib (1964).

Free and protein amino acids and tryptophan were estimated as described by Farhangi & Valadon (1982) and by Kashani (1982).

The separation and identification of the amino acids were carried out on a Joel Model JLC 6 AH fully automatic amino acid analyser. The amount of amino acid in each sample was calculated by comparison of peak areas with those obtained using a calibration mixture as described by Eveleigh & Winter (1970). The results are expressed as mg/g fresh weight.

Roasting

Five hundred g pistachio nuts were soaked in 15% NaCl (99.998% pure) in deionized water (w/v) for 5 hr with slow stirring. The salted nuts were then dried in a rotary drier at 70°C for 1 hr until the moisture taken up by the salting was removed. The temperature was then raised at 145°C over a 30 min period and held for 20 min (Bloch & Brekke, 1960). After roasting, samples were cooled at room temperature. They were put in polythene bags separately and were stored in the deep freeze (−10°C).

The solvents were from AR stock, redistilled where necessary.

All the experiments were repeated four times and the results are the average ± s.d. of these four determinations.

Table 1. The effect of heat treatment (salting and roasting) on total available carbohydrates, free sugars, total starches and dextrins and on individual sugars of the pistachio var. Badami*

	Control	Roasted
Total available carbohydrates	101.3 ± 3.2	86.5 ± 2.3
Total free sugars	27.7 ± 1.3	22.1 ± 1.4
Total starches and dextrins	73.6 ± 1.9	64.4 ± 2.1
Fructose	1.3 ± 0.2	+
Glucose	1.7 ± 0.2	+
Sucrose	13.5 ± 0.6	11.3 ± 0.3
Maltose	1.4 ± 0.1	2.1 ± 0.1
Isomaltose	0.3 ± 0.02	+
Cellobiose	0.3 ± 0.02	+
Raffinose	6.2 ± 0.2	5.8 ± 0.4
Stachyose	1.4 ± 0.1	1.6 ± 0.2
Unknown	1.6 ± 0.2	1.3 ± 0.3

*The results are expressed as mg/g fresh weight ± s.d.
+ indicates a trace.

Results and discussion

Total available carbohydrates, total free sugars, and total starches and dextrins of the pistachio var. Badami were 101.3, 27.7 and 73.6 mg/g fresh weight respectively (Table 1). After salting and roasting, all three carbohydrate components decrease significantly especially total free sugars which are therefore reflected in total available carbohydrates. These results are in agreement with those of Salem (1975) who showed that there was a reduction in total carbohydrates, sugars and starches after cooking broad beans.

The free sugars identified in pistachio kernels of the variety Badami were the same as those of the other five varieties studied by Kashani (1982), namely, glucose, fructose, sucrose, maltose, iso-maltose, cellobiose, raffinose and stachyose. Cellobiose and iso-maltose were found in small amounts compared to sucrose and raffinose which were the major sugars. Crane & Al-Shalan (1974) have already reported the presence of large amounts of sucrose generally followed by fructose, glucose and inositol in the American cv Kerman. However, inositol was not identified in Iranian pistachios. Neither Crane & Al-Shalan (1974) nor Clarke *et al.* (1976) had identified maltose, iso-maltose, cellobiose, raffinose or stachyose in their cv of American pistachio nuts. It is even more surprising in that the cv Badami contained as much as 6 mg/g raffinose which previous workers had failed to observe. This may be varietal or geographical or both.

Salting and roasting has a very marked effect on the individual sugars: most of them show a decrease, while maltose an increase and stachyose and raffinose do not change (Table 1). The most obvious decreases are the reducing sugars fructose and glucose which disappear almost completely. As maltose may be produced by starch hydrolysis, the fact that it increases after heat treatment is evidence that during roasting there is hydrolysis of starch. There is no evidence of direct involvement of raffinose and stachyose in Maillard reactions and they must undergo hydrolysis into their constituent monomers before they can combine with proteins and amino acids. Before roasting, the nuts were soaked in a salt solution for 5 hr when the moisture content increased to a maximum 15%, compared to the average moisture content (5.5%). In the first stage of roasting (drying stage), although the inside temperature of the nuts does not exceed 100°C, the temperature is high enough to bring about the hydrolysis of starches and dextrins to free sugars in the presence of increased moisture. In the roasting or drying of a food stuff as long as there is free escape of steam, and moisture is present the temperature of the heated food cannot rise above 100°C regardless of how rapid the heat input is. Once the material has dried, its temperature will then rapidly rise to that of the heating surface (Bender, 1978), and this will cause hydrolysis of starches and dextrins. At the same time, some of the free sugars are used up by combining with proteins and amino acids to produce brown pigments. In the second stage of roasting, when all the water has evaporated, the temperature is increased to 145°C for 30 min. At this stage the hydrolysis of starches is almost non-existent while the rate of Maillard reactions increases which brings about a sharp reduction in free sugars (especially reducing sugars) together with an improvement in the colour, odour, flavour and texture of the nuts.

Total proteins and free amino acids of the pistachio var. Badami were 184 and 48 mg/g fresh weight respectively. After salting and roasting, there was no significant effect on total proteins while there was a marked decrease in the free amino acids, showing a 40% loss (Table 2). There were the same seventeen amino acids both free and

Table 2. The effect of heat treatment (salting and roasting) on total proteins, on total free amino acids and on individual amino acids of the pistachio var. Badami*

	Free amino acids		Protein amino acids	
	Control	Roasted	Control	Roasted
Amino acids				
Lysine	5.4±0.4	3.1±0.1	20.4± 1.9	20.4± 1.7
Histidine	0.9±0.1	+	3.6± 0.2	3.6± 0.2
Arginine	0.9±0.1	+	3.5± 0.2	3.5± 0.3
Aspartic acid	4.6±0.4	3.3±0.2	17.6± 1.1	17.6± 1.4
Threonine	1.9±0.2	1.2±0.1	7.3± 0.9	7.3± 0.5
Serine	1.8±0.3	1.2±0.1	6.8± 0.7	6.8± 0.6
Glutamic acid	11.0±0.9	7.7±0.6	41.8± 3.3	41.4± 3.0
Proline	2.2±0.2	1.6±0.1	8.6± 0.7	8.6± 0.5
Glycine	2.5±0.3	1.5±0.1	9.6± 0.6	9.8± 0.7
Alanine	2.7±0.3	1.8±0.1	0.1± 0.9	10.0± 0.8
Cysteine	0.4±0.1	+	1.5± 0.2	1.5± 0.2
Valine	3.1±0.2	2.0±0.1	1.9± 0.9	11.6± 0.8
Methionine	1.0±0.1	+	3.8± 0.2	3.7± 0.2
Iso-leucine	2.3±0.3	1.6±0.1	8.8± 0.6	8.7± 0.7
Leucine	3.9±0.2	2.9±0.2	5.0± 1.1	15.1± 1.2
Tyrosine	1.4±0.2	+	5.2± 0.4	5.2± 0.5
Phenylalanine	2.3±0.3	1.5±0.1	8.7± 0.6	8.8± 0.7
Total free amino acid (mg/g)	48.3±3.9	29.4±2.6	—	—
Total protein amino acid (mg/g)	—	—	184.2±14.3	183.5±12.9

*The results are expressed as mg/g fresh weight ± s.d.
+ indicates a trace.

as proteins as already reported for other varieties (FAO, 1970, Clarke *et al.*, 1976); thus tryptophan, asparagine and glutamine were missing altogether (Table 2). The loss in total free amino acids is reflected by a loss in all individual amino acids: some more than others. Here, cysteine, histidine, arginine, methionine and tyrosine disappear almost completely after salting and roasting. These results are not unexpected, since during the roasting period all the promoting factor of the Maillard reactions such as reducing sugars, moisture (approximately 15%) and high temperature were present. No effect on protein amino acids was observed. These results are very different from those of Pieniazek, Rakowska & Kunachowicz (1975) who reported a heavy loss of total amino acids, and a considerable reduction in availability of eleven amino acids when casein was heated with glucose for 24 h at 90°C in the presence of 4% moisture. Pieniazek *et al.* (1975) had worked with a model system at a high temperature for a longer time than was used in the present study. Thus it is not surprising that the results are so different especially as the natural state of a complex food such as pistachio nut contains other compounds which may have an inhibitory effect on the Maillard reactions.

Kashani & Valadon (1983) have shown that after salting and roasting, total lipids, total simple and total complex lipids were not significantly affected although free fatty acids were. Similar observations may be seen with amino acids. Although protein amino acids are not affected by salting and roasting, free amino acids are. So, the free components are usually hydrolysed or subjected to other enzymic or non-enzymic activities first, and then the more complex molecules are attacked if at all. With carbohydrates on the other hand, the larger as well as the smaller molecules are subjected to hydrolysis at the same time. There were therefore decreases in total

available carbohydrates, in starches and dextrins, and in free soluble sugars, although the latter were more severely affected. So, although carbohydrates are generally regarded as less vulnerable to heat processing as certain labile nutrients such as vitamins, they may be affected as well depending on the temperature and period of heating, the moisture content and the pH of the solution (Williams, 1976; Kashani, 1982).

In conclusion then, the common method of roasting pistachio nuts does cause decreases in total available carbohydrates, in total free sugars and in total starches and dextrins. The individual sugars too are severely affected, especially reducing sugars. Similarly, free amino acids but not protein amino acids decrease significantly after salting and roasting. These decreases may in part be due to Maillard reactions which occur between reducing sugars and nitrogenous compounds, in this particular case with certain free amino acids but not with protein amino acids.

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Effect of cloudy agents on the stability and opacity of cloudy emulsions for soft drinks

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Summary

Droplets with different sizes scatter light at different wavelengths. A droplet size index (R) was developed for predicting emulsion stability and absorbance of light at 660 nm was used to evaluate the opacity of the system.

Various cloudy agents were tested on both stability and cloudiness of emulsions. The presence of emulsifiers and gums affected both the stability and opacity.

Best conditions for high stability and opacity were found using combinations of ester gums and emulsifiers.

Introduction

Brominated vegetable oils (BVO) have been successfully used since the 1940s, therefore there has been little need for alternatives in preparing cloudy emulsions for orange drinks. Moreover, BVO set the standard for the cloud expected by the trade and the consumer. In 1970, the authority to use edible brominated vegetable oil was withdrawn in the U.K., and a limit of 15 ppm in the finished beverage was set for the U.S.A. Since then the soft drink industry has been faced with finding alternative means of producing stable emulsions. Such alternatives include: sucrose esters, e.g. sucrose diacetate hexaisobutyrate (SAIB); rosin esters, e.g. glycerol ester of wood rosin, (Ester Gum-EG); polyol benzoates; polyglycerol esters; sorbitan esters; polysorbates; modified starches; gums; celluloses and others (Green, 1978; Babayan, 1978; Garti, 1979).

The final cloudy emulsion should remain stable for at least 3–6 months without 'ringing', 'creaming' or 'separation'; should not interfere with the colour, taste and odour of the finished beverage; should give, at least, the standard cloudiness (opacity); and should not change properties or performance upon dilution.

A stable emulsion is one with small droplet size (Sherman, 1968). On the other hand, opacity or cloudiness are increased by large droplets, which lead, evidently, to instability. The rates of creaming and separation depends on the droplet size and the difference between the specific gravity of the oil and the water phase. Thus, a stable emulsion, prepared of emulsifiers only, will be less opaque than the desired one, with large droplets, sufficient opacity and considerable instability. The formulator, when trying to prepare cloudy emulsions, based on food grade emulsifiers, is facing a dilemma, which probably can be solved by: (i) adding compounds with high refractive index to either the oil phase, the water phase or better to the interface between them. Such operation will increase the difference between the refractive indices of the two

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phases and as the consequence also the opacity. (ii) Adding materials with high specific gravity to the oil phase, in order to reduce the difference between the phases and the tendency for creaming.

The light scattering from uniform particles depends upon size, wavelength, and refractive index, or, in the case of an emulsion having droplets with non-uniform size distribution the light scattering is an integration over the whole size range. Droplets with different size scatter light at different wavelengths (Kerker, 1969; Goulden, 1958). From the rato of the absorbance at two wavelengths, a size index (R) was developed (Kaufman & Garti, 1981; Horie, Tanaka & Akabori, 1978) for predicting emulsion stability. On the other hand, absorbance of light at 660 nm is a valuable measure for the opacity of the system. The combination of these two measurments will provide the correlation between emulsion opacity and stability at a given system.

The following study is an attempt to evaluate the effect of various cloudy agents on both stability and cloudiness of emulsions prepared with food grade emulsifiers.

Materials and methods

Materials

Sorbitan monolaurate (Span 20), sorbitan monooleate (Span 80) and ethoxylated sorbitan monooleate (Tween 80), were purchased from Atlas Europol (Italy). Ester gum 8BG was obtained from Hercules Inc., U.S.A. Sucrose diacetate hexaisobutyrate (SAIB); xanthan gum (XG) and tragacanth gum (TG) were analytical grade samples from Sigma Chemicals. Essential oil and limonene were extracted from Israeli oranges using standard procedures. Decaglycerol monooleate (10.G.1.0) and decaglycerol distearate (10.G.2.S) were prepared in our laboratory (Kaufman & Garti, 1982; Garti, Aserin & Zaidman, 1981).

Preparation of oil in water emulsions

Weighted amounts of EG or SAIB were dissolved in the essential oil using the suppliers' recommended methods (solution A). The XG was dissolved separately in distilled water in the presence of the emulsifiers (solution B), and heated to 75°C prior to its addition to solution A with gentle stirring. A high speed mixer was used for 30 sec to achieve preliminary homogenization followed by an additional 10 min homogenization using a Silverson homogenizer. A typical example was prepared by dissolving 10 g of EG in 10 g of orange oil without heating, to prevent masticating of the EG. Separately 0.5 g of XG were dissolved in 71.5 g of hot water (50–60°C). To the XG solution 5 g of Span 20 and Tween 80 (at HLB = 10, 3.2 and 1.8 g respectively), were added, and heated to 75°C, while adding the oil.

Method of measurement

Every emulsion was diluted 1 part to 1000, prior to the absorbance measurements. The absorbances were taken at 400, 660 and 800 nm, using a Spectronic 2000 spectrophotometer (Bausch and Lomb). From the absorbance values at 660 nm the opacity was determined, and from the ratio of the absorbance at 800 to 400 nm, the size index (R) and the stability were predicted.

Results and discussion

The required HLB for limonene or essential oil of orange was determined from a series

of experiments using a variety of food emulsifiers (Kaufman & Garti, 1981) and was found to be 10±0.5. Preliminary work has shown that there is an indirect correlation between the required HLB of the o/w emulsion, its stability and the measured opacity. Table 1 summarizes part of these results. For a series of emulsions prepared with Span 20–Tween 80 as emulsifiers the best emulsion having the smallest droplet size (*R* value of 0.134) was that of HLB = 12. By adding 0.5 wt % of XG, all the emulsions lost stability in comparison to those prepared without the gums and the one having HLB = 10 was the most stable with stability index of 0.375. The emulsion having an HLB = 12 was formed most easily (best ease of formation) but relatively less stable (for details on the relationship between ease of formation and emulsion stability see Kaufman & Garti, 1981). The opacity was gradually decreased with the increase in the HLB values of the emulsions. Similar trend was obtained for other combinations of emulsifiers (Span 80–Tween 80).

Table 1. Values of *R* (stability index) and opacity (OP) as function of HLB, for emulsions prepared with Span 20–Tween 80 and Span 80–Tween 80 without and with 0.5 wt % – XG

HLB		7	8	9	10	11	12	13	14	15
SP 20+TW 80	*R*	—	—	0.250	0.229	0.243	0.134	0.199	0.323	0.502
no XG	Opacity	—	—	0.221	0.175	0.125	0.073	0.054	0.049	0.035
SP 20+TW 80	*R*	—	—	0.394	0.375	0.392	0.427	0.445	0.497	0.528
with XG	Opacity	—	—	0.259	0.228	0.207	0.184	0.162	0.131	0.102
SP 80+TW 80	*R*	0.349	0.410	0.229	0.223	0.216	0.256	0.338	0.458	0.502
no XG	Opacity	0.256	0.247	0.185	0.173	0.114	0.098	0.072	0.035	0.025
SP 80+TW 80	*R*	0.411	0.425	0.384	0.371	0.395	0.422	0.464	0.504	0.574
with XG	Opacity	0.348	0.317	0.255	0.232	0.214	0.194	0.172	0.142	0.112

Table 2. The effect of emulsifier and EG concentrations on stability and opacity at HLB = 10, with Span 20–Tween 80 as emulsifiers

		0.5	1.0	2.0	2.5	3.5	5.0	8.0	10.0
No XG	*R*	1.00	0.943	0.739	0.65	0.532	0.229	0.168	0.143
No EG	Opacity	0.524	0.491	0.420	0.347	0.213	0.175	0.102	0.077
0.5% XG	*R*	1.23	1.07	0.792	0.684	0.572	0.375	0.351	—
No EG	Opacity	0.473	0.431	0.345	0.282	0.255	0.288	0.175	—
0.5% XG	*R*	0.984	0.866	0.602	0.493	0.327	0.232	0.252	0.284
1% EG	Opacity	0.526	0.471	0.387	0.352	0.317	0.289	0.264	0.255
0.5% XG	*R*	1.12	0.972	0.749	0.524	0.439	0.278	0.348	0.421
2.5% EG	Opacity	0.827	0.704	0.619	0.527	0.472	0.427	0.384	0.380
0.5% XG	*R*	—	—	0.934	0.606	0.487	0.35	0.453	0.579
5% EG	Opacity	—	—	0.973	0.864	0.759	0.615	0.624	0.653
0.5% XG	*R*	—	—	0.824	0.774	0.654	0.512	0.547	0.613
10% EG	Opacity	—	—	1.24	1.16	1.09	1.03	1.10	1.26

EG as an opacifier

Some representative results concerning both stability and opacity indices for several emulsions containing the EG of various levels of concentration are presented in Table 2 and Fig. 1. These series were prepared at a given HLB = 10 and varying the concentrations of emulsifiers. High concentrations of emulsifiers formed more stable emulsions with smaller droplets and lower opacity. With 0.5 wt % emulsifier, R = 1.00 and an opacity of 0.524 is reached. The emulsion with 10 wt % emulsifier has a stability index of 0.143 and opacity of 0.077 (very small droplets, 0.5–1.5 μm). The addition of 0.5 wt % XG causes a general decrease in the stability as well as in the opacity of the system but is essential for the emulsions in which EG of SAIB are to be used. No stabilization was reached when EG or SAIB were used in the absence of XG. Addition of 1 wt % EG has a small effect on both stability and opacity. The influence of 2.5 wt % EG is quite pronounced; there is a small decrease in the emulsion stability but significant increase in the opacity. Emulsions prepared with 5 wt % emulsifier without EG will have an opacity of 0.228 while in the presence of 2.5 wt % EG the opacity will be doubled (0.427). Addition of 5 and 10 wt % of EG will increase the opacity sharply up to 1.03. Unfortunately these emulsions will show a severe loss of their stability. The emulsions with 0.5–1.0 wt % emulsifier are too unstable even in the presence of large quantities of EG. The large droplets (as shown from the R index) indicates loss of stability but nevertheless does not cause an oil separation because EG adds to the specific gravity of the oil and as a result decreases the difference between the specific gravities of the oil and the water phases. Despite the considerable number of large droplets now present in the emulsion, no coalescence occurs due to the presence of XG and the emulsifiers forming a firm protected interface.

Similar behaviour was found for a series of emulsions prepared with Span 20–Tween 80 at HLB = 12 (Table 3). As the emulsifier concentration increases, the stability increases and the opacity decreases. The added XG caused a small decrease in the stability due to possible incorporation of the gum into the interface network occupied by the emulsifiers, decreasing the synergistic effect of the emulsifiers interaction. When EG is added to the oil, the opacity increases significantly. At too high EG concentrations there is quite a severe loss of stability.

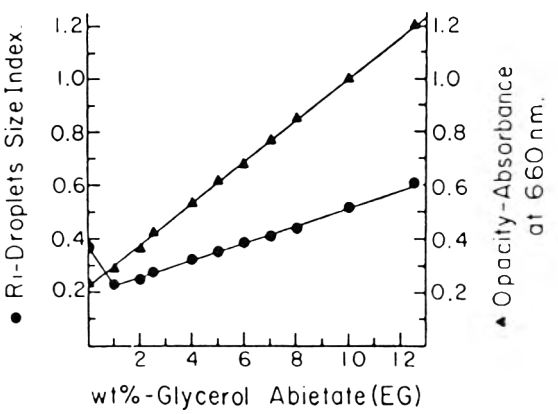

Figure 1. Droplet size index (R_1) and opacity measurements for emulsions prepared with glycerol ebicate (Eb).

Table 3. The effect of emulsifier and EG concentrations on stability and opacity at HLB = 12, with Span 20–Tween 80 as emulsifiers

		0.5	1.0	2.0	2.5	3.5	5.0	8.0	10.0
Emulsifiers only	*R*	0.641	0.432	0.277	0.231	0.196	0.134	0.102	0.084
	Opacity	0.304	0.296	0.273	0.237	0.162	0.125	0.074	0.031
0.5% XG	*R*	0.606	0.527	0.457	0.411	0.372	0.224	0.197	0.184
	Opacity	0.445	0.338	0.294	0.271	0.235	0.209	0.165	0.105
1% EG	*R*	0.681	0.611	0.529	0.437	0.342	0.234	0.211	0.194
	Opacity	0.497	0.445	0.361	0.309	0.287	0.237	0.200	0.182
2.5% EG	*R*	0.948	0.874	0.703	0.594	0.473	0.349	0.312	0.324
	Opacity	0.822	0.711	0.598	0.534	0.419	0.359	0.321	0.304
5% EG	*R*	1.15	0.972	0.883	0.804	0.649	0.524	0.579	0.643
	Opacity	1.27	1.13	0.925	0.784	0.702	0.627	0.669	0.723
10% EG	*R*	—	—	0.975	0.849	0.714	0.592	0.628	0.697
	Opacity	—	—	1.18	1.10	0.984	0.920	0.947	1.11

Table 4. The average effect of 1% —EG on the opacity*

Amount of EG (%)		1.0	2.5	5.0	10.0	Average value†
3.5% emulsifier	HLB 10	0.104	0.104	0.109	0.087	0.101
	HLB 12	0.125	0.103	0.108	0.082	0.104
5.0% emulsifier	HLB 10	0.106	0.101	0.088	0.085	0.095
	HLB 12	0.112	0.094	0.100	0.079	0.096
8% emulsifier	HLB 10	0.167	0.113	0.104	0.0998	0.119
	HLB 12	0.126	0.114	0.119	0.087	0.111

*Calculated by subtracting the value of opacity with no EG from the one with EG and dividing by the percent of EG presented. The data are from Tables 2 and 3.

†The average effect of 1% EG is 0.104 units.

From the data in Tables 2 and 3 it is possible to calculate the average amount of increase in opacity caused by 1% EG. Since scattering and absorbance are functions of concentration, it is possible to formulate an emulsion, with accordance to the needs, with only few tests. By calculating the average effect of 1% EG on the opacity, prediction of the opacity (and stability) of a designed formulation can be achieved. Some of the calculations are summarized in Table 4. For 3.5 wt % emulsifier, at HLB = 10, the average contribution to the opacity of 1 wt % EG is 0.101 units. At HLB = 12 the effect is of 0.104 units. With 5 wt % emulsifier at HLB = 10 and 12 respectively, an increase of 0.095 and 0.096 units has been calculated and 0.119 and 0.111 with 8% emulsifier. The average increase in opacity caused by 1% EG is 0.104 units. The increase in the opacity is affected very little by the concentration of emulsifier, HLB and the oil.

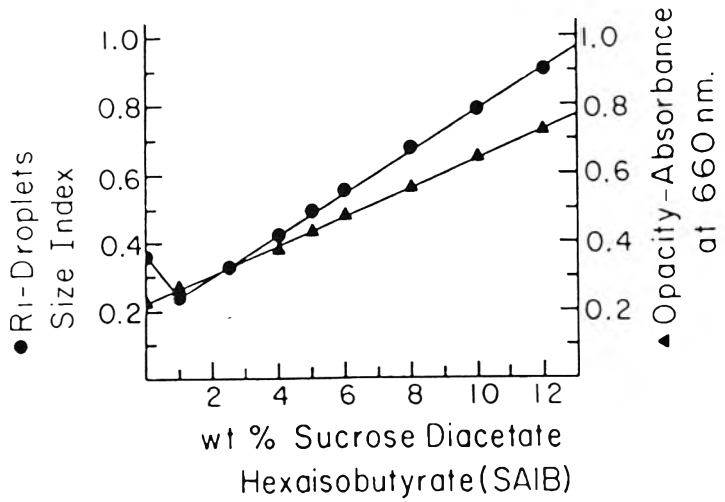

Figure 2. Droplet size index (R_1) and opacity measurements for emulsions prepared with SAIB.

SAIB and polyglycerol esters as opacifiers agents

When EG is changed to SAIB, similar trends can be observed as can be seen from Fig. 2. The opacity of the emulsions increases and the stability decreases, as the SAIB concentration rises. Comparing the results obtained with SAIB to those of EG, it can be noted that for the same amount of additive, EG causes a significantly large increase in opacity and the emulsions are more stable with EG than with SAIB.

Table 5. The effect of decaglycerol monooleate distearate on the opacity and stability at HLB 12

Emulsifier (wt %)	Emulsions with decaglycerol esters		Emulsions with 0.5% TG and decaglycerol esters	
	R	Opacity	*R*	Opacity
1.0	0.623	0.543	0.562	0.562
2.0	0.459	0.474	0.407	0.471
2.5	0.440	0.325	0.396	0.434
3.5	0.412	0.302	0.352	0.394
5.0	0.394	0.272	0.298	0.357
6.0	0.327	0.235	0.274	0.395
8.0	0.479	0.349	0.305	0.423
10.0	Multiple emulsion		0.336	0.492
12.0	Multiple emulsion		0.389	0.584
15.0	Multiple emulsion		0.423	0.701
20.0	Multiple emulsion		0.548	0.962

Similar behaviour was found with polyglycerol esters used both as emulsifiers and opacifiers. This unique behaviour is possible due to its high specific gravity (1.01–1.03) and high refractive index (1.4731–1.4875). Some of the results are summarized in Table 5. The dual function of the decaglycerol monooleate-distearate requires large quantities of it to be used, and stresses the need for TG to stabilize the emulsions.

Without TG, up to 6% emulsifier, the normal behaviour is found, that is to say, the higher the emulsifier concentration the higher the stability, with relatively low opacity. But, as the decalgycerol esters concentration increases, its dual behaviour is revealed, the opacity is increased drastically up to 0.962 (for 20% decaglycerol esters) while the stability index increased to 0.548.

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Optimization of the heat stability of protein-rich concentrates prepared by ultrafiltration of skim-milk

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Summary

Methods of improving the heat stability of protein-rich concentrates prepared by ultrafiltration of skim-milk have been evaluated. It has been found that concentrate diafiltration promotes large increases in heat stability with little penalty in terms of processing difficulties.

Introduction

Laboratory studies have suggested that a new range of sterilized milk products can be formulated using concentrated skim-milk fractionated by ultrafiltration (Sweetsur & Muir, 1980a, b). During the ultrafiltration process, the milk protein is concentrated and a proportion of the soluble salts is removed. It was found that the concentrations of the soluble milk salts were more important in determining heat stability than were the concentrations of the proteins (Sweetsur & Muir, 1980a). The assessment of the heat stability of these products was based on the time taken for visible clots to form in a standardized test (Sweetsur & White, 1974). However, subsequent work with evaporated milks have shown that the measurement of visible coagulation time provides an overestimate of the suitability of a concentrate to withstand sterilization. For example, it has been found that undesirable grain formation and excessive increases in viscosity precede visible coagulation in conventional full-cream evaporated milk.

As a result of this new information, it was necessary to re-assess the results of our earlier work (Sweetsur & Muir, 1980a, b) before pilot scale studies could be undertaken with the new range of concentrates. This paper describes efforts to further improve heat stability of milk concentrates prepared by ultrafiltration.

A large number of potential methods are available for manipulation of heat stability and these are reviewed by Fox & Morrissey (1977). However, these techniques are often complex and may involve the use of reagents which are undesirable in food. For these reasons, we have concentrated our attention on three potentially useful techniques of undoubted safety from a toxicological standpoint but of unknown practical value. The processes evaluated were: manipulation of the colloidal calcium phosphate content of milk by pH adjustment, selective fractionation of whey proteins during ultrafiltration and three types of soluble solute removal based on dialysis.

Materials and methods

Milk samples were collected from the Hannah Research Institute Farm bulk tank. Skim-milk was prepared as described by Davies & White (1966) and the skim-milk was stored in the dark at 5°C until used.

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The pH of milk was adjusted prior to ultrafiltration by the addition of hydrochloric acid (2 M).

Skim-milk was concentrated at 40°C in a laboratory rotary evaporator. The total solids content of the milk were measured by the rapid method of Wilson detailed in Muir & Sweetsur (1978).

Skim-milk was concentrated by ultrafiltration at 50°C using a laboratory scale apparatus with flat 90 mm membranes (a TCF-10 ultrafiltration unit from Amicon Ltd, Stonehouse, Glos.). Membranes of type PM30 (nominal cut-off 3×10^4 MW) and DPO.45 (nominal pore size 0.45 μm) were employed.

Total nitrogen, non-protein nitrogen and β-lactoglobulin nitrogen in the skim-milks were estimated by methods devised by J. C. D. White and described in Muir & Sweetsur (1978).

The concentrations of calcium, magnesium, sodium, potassium, and phosphate were measured in the skim-milk and ultrafiltrate by the methods detailed in Holt & Muir (1978, 1979).

Coagulation time (CT) pH profiles for milk and UF concentrates were measured as described in Sweetsur & White (1974).

Results

Manipulation of soluble salts in milk by pH adjustment

There is ample evidence that a change in the calcium concentration of milk will lead to profound effects in the heat stability (e.g. Pyne & McHenry, 1955). In addition, the formation of milk free from colloidal calcium phosphate results in marked increase in stability (Pyne & McGann, 1960; McGann & Pyne, 1960; Rose, 1962). Therefore by deduction, a reliable method of reducing both the soluble calcium concentration and the colloidal calcium phosphate level will result in significant increases in the heat stability of concentrate.

The calcium phosphate complex in milk is partitioned into a soluble and a colloidal phase and the equilibrium is highly pH dependent (Pyne & McGann, 1960). Slight increases in acidity between pH 7.0 and 5.0 result in significant solubilization of calcium phosphate. Since soluble salts pass freely through standard ultrafiltration membranes (PM30 type), adjustment of the pH of milk prior to ultrafiltration is a simple device for manipulation of the calcium phosphate to protein ratio in concentrates. The equilibrium between the colloidal and soluble phases of the minerals in skim-milk at pH

Table 1. The effect of pH on mineral equilibrium of milk

	Concentration (mM)*						
	Calcium		Phosphate		Citrate		Total
pH of milk	Total	Soluble	Total	Soluble	Total	Soluble	solids (%)
6.6	33.9	8.7	22.6	10.4	10.2	9.1	9.19
6.0	32.9	13.1	20.8	12.8	10.7	9.6	9.06
5.5	32.0	21.0	20.3	16.3	10.1	9.8	9.00

*The values for soluble constituents given are mean values of five samples of ultrafiltrate collected at equal intervals during concentration of the milk by a factor of ×3.

values of 6.6, 6.0 and 5.5 are shown by typical data in Table 1. As expected, acidification resulted in marked solubilization of calcium phosphate with comparatively little change in the soluble citrate levels (cf. Pyne & McGann, 1960). However, the effect of changing milk pH had other consequences. At 50°C, the optimal temperature for processing, the milk became unstable at pH values below 6.0. This effect was mediated by lowering the temperature at which ultrafiltration was carried out, but this resulted in a general lowering of the flux (Glover *et al.*, 1978). Typical concentration curves are shown in Fig. 1, where permeate flux was measured at 25°C during concentration of skim-milk (initial pH values 6.6, 6.0 and 5.5) to one-third volume. Under standard conditions, the time taken to reach a concentration factor of ×3 was 85 min at pH 6.6 increasing to almost 160 min at pH 5.5. This reduction in flux rate with decreasing pH was confirmed in subsequent experiments with other milk samples.

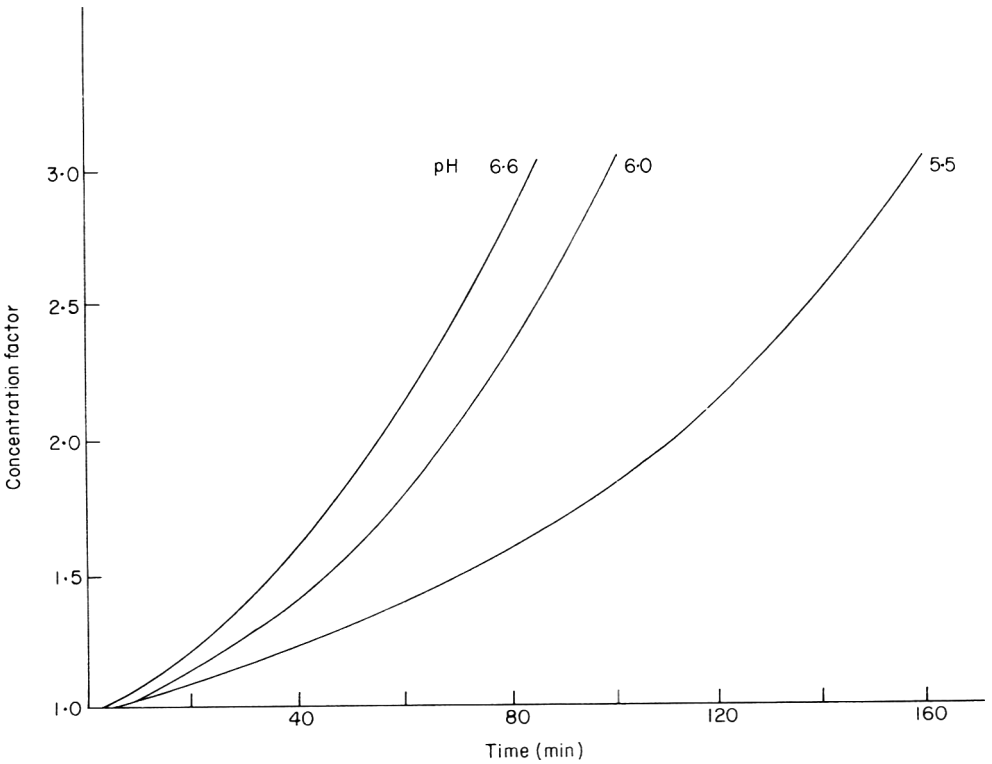

Figure 1. The effect of pH on permeate flux during ultrafiltration of skim-milk at 25°C.

In addition to the reduction in flux as initial pH was lowered, very large increases in viscosity of the concentrate occurred when the pH was adjusted back to 6.6. by the addition of sodium hydroxide. The effect was a consequence of the net exchange of protein-bound calcium ions for sodium ions during the processing and subsequent pH adjustment and the increase in viscosity rendered further processing of the concentrates very difficult.

Coagulation occurred when concentrate prepared from milk with an initial pH of 6.0 or less was heated without neutralization.

Manipulation of the whey protein content of milk by the use of macroporous membranes

There is a significant body of evidence which shows that interactions between sulphydryl group containing proteins in milk results in destabilization during heating (for a review see Fox & Morrissey, 1977). With unconcentrated milk the extent of destabilization is very pH dependent (Rose, 1961; Tessier & Rose, 1964; Fox & Hoynes, 1975) but, after concentration of milk, the addition of whey protein results in a general lowering of coagulation time (Muir & Sweetsur, 1978; Newstead, Sanderson & Conaghan, 1977). Most studies have focussed attention on the effects on heat stability of adding β-lactoglobulin to milk but more recent work also demonstrated destabilization of heated milk by α-lactalbumin (Fox & Hearn, 1978b). Thus there is a good deal of evidence that selective removal of whey protein from milk will promote a significant increase in heat stability.

A large number of membrane types are currently available for membrane fractionation with nominal cut-off points ranging in terms of molecular weight from a few tens of Daltons to macromolecular filters which can allow passage of material up to several μm diameter (Glover *et al.* (1978) have reviewed reverse osmosis and ultrafiltration membrane types). In milk, β-lactoglobulin (molecular weight = 18300) exists as a polymer and thus very porous membranes are required to allow passage of the protein during ultrafiltration. However, as the pore size—and hence the cut-off point—increases, the membrane becomes increasingly permeable to casein and a compromise must be reached between the selective reduction of β-lactoglobulin content and the loss of casein. By experiment, such a compromise was found when macroporous filters of nominal pore size 0.45 μm were used for selective ultrafiltration.

A comparison of the heat stability of concentrations produced using normal membranes (Amicon type PM30) and diaporous filters (Amicon type DPO.45) is shown in

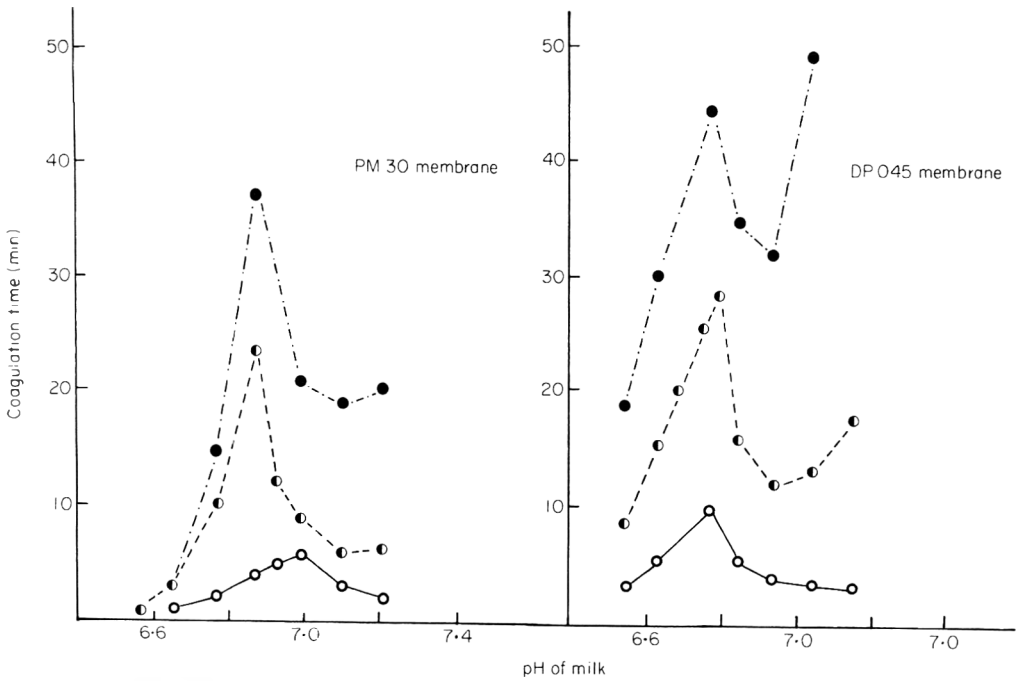

Figure 2. The effect of membrane type on the heat stability of skim-milk concentrated to 16.1% total solids. ●, 120°C; ◐, 130°C; ○, 140°C.

Fig. 2. Both samples were concentrated to a total solids content of 16.1%. At the three heating temperatures examined—120, 130 and 140°C—the concentrate produced using the macroporous filter was more stable, the difference being greatest at the highest heating temperature. These increases in stability were significant although the differences in the relative proportions of whey protein (Table 2) were fairly small.

At first sight, the results suggested that this approach might have practical merit but two problems were encountered. First, there were marked differences in performance of individual membranes from the same and from different production batches. In some cases, the selective reduction in the proportions of whey proteins was slight and this was reflected by small increases in heat stability. Secondly, the permeate flux using the macroporous filters was very variable and poor in comparison to that achieved using type PM30 membranes. Initial fluxes were very high, but by the time a concentration factor of between ×1.5 and ×2.0 was attained, the flux had fallen markedly. This fact probably reflected partial blockage of the membrane pores by protein during processing and is supported by observations of the turbidity of the permeate. At the start of processing, some micellar casein passed through the filter (albeit in small amounts) but as the flux dropped the permeate became clear.

Table 2. A comparison of the nitrogen distribution of concentrates prepared using different membrane types

	Treatment*		
	Skim-milk	PM30	DPO.45
Casein N (%)	79.6	82.3	83.7
β-lactoglobulin N (%)	8.6	8.0	7.6
Residual albumin N (%)	3.7	4.1	3.5
Globulin N (%)	0.2	0.4	0
Proteose-peptone N (%)	2.6	2.3	2.9
Non-protein nitrogen (%)	5.3	2.9	2.3

*The concentrates prepared by the different membrane types were diluted with water prior to assay. Values are normalized as % of total nitrogen.

The use of dialysis to reduce soluble salt levels in concentrates

It has already been noted that reductions in soluble salt levels, especially that of calcium, resulted in changes in heat stability. For example, Fox & Hearn (1978a) found that dialysis of milk resulted in reductions in the soluble salt levels and increases in coagulation time though the extent of the increase was highly dependent on the pH of the milk. The effect of dialysis of milk against distilled water for 30 min followed by concentration to 22.5% solids (by conventional evaporation) on heat stability is shown in Fig. 3. In this case the coagulation time increased after dialysis at all pH values of practical significance. Only low molecular weight material such as urea was lost from the milk during dialysis whilst the level of β-lactoglobulin remained unchanged (Table 3). The increases in heat stability after short-term dialysis were of such magnitude that the technique was adapted to ultrafiltration.

The effect on heat stability of dilution of milk prior to ultrafiltration

Distilled water was added to milk prior to ultrafiltration in volume ratios ranging from 0.5 to 2.0 and the milk was then concentrated to over 21% total solids. The solids

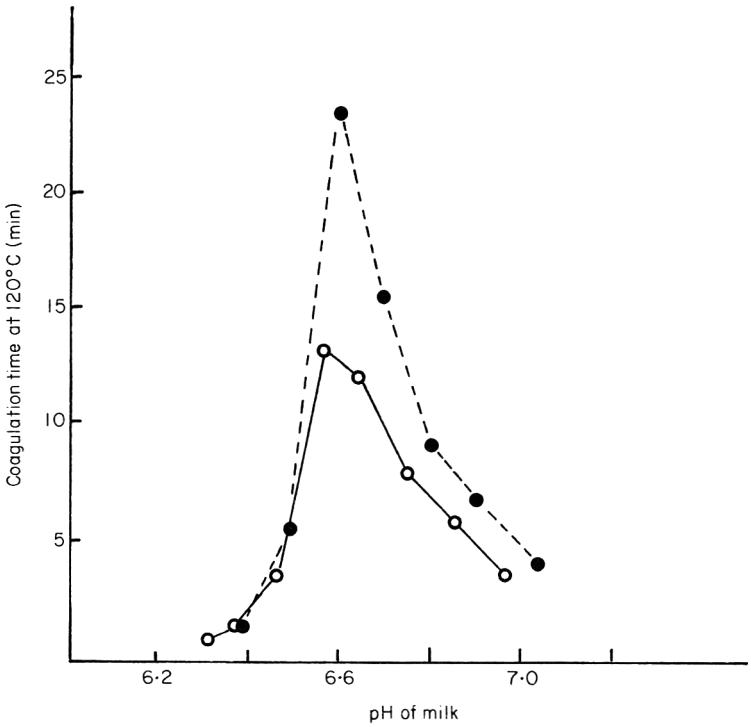

Figure 3. The effect of partial dialysis of skim-milk followed by concentration by evaporation to 22.5% total solids on the heat stability of the milk. O, control; ●, Dialysed against distilled water for 30 min.

Table 3. The effect of partial dialysis on soluble material in milk

	Urea (mg/100 ml)	β-lactoglobulin (% N)
Control milk	45.4	8.6
Dialysed milk*	27.3	8.6

*Milk was dialysed against water for 30 minutes.

Table 4. The effect of dilution with water before concentration on heat stability of concentrated milk

Dialysis ratio (Milk : Water)	Total solids (%)	Coagulation time* (min, 120°C)	pH
1:0	23.7	25.7	6.81
1:0.5	22.4	31.5	6.88
1:1	21.1	35.5	6.91
1:2	22.4	40.2	6.93

*Samples were adjusted to 21.1% solids before evaluation of CT.

contents were then standardized to 21.1% by addition of the relevant permeate. As shown in Table 4, this technique promoted significant increases in heat stability. Although there were slight increases in the pH of the concentrate as the initial dialysis ratio increased, these changes could not explain the increases in stability. Notwithstanding the significant increase in stability, the total process time became longer as the initial ratio of water to milk was increased. For example, when a dilution ratio of 1 : 1 was used the total manufacturing time was almost doubled.

The effect of concentrate diafiltration

Skim-milk was concentrated to one-fifth volume by normal ultrafiltration (total solids 19.8%), water was then added in various ratios of the concentrate volume (from 1 : 1 to 1 : 4) and the mixture re-concentrated to 19.8% solids. It should be noted that this treatment resulted in a further concentration of the protein (and protein-bound salts) for most of the lactose is removed by this process. The effects of this treatment on heat stability are shown in Fig. 4 and the corresponding changes in total salt content in Table 5. The conventional and ultrafiltrate samples were concentrated by factors of ×2.1 and ×5 respectively. Thus differences in the salt concentrations shown in Table 5 were consequences of these concentration factors and the extents to which the salts were soluble (i.e. dialysable) or protein bound. As expected, the concentrate prepared by normal ultrafiltration was more stable than its analogue made by conventional evaporation (Fig. 4a). After diafiltration of the concentrate even greater increases in heat stability were observed (Fig. 4b) and these improvements in stability occurred at pH values over 6.8. These results reinforce the view that the improvements in stability recorded by the alternative technique in Table 4 are a consequence of the diafiltration process, not of the slight change in final pH. The increases in heat stability shown in Fig. 4 are not related to total mineral content for the most stable samples had very similar

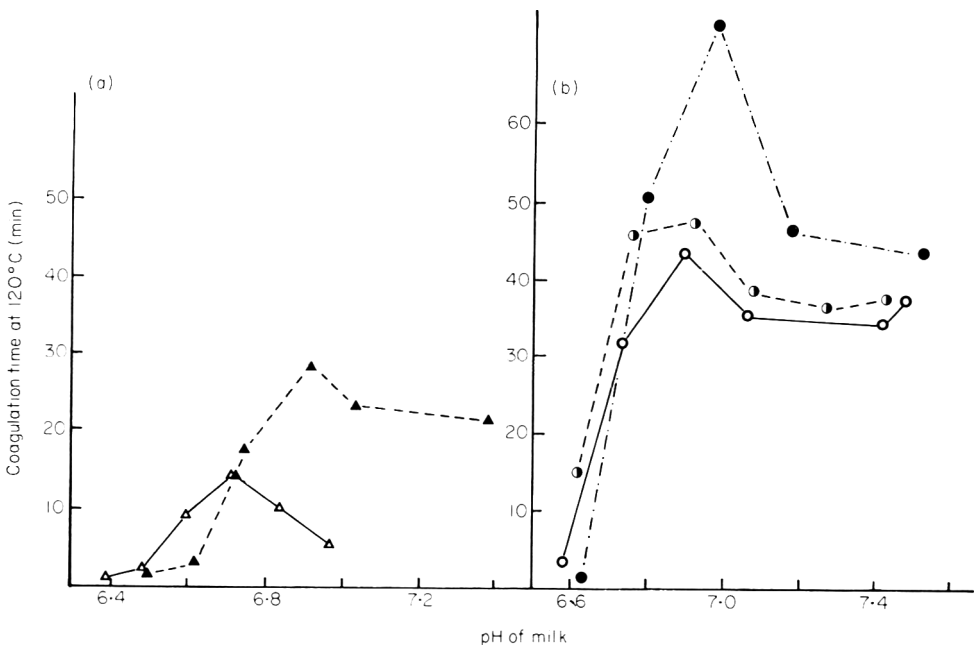

Figure 4. A comparison of diafiltration with other concentration processes on the heat stability of skim-milk concentrated to 19.8% total solids. (a) △, normal evaporation, ▲, normal ultrafiltration. (b) Diafiltration: O, dialysis ratio 1 : 1; ●dialysis ratio 1 : 2; ◑, dialysis ratio 1 : 4.

Table 5. The effect of diafiltration on mineral content of concentrate prepared by ultrafiltration

Sample	Concentration (mM)				
	Phosphate	Calcium	Magnesium	Sodium	Potassium
Milk	20.8	33.0	4.8	22.5	37.6
conventional concentrate	43.9	69.6	10.0	47.5	78.3
UF concentrate	49.8	95.1	5.9	24.2	36.1
Dialysed 1 : 1	51.9	105	7.7	13.2	19.7
Dialysed 1 : 2	54.9	111	7.9	10.8	15.4
Dialysed 1 : 4	51.8	110	7.4	8.0	10.4

levels of calcium and phosphate (Table 5). Clearly the changes are a result of loss of the soluble ions, though the relative importance of individual species is not known. A further point of interest is that the results in Table 5 imply that the micellar based calcium phosphate does not dissolve quickly at near neutral pH, for there was little change in total calcium phosphate between the normal UF concentrate and those subject to diafiltration.

In contrast to the earlier techniques of dialysis and diafiltration, process time was not greatly extended by the concentrate diafiltration method. For example, in the 1 : 1 treatment the concentration time was increased by less than 25% for it has been found that the average flux was significantly higher than usual.

Discussion

The results presented in this paper show that although a number of potential methods might be expected to improve the heat stability of milk concentrated by ultrafiltration, not all live up to expectation. Conversion of the calcium phosphate in milk from the colloidal to the soluble form by pH adjustment is simple and effective. Nevertheless, during concentration of the pH-adjusted milk excessive fouling of the membranes occurs rendering the process of reduced practical value. Selective fractionation of the whey proteins was more successful and when the ratio of casein to whey protein was increased by the use of macroporous filters, increases in stability were observed. Unfortunately, the performance of this type of membrane was very variable and, until a more reliable filter system is produced, the technique could not be applied in pilot plant. In contrast, all the methods based on dialysis and diafiltration were simple to use, did not reduce membrane performance and promoted significant increases in heat stability. From an economic viewpoint, concentrate diafiltration was most successful since it combined effectiveness in increasing heat stability with economy in terms of processing time. In conclusion, concentrate diafiltration is a practical option for use when the heat stability of concentrates prepared by conventional ultrafiltration is insufficient to allow sterilization.

Acknowledgments

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Book Reviews

Unit Operations in Food Processing, 2nd ed. By R. L. Earle.
Oxford: Pergamon, 1983. Hardback: ISBN 0 08 025537 X; £20.00. Flexicover: ISBN 0 08 0255361; £9.95.

This is the long awaited second edition of a book intended for students of food technology. The author applies the chemical engineering approach of unit operations to food processing. As he stated in his preface to the first edition in 1966, 'Principles of operation are discussed rather than specific applications'. Using this approach, areas covered include fluid flow, heat transfer, drying and evaporation, separation processes, size reduction and mixing. This edition has been extended with the inclusion of a chapter on material and energy balances and shorter additions on refrigeration and psychometry. Each area covered is outlined from basic principles with each concept being developed clearly. It therefore establishes the basic criteria for design of an operation when it is applied to food. The text incorporates many worked examples to illustrate how the equations which are derived can be used in food processing.

The major change in the text has been the complete conversion to S.I. units. The adoption of S.I. units is total with only a short appendix of conversion factors included for the benefit of those still using Imperial measurements. Although this change is welcome, it may be too sweeping for many working with traditional equipment. Students wishing to be confident in both systems of units should be aware of this limitation.

Apart from these alterations, the book's contents remain substantially the same as the first edition. The presentation though is much improved. A larger page enables the text and mathematical calculations to be followed with ease. The text is legible and both the illustrations and graphs are clear and well presented.

The author's statement in this edition that 'those wishing to become specialists in food engineering will need to study the subject more deeply', is certainly true. Indeed, I believe that most food technologists will require greater depth in many of the areas covered. Whilst the many examples of process calculations given in the text are related to food products, the final chapter on applications of process engineering in the food industry is disappointing. Having successfully covered the details of unit operations, the inclusion of this brief chapter provides a rather unsatisfactory conclusion to the book.

The above criticisms are not meant to detract from the useful introduction the book provides to the unit operations of chemical engineering considered in the context of food processing. The book can be recommended to students of food technology wanting to understand the basic concepts of these operations.

D. J. Jukes

Edible Oils and Fats: Developments since 1978 (Food Technology Review No. 57). Ed. by S. Torrey.
New Jersey: Noyes Data Corporation, 1983. Pp. xiii + 402. ISBN 0 8155 0923 5. U.S.$44.00.

Noyes Data Corporation books summarize U.S. patent specifications published in defined areas during the recent period under review. In this volume, the 240 specifications published during the period 1978–1982 are reported as a sequel to a similar volume which appeared in 1979. Each patent is introduced by a brief introduction indicating the background and objectives of the invention and there are helpful critical comments which assist the reader. Taken together with an indication of the scope of the claims and with selected examples the publishers' claim that the book presents an advanced commercially oriented review of recent developments is amply justified.

Except in sophisticated commercial R and D organizations the patent literature is not known or used as much as it should be, especially by academically oriented scientists and technologists. This is possibly because material is difficult to locate and interpret and the underlying commercial significance of the inventions is obscure to the average R and D operator. Further, it is often not appreciated that work described in patents is usually of a very high scientific standard: no patentee wants to see his claims demolished as invalid. This book meets a distinct need by avoiding the use of legal jargon and giving full details of key examples so that the reader will have no difficulty in repeating experimental work if he wishes.

Coverage is thorough and surprisingly wide. Processing technology and speciality oil and fat products come first, followed by composite products containing major amounts of oil or fat. These last include margarines, dairy products, infant formulas, meat analogues, sweets and snack products. Even feed compounds are included. Finally modified fatty products such as emulsifiers and pan release agents find a place.

This book is of obvious importance to food scientists and technologists in this very competitive sector of the food industry, whether they are mainly concerned with research and development or with technical aspects of marketing. It could also be studied with advantage by academics concerned with edible or products research since it complements very neatly the standard literature based on research papers and reviews.

The book is well produced and remarkably free from errors. Indexes of patent numbers, inventors and companies are provided, but, though there is a comprehensive contents list, a subject index would be helpful. Occasionally one also notes that, though dates of publication are quoted, dates of filing would be helpful. The British reader may also ask, has a British equivalent been filed or published?

B. J. F. Hudson

Biotechnology. Vol. 3. Biomass, Microorganisms for Special Applications, Microbial Products I, Energy from Renewable Resources. Edited by H. Dellweg.
Weinheim: Verlag Chemie, 1983. Pp. xix + 642. ISBN 3 527 25765 9. DM495.

Volume 3 of the eight volume biotechnology treatise is concerned with a range of topics grouped under four general headings: Biomass Production, Microbial Products, Micro-

organisms for Special Applications and Energy from Renewable Resources, although the different sections receive far from equal weighting. Incidentally, Volume 2 is yet to be published.

Microbiological, biochemical, engineering and economic aspects of biomass production from carbohydrates, higher *n*-alkanes, methane, methanol and sunlight are concisely outlined. The final chapter in this section deals with an interesting though generally neglected topic—edible mushrooms. The cultivation of higher fungi provides a system both for disposing of cellulosic and lignocellulosic waste and producing fertilizers and food. Under the heading of Microbial Products the biotechnology of ethanol, acids, including acetic, lactic, citric, gluconic, amino acids, extracellular polysaccharides and emulsifier and deemulsifier production is summarized. This section indeed forms a major part of the volume. The remaining hundred pages cover the other two sections—Energy from Renewable Resources and five comparatively short chapters on Microorganisms for Special Applications.

This volume forms part of a reference work which provides introductions to a range of different topics and so furnishes the reader with the basics required to tackle more exacting reviews and research literature. To the interested general reader the volume also provides an excellent indication of the scope of biotechnology. The text will therefore form a useful addition to both academic and industrial libraries.

The authors and editor are to be commended for producing a volume which is readable and informative. The organization of the material leaves the reader with a feeling of imbalance, however. The section on Microorganisms for Special Applications sits uneasy in the context of this volume and indeed the brevity of certain chapters within this section suggests that a collaborative approach may have resulted in greater cohesion. It must be stressed, however, that lack of cohesion does not detract from the individual contributions within this section which include starter cultures for milk and meat processing, the use of microorganisms for soil amelioration, nitrogen fixation and pest control.

Although the volume does not present any new material it does bring together biochemical, microbiological and engineering aspects of a range of topics in a readable manner. Since the aim of the authors was to produce a series of general theses the reference lists are not extensive but are recent, relevant and provide a source of material or further reading.

In concusion, Volume 3 of the Treatise on Biotechnology will be of interest to both specialists and the general readers and would be a useful addition to any library.

K. L. Jones

Food Research and Data Analysis. Ed. by H. Martens and H. Russwurm. London: Applied Science, 1983. Pp. ix + 535. ISBN 0 85334 206 7. £40.00

This volume contains eighteen invited papers presented at the IUFoST symposium on Food Research and Data Analysis held in Oslo in September 1982. It was an inter-disciplinary meeting for scientists interested in the computer based analysis of multivariate data encountered in food research. The general aim of the symposium was to cover both theoretical and practical aspects of relevant methods of data analysis with the declared intention that it should 'increase the number of food scientists benefiting from multivariate analysis'.

Many of the papers discuss the use of multivariate techniques through case studies. Naturally enough, the technique of multidimensional scaling features particularly prominently but there are also papers that include material on principal components analysis, factor analysis and the technique of correspondence analysis, which until recently has rarely been described outside French statistical literature. A few expository papers have also been included.

There is inevitably a large variation in the quality of the papers, but although some are quite poor, the majority are worthy of serious study. The volume has several characteristics which add to its value. The general overview of multivariate analysis by Gower entitled 'Data Analysis: Multivariate or Univariate and Other Difficulties', coupled with the 'Layman's Guide to Multivariate Data Analysis' by Martens, Wold & Martens will be useful to the newcomer to the field. Such a reader will doubtless need to refer to the self-contained section on 'Matrix Algebra for Data Analysis' written by Digby. Other attractive features of this volume include the twenty-one one-page summaries of the 'poster sessions', the extensive (though individual) bibliography of multivariate methods in food science and technology, and the indexing.

My main criticism of this book is that the food scientist who thumbs through it might well get the impression that the analysis of food science data is likely to involve the use of sophisticated statistical techniques; perhaps a symposium on the use of univariate methods should be held in the interests of balance!

The book has been produced by photographic reproduction from typescripts submitted by individual authors. The quality of presentation is high but some papers contain more than their fair share of typographical errors. Finally, whilst the editors are to be congratulated on producing this interesting volume in such a short period of time, it must be said that a food scientist wishing to learn something about multivariate data analysis would find it more useful (and cheaper) to purchase an introductory text on the subject such as: Chatfield, C. & Collins, A.J. (1980). *Introduction of Multivariate Analysis*. Chapman and Hall.

D. Collett

Handbook of Lethality Guides for Low-Acid Canned Foods. Vol. I. Conduction Heating and Vol. II. Convection Heating. By C. R. Stumbo, K. S. Purohit, T. V. Ramakrishnan, D. A. Evans and F. J. Francis.
Boca Raton, Florida: CRC Press, 1983. Pp. vi + 540 (Vol. 1), v + 522 (Vol. II). ISBN 0 8493 2961 2 (Vol. I), 0 8493 2962, 0 (Vol. II). U.S.$85.00 each volume.

These two books present lethality guides for low-acid canned foods by conduction (Vol. I) and convection (Vol. II).

In Vol. I, detailed tables are presented, giving processing times required for production of a 'safe' product, based on the chance of finding one surviving *C. botulinum* spore in every 10^{12} cans, and a 'commercially sterile' product, based on the chance of finding one surviving spore of *C. sporogenes* in every 10^4 cans.

The processing times are derived for forty-one can sizes, five heating rates (f_h), two test organisms, seven z values for *C. botulinum* and four for *C. sporogenes*, four retort temperatures and eleven filling temperatures. They are evaluated using Ball's equations and the steps involved in the evaluation are detailed.

The total integrated lethal effects for each combination of experimental conditions (*F* values) are expressed in two ways: firstly an F_c value based on heat penetration to the slowest heating point and secondly, an F_s value based on the sum of the total lethal effects at all points in the container, using a procedure suggested by Stumbo. With conduction heating packs the latter can be significantly higher than the former, due to the existence of considerable temperature gradients within the food.

The second volume deals with process time evaluation in convection packs, for producing 'safe' and 'commercially sterile' products. The calculations are simpler than those for conduction, as they are based on the slowest heating point, assuming that the contents are reasonably well mixed. The tables show that the processing times are strongly dependent on the f_h value of the product, and for products with similar f_h values the processing time is almost independent of the can size. (Note that the f_h value for a particular product will increase as the can size increases.)

In both volumes the introduction to thermal processing of low-acid foods and the thermal process evaluation procedures are clearly explained. The computer processing times are recorded in 984 tables. The tables themselves are well formatted and clearly presented; useful instructions are provided for interpreting the data therein.

It is claimed that the criteria selected for a 'safe' process and a 'commercially sterile' process are reductions of 12 log cycles for *C. botulinum* and 4 log cycles for *C. sporogenes*, respectively. However, the specimen calculations show that they are actually based on between approximately 12–15.5 log cycles for *C. botulinum* and 6–7.5 log cycles for *C. sporogenes*.

On several points, however, little in the way of guidelines is given. In connection with the production of a safe product, what *z* value should be used for *C. botulinum*? Tables are given for values ranging from 12 to 18°F and from 18 to 24°F (note the repetition), whereas most of the literature values for the different types of *C. botulinum* spores range between 12 and 18°F. Therefore one could argue that 143 of the tables are redundant. To err on the side of safety, the lowest *z* value should be chosen when selecting a processing time.

I would also be interested to know on what occasions it may be desirable to produce a safe product that is not necessarily commercially sterile. The tables clearly show that if a product is commercially sterile, there should be no problems arising due to insufficient heating of *C. botulinum* spores; in fact the reduction of these spores would be in the order of 48 log cycles. Therefore if commercial sterility is the objective, the tables for production of a safe product, although of academic interest, would be largely irrelevant to the needs of the commercial processor.

In addition, these tables are only of use to the processor who knows the heating rates (f_h) values for his particular products. Fortunately there is sufficient information required to allow f_h values to be determined experimentally. However, I feel that slightly more attention could have been devoted to f_h values with some typical values for a selection of foods, if such data are available.

Finally, in the examples for using the process lethality guide tables, it should be made clear that the computed processing time is not the operator's processing time, i.e. the time that elapses between the retort reaching the operating temperature and the steam being switched off. The computed processing time is longer than the operator's processing time by an amount equal to 0.4 times the 'come-up' time (CUT). Therefore if the operator's processing time is made equal to the computed processing time, an additional margin of safety will be introduced.

It would appear that most of the work involved in the production of these tables, has

been done by the computer. Therefore, I am slightly surprised that it has taken five people to edit this work. The two volumes cost a total of $170. For an almost similar price it is now possible to buy a micro-computer with the capacity to generate this type of processing data, although probably not so neatly.

M. J. Lewis

Lehrbuch der Lebensmittelchemie. By H.-D. Belitz and W. Grosch. Berlin: Springer Verlag, 1982. Pp. xxxviii+788. ISBN 3 540 10935 8. DM124, U.S. $49.60.

The present reviewers feel that they must preface their comments by congratulating Professors Belitz and Grosch on the production of an outstanding book on food chemistry. This book is not unique in terms of the topics covered but the authors have managed to bring together more detailed information than can be found in any other single volume.

The book is effectively divided into two sections, although they have not been designated as such, with chapters 0–9 dealing with the more important food components and chapters 10–22 covering the major commodity areas.

Chapter 0 (an unusual notation) starts sensibly with a short discussion of water and its role in food systems. This could perhaps have been expanded to include more detail of hydration mechanisms, e.g. of polysaccharides and proteins, especially as this aspect is not covered again in subsequent chapters.

The next chapter covers amino acids peptides and proteins and here the attention to detail which characterizes the remainder of the book becomes apparent. Chemical, physical and analytical aspects are all discussed with even a short section on protein sequencing. Chapter 2 extends the discussion of proteins to enzymes which is a topic which often does not receive the attention its importance in food system demands. It is refreshing to see a well explained quantitative approach to enzyme kinetics, a subject which is often avoided by authors and students alike. Analytical applications of enzymes and immobilized enzymes also receive attention. Chapter 3, on lipids, is probably the best in the book, as might be expected from the research interests of the authors. Even relatively complex reactions, such as autoxidation, are discussed in a clear and precise manner with well drawn diagrams.

In chapter 4 attention is turned to carbohydrates, which are discussed under the headings monosaccharides, oligosaccharides and polysaccharides. The chemical reactions of these sugars are discussed in detail but perhaps those reactions that lead to analytical methods could have been stressed, e.g. the inversion of sucrose.

Aroma compounds form the subject matter for chapter 6, and although the mechanisms of aroma production, both enzymic and chemical, are well covered the section on analysis is relatively limited. It is fully appreciated that this is not a book on food analysis but the single illustrative chromatogram shown (p. 268) does give the impression that aroma mixtures are relatively simple; perhaps reference to capillary gas chromatography/mass spectrometry, where hundreds of compounds have been isolated from beverages (e.g. coffee), would have helped balance the picture.

The next two chapters on vitamins and minerals appear rather short in relation to their importance. The major vitamins are discussed in terms of function, occurrence

and stability but only in outline. In the section heading for vitamin B_6 pyridoxin is erroneously equated to pyridoxal rather than pyridoxol. Chapter 7 on minerals simply outlines the major essential elements. The final two chapters in this section turn to food additives and contaminants. Here again excellent coverage is achieved and perhaps it is unreasonable to expect a detailed discussion of all components. However, ten pages on pesticide residues does seem out of balance with a mere page and a half on mycotoxins.

Chapter 10 starts the unstated part 2 of the book where commodities are considered. There is not space in a review of this kind to describe these chapters in detail other than to list the areas covered: milk and milk products, meat, fish, oils and fats, cereals, pulses, vegetables, fruits, sugar, alcoholic beverages, coffee, tea and cocoa and condiments. In general the material selected for these areas is excellent, though in some cases more data are presented than are necessary, for example amino acid compositions of caseins (Table 10.6) or of cereals (Diagram 15.4). The chapter on meat contains a particularly lucid description of muscle function together with some elegant photomicrographs. The chapter on alcoholic beverages is also well written with much background material, although the nomenclature of the grape types could have been clarified.

The above comments must be taken within the overall opinion that this is an excellent well written book, which will be ideally suited to those students, probably in the second or third year of an Honours Course, who require a single text to cover most aspects of food chemistry. In addition to well written accounts of the chemistry of the major food components, both in general terms and also in specific food areas, there is much peripheral material, such as physical properties, physiological roles, sensory attributes, etc., which helps to emphasize their significance in food systems.

The book is expensive for a student text and the publishers are strongly urged to consider publication in paperback form when it is translated into English, something which must be undertaken as a priority.

R. Macrae & Jane Robinson

Books received

Atlas der Gärungsorganismen. By M. Glaubitz and R. Koch.
Berlin: Paul Parey, 1983. Pp. 91. ISBN 3 489 61614 6. DM 48.

This book consists of ninety-nine diagrammatic representations of the microscopic appearances of micro-organisms considered to be commonly encountered in the fermentation and food industries, together with a brief paragraph describing some of the key characteristics of each. In some cases pure cultures are depicted, in other cases—especially relating to naturally fermented products such as sauerkraut, yoghurt and kefir—the total microbial association is shown.

Recommended Health-based Occupational Exposure Limits for Selected Vegetable Dusts. Report of a WHO Study Group (Technical Report Series No. 684). Geneva: World Health Organization, 1983. Pp. 78. ISBN 92 4 120684 5. SFr 6.

Information and data are presented on the effects of, responses to, and environmental health criteria on the various vegetable dusts (cotton, flax and soft hemp) which cause byssinosis.

Teach Yourself Chemistry, 4th ed. By J.S. Clarke.
Sevenoaks: Hodder and Stoughton, 1983. Pp. viii+192. ISBN 0 340 27583 9. £2.50 (paperback).

The object of this book is to provide a text suitable for those studying the subject at an elementary level and for those who 'in later life wish to gain some appreciation of the subject as a whole'.

R & D at the CFTRI: The First Three Decades 1951–1980. Ed. by M. R. Raghavendra Rao, K. R. Bhattacharya and J. V. Shankar.
Mysore, India: Central Food Technological Research Institute, 1982. Pp. vii+360. Rs 100.

An account is given of the research and development work carried out in the CFTRI since its inception. The work is discussed under four main headings: Commodity Research (foodgrains, oilseeds and unconventional sources of protein and fat, foods of animal origin, fruits and vegetables, plantation products and food flavours): Multi-commodity Research (control of pests of stored foods, food packaging, food engineering, sensory evaluation, microbiology, biochemistry, nutrition); Formulated Foods and Beverages (infant foods, weaning foods, supplementary foods, protein-rich foods, convenience foods, sweets and snacks, beverages, vegetable milks and soft drinks); 'Other Essential Activities' (information centre, animal house, special instruments and analytical facilities, training). There is a thirty-page index and references are provided to the papers published.

Report of the Hannah Research Institute, 1982.
Ayr: Hannah Research Institute, 1983. Pp. xx+128. ISSN 0301 6315.

This includes research reports on milk production, milk composition, milk utilization and analytical methods and standards. Aspects covered include the hygienic quality of raw milk, heat stability of milk, cheese yield in relation to milk composition, milk calcium phosphate, factors affecting casein aggregation, rennet-induced aggregation of casein-micelles, chemistry of heated milk proteins, and HPLC of milk proteins. There are four review articles, one of which concerns the role of insulin receptors in insulin action.

Report of the Houghton Poultry Research Station, 1981–82.
Huntingdon: Houghton Poultry Research Station. Pp. 95.

Topics covered in research reports include: immune responses in the intestine to *Escherichia coli*; rapeseed hepatotoxicity; factors influencing excretion and spread of *Salmonella*; transmissible plasmids in *E. coli*; and egg taint from fish meal.

Report of the Moredun Research Institute, 1981–82.
Edinburgh: Moredun Institute (Animal Diseases Research Association). Pp. 108.

This includes reports on rotavirus and *E. coli* as causes of diarrhoea in calves, and *Toxoplasma gondii* in sheep.

Report of the Rowett Research Institute, 1981.
Aberdeen: Rowett Research Institute, 1982. Pp. 137. ISBN 0 7084 0261 5. £4.00.

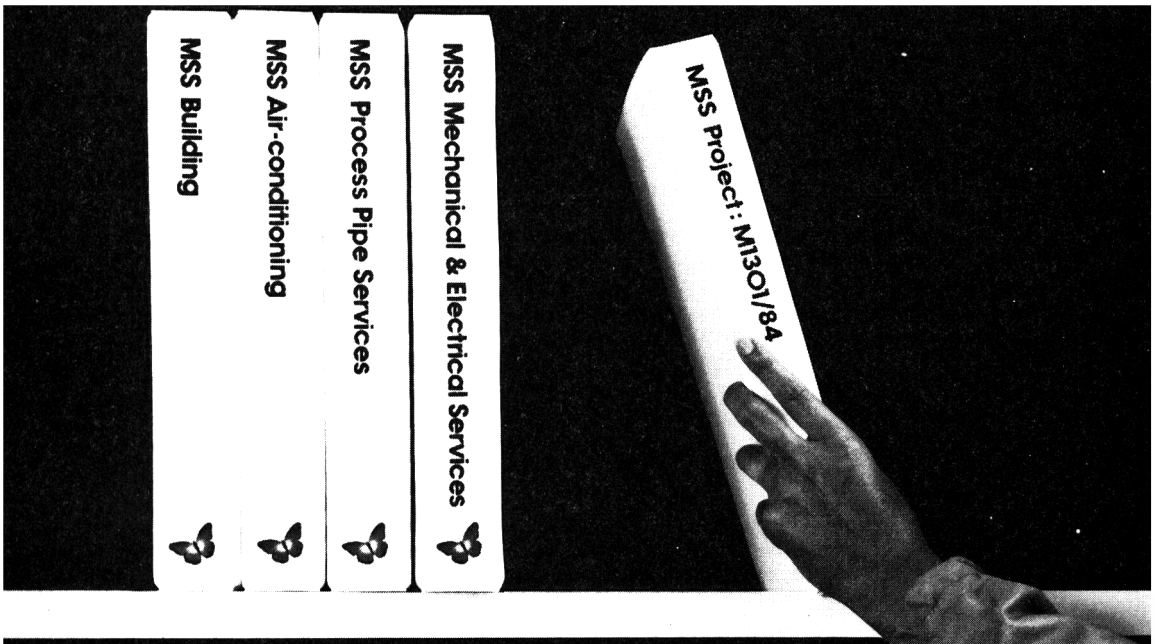
Topics covered in the research reports include: fungal cellulase and xylanase; lectins in seeds, and nutritional toxicity and cytopathology of bean lectins.

Report of the British Food Manufacturing Industries Research Association, 1982.
Leatherhead: Leatherhead Food R.A., 1982. Pp. 76.

Research work summarized in this report includes: amino acid analysis of citrus juices; use of photoelasticity in texture research; use of milk proteins in chocolate couvertures; tempering characteristics of chocolate; HPLC analysis of vitamins; HPLC of natural colours in foods; effects of food processing on ochratoxin A; adhesion of food to heated surfaces; pulsed NMR studies of water in meat and polymorphism in cocoa butters; and mathematical modelling of process control systems.

Report of the Letcombe Laboratory, 1981.
Wantage: Letcombe Laboratory, 1982. Pp. ix + 90. ISBN 0 7084 0257 7. £2.50 (from H.M.S.O.).

Report of the National Vegetable Research Station, 1982.
Wellesbourne, Warwick: British Society for the Promotion of Vegetable Research, 1983. Pp. 176. £3.25 (post paid).



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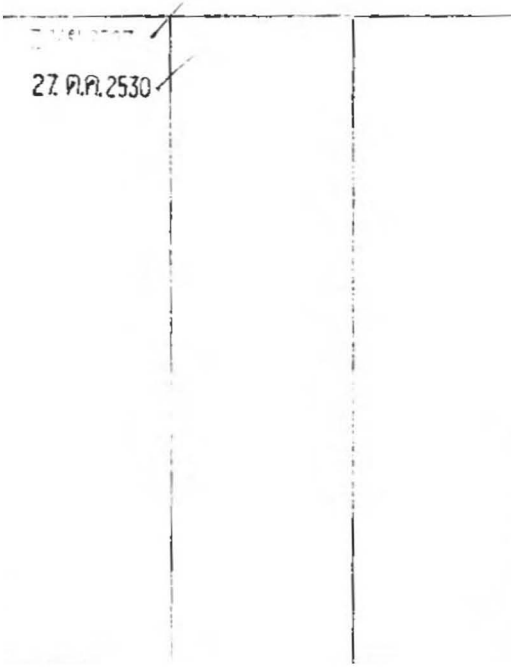
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JOURNAL OF FOOD TECHNOLOGY: NOTICE TO CONTRIBUTORS

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Steiner, E. H. (1966). Sequential procedures for triangular and paired comparison tasting tests. *Journal of Food Technology*, , 41–53.

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Hawthorn, J. (1980). Scientific basis of food control. In *Food Control in Action* (edited by P. O. Dennis, J. R. Blanchfield & A. G. Ward). Pp. 17–33. Barking, Essex: Applied Science.

Standard usage. The *Concise Oxford English Dictionary* is used as a reference for all spelling and hyphenation. Statistics and measurements should always be given in figures, i.e. 10 min, 20 hr, 5 ml, except where the number begins the sentence. When the number does *not* refer to a unit of measurement, it is spelt out except where the number is one hundred or greater.

Abbreviations. Abbreviations for some commoner units are given below. The abbreviation for the plural of a unit the same as that for the singular. Wherever possible the metric SI units should be used unless they conflict with generally accepted current practice. Conversion factors to SI units are shown where appropriate.

SI UNITS

gram	g	Joule	J
kilogram	kg = 10^3 g	Newton	N
milligram	mg = 10^{-3} g	Watt	W
metre	m	Centigrade	°C
millimetre	mm = 10^{-3} m	hour	hr
micrometre	μ = 10^{-6} m	minute	min
nanometre	nm = 10^{-9} m	second	sec
litre	l = 10^{-3} m^3		

Chemical formulae and solutions. The correct molecular formula should be used indicating whether the substance is anhydrous or hydrated. Solutions should be given in terms of molarity. The arithmetic basis of other expressions of concentration must be clearly defined.

Species names. In addition to common or trivial names of plants, animals or microorganisms, the species should normally also be indicated by use of the latin binomial at least once. This should be given in full and italicized (underlined once in typescript). In subsequent use, the generis name may be abbreviated to a single letter provided that this does not result in ambiguity.

Figures. In the text these should be given Arabic numbers, e.g. Fig. 3. They should be marked on the backs with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of a figure the top should be marked with an arrow. Each figure must be the reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Line diagrams should be on separate sheets; they should be drawn with black Indian ink on white paper and should be about four times the area of the final reproduction. Lines and lettering should be of sufficient thickness and size to stand reduction to one-half or one-third. Whenever possible, the originals of line diagrams, prepared as described above, should be submitted and not photographs. The legends of all the figures should be typed together on a single sheet of paper headed 'Legends to figures'.

Tables. There should be as few tables as possible and these should include only essential data; the data should not be crowded together. The main heading should be in bold with an Arabic number, e.g. **Table 2.** Each table must have a caption in small letters. Vertical lines should not be used.

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