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The effect of glutathione on the cohesiveness of pounded cassava, cocoyam, gari and yam

G. O. OSUJI

Summary

In order to understand the part played by glutathione on the cohesiveness of the tropical staple items of food, pounded cassava, cocoyam, gari and yam, varying amounts of glutathione were added to the foods after which the resistance to shear stress (cohesiveness) of each food was determined. In each pounded food the added glutathione immediately increased the food's cohesiveness. There was also a linearly increasing relationship between the foods' cohesiveness and their endogenous glutathione contents on one hand and between the foods' cohesiveness and foods' protein contents on the other hand. Glutathione, therefore, increases the cohesiveness of tropical pounded foods by interacting with their proteins which then assume new conformations that increase the cohesiveness of the foods.

Introduction

The cohesiveness of pounded cassava, cocoyam, gari and yam determines the acceptance of these tropical staple items of food by their consumers. Whereas pounded cassava and gari can retain their cohesiveness many hours after they have been prepared, pounded cocoyam and yam lose theirs a few hours after their preparation. In many African homes, if the latter pair of foods are not eaten soon after their preparation, they are discarded as a result of the loss of cohesiveness thus leading to food wastage.

When it is realized that the tubers of cocoyam (*Xanthosoma sagittifolium* (L.) Scott) and especially of yam (*Dioscorea* spp.) suffer a phenomenal post-harvest decay (Coursey, 1961; Booth, 1974; Passam, Read & Rickard, 1976a) the additional wastage of tropical staple foods after they have been pounded shows that the overall wastage of food in the tropics could be alarming. Whereas concerted efforts are being made to understand the causes

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of the post-harvest decay of tropical root tubers (Passam, Read & Rickard, 1976b; Ige & Akintunde, 1981; Osuji, 1981) only little attempt has been made to study the causes of the loss of cohesiveness of pounded cocoyam and yam (Osuji, 1980a; Cruz-Cay & Gonzalez, 1974).

One of the attempts made to understand the biochemical reactions associated with the loss of cohesiveness (softening) in pounded cocoyam and yam implicated the tubers' thermostable γ-glutamyl transpeptidase, which continued to degrade the glutathione (GSH) in the pounded foods, thereby softening the foods and encouraging souring (Osuji, 1980a). In the present investigation, the aim was to find out the exact effect of glutathione on the cohesiveness of pounded foods.

Materials and methods

The tropical staple root tubers, *Dioscorea rotundata* Poir (white yam) and *X. sagittifolium* (cocoyam) were freshly harvested from the University of Nigeria farm and sometimes purchased from a local market. Gari and fermented cassava (which are prepared from the tubers of *Manihot esculenta* Crantz) were purchased from a local market.

Preparation of pounded foods

The methods used for preparing pounded yam, cocoyam, gari and cassava are those traditionally used in eastern Nigeria. Yam and cocoyam tubers were peeled, washed, cut into small lumps, and after weighing (100 g) were boiled separately for between 15 and 30 min. They were pounded separately with pestle and mortar, water being added during the pounding until adequate cohesiveness of the food was achieved. The weight of the pounded food was 150 g in each case.

For pounded gari, known weight of the gari (100 g) was stirred in appropriate volume of boiling water until adequate cohesiveness was achieved. The weight of the gari food was 200 g.

Fermented cassava (100 g) was moulded into apple-sized balls, boiled for about 15 min and pounded with pestle and mortar to improve its cohesiveness; then moulded into balls again, boiled for about 30 min and pounded to achieve the required final cohesiveness. The weight of the cassava food was 300 g.

The pounded foods were immediately used for the experiments described below.

Extraction and assay of the glutathione and proteins of the pounded foods

The extraction of the glutathione and soluble proteins were as described earlier (Osuji, 1980a). Essentially known weight of each pounded food was

blended with four times its volume of ice-cold 0.05 M $NaHCO_3$ in a food blender at maximum speed for 3 min. The blended food was centrifuged at 5000 *g* for 15 min and the supernatant used for the assay of glutathione and proteins. The glutathione was assayed by the method of Owens & Belcher (1965). Protein was assayed by the method of Lowry *et al.* (1951), using bovine serum albumin as standard.

Determination of the cohesiveness of the pounded foods

For the cohesiveness of each pounded food, its resistance to shear stress was determined on a pully-type shear frame. Each pounded food was compressed to a thin film between the two plates of the pully frame; any pieces of food protruding outside the plates were cleanly removed from the edges of the plates. Then the upper movable plate was connected via the pulley to a variable weight load. The food's resistance to shear stress was calculated in Newtons/m^2 from the weight loads that produced the shearing of the food.

Then reduced glutathione (10, 30, 60 and 100 nmol/ml) were added to known weights (10 g) of each food and thoroughly mixed by pounding. Parallel control experiments were carried out and to each of these, 1 ml distilled water was added for every 10 g pounded food. The resistance to shear stress of each pounded food was determined immediately after glutathione addition.

Results and discussion

The African traditional methods which were used for preparing the pounded foods in this investigation made it necessary to use the pully-type shear frame instead of the Brabender texture instruments for determining the resistance to shear stress of the pounded foods. It was not practicable for instance to prepare the pounded cassava in the Brabender bowls. The traditional method involves a thorough manual pounding of the food in order to achieve the required cohesiveness.

Figure 1 shows that the resistance to shear stress (cohesiveness) increased linearly with the endogenous glutathione contents of the pounded foods. Thus, pounded yam with the highest content of glutathione (3.2 μmol/g dry tuber) gave the highest resistance to shear stress while pounded cassava with the lowest glutathione content (0.99 μmol/g dry fermented cassava) gave the lowest resistance to shear stress. The yam and cocoyam tubers used for the experiments in Fig. 1 were freshly harvested. With tubers which were not freshly harvested, the glutathione and also the resistance to shear stress of their pounded foods were considerably lower as shown at the zero concentration of added glutathione in Fig. 2.

The changes that occurred in the cohesiveness (resistance to shear stress) of pounded food when glutathione was added are shown in Fig. 2. In each

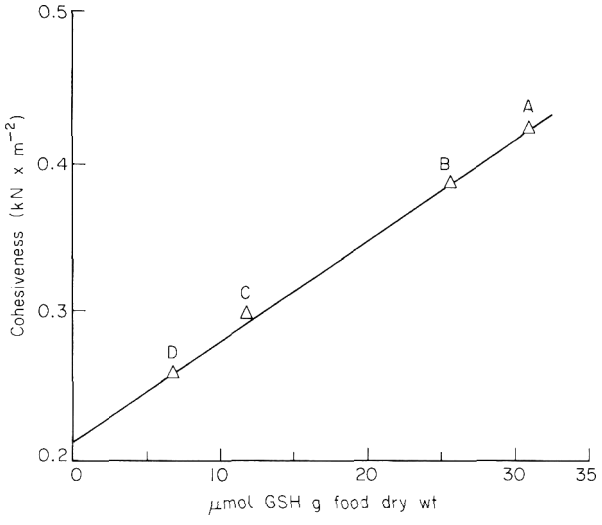

Figure 1. The effect of the level of the endogenous glutathione on the cohesiveness of (A) pounded yam; (B) cocoyam; (C) gari and (D) cassava. Food's cohesiveness was measured as its resistance to shear stress.

pounded food the resistance to shear stress was elevated by the added glutathione. As little as 1–2 μmol added to 1 g pounded food was sufficient to give the maximum elevation of the resistance to shear stress.

Glutathione has been shown to affect the rheological properties of bread dough (Jones & Carnegie, 1969) and it does this by interacting with the proteins of the dough (Coventry, Carnegie & Jones, 1972). A similar influence by glutathione could be responsible for the elevation of the cohesiveness of the pounded cocoyam, cassava, gari, and yam. The interaction of glutathione with proteins causes the cleavage of protein

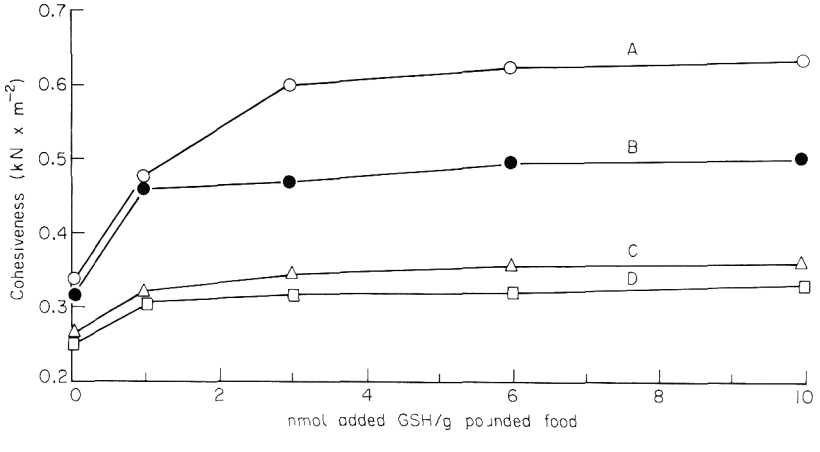

Figure 2. The effect of added glutathione on the cohesiveness (resistance to shear stress) of (A) pounded yam; (B) gari; (C) cassava and (D) cocoyam.

disulphide bonds and the formation of mixed disulphides (Proteins S–SG). Such glutathione-bound proteins unfold themselves thereby exposing their hydrophobic regions (Jones & Carnegie, 1969). In the pounded food therefore it is the exposure of the hydrophobic regions of its proteins upon the addition of glutathione that leads to the elevation of the resistance to shear stress. Thus glutathione exerts its effect in pounded food as in bread dough by maintaining proteins in conformation in which intermolecular hydrophobic interactions are maximized.

Figure 3 shows that the resistance to shear stress increases linearly with the protein contents of the pounded foods similar to the results in Fig. 1. This therefore supports the interrelationship between glutathione, protein and the cohesiveness of pounded foods. This protein–glutathione interaction in the foods is also shown in Fig. 2, each curve representing a titration plot between the glutathione and proteins.

The earlier result (Osuji, 1980a) which implicated yam tuber and cocoyam tuber γ-glutamyl transpeptidase in the softening of pounded yam and cocoyam now becomes easily explained in that the thermostable γ-glutamyl transpeptidase by continuing to degrade the glutathione in the food decreased the concentration of Proteins S–SG thereby lowering the hydrophobic changes, the factor responsible for maximizing the cohesiveness of the pounded foods. This reason also explains why pounded cassava and gari do not lose their cohesiveness as quickly as pounded cocoyam and yam because whereas the latter pair of foods have active γ-glutamyl transpeptidase, the former pair have no active γ-glutamyl transpeptidase. The fermentation process through which cassava tubers are passed during the preparation of gari and fermented cassava (Collard & Levi, 1959) deactivates the cassava tuber γ-glutamyl transpeptidase. So pounded cassava and gari do not have any active γ-glutamyl transpeptidase and consequently their glutathione contents are not degraded and they do not suffer a significant decrease of their cohesiveness.

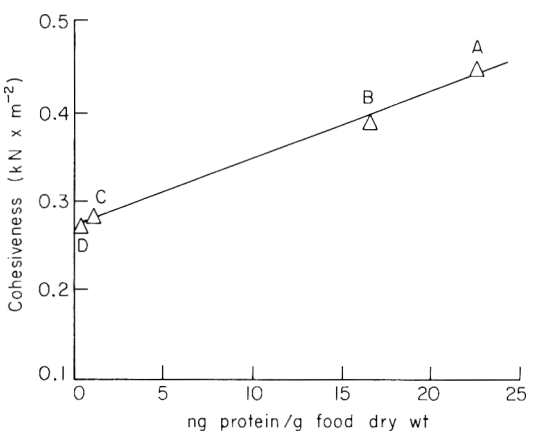

Figure 3. The relationship between the level of endogenous protein and the cohesiveness (resistance to shear stress) of (A) pounded yam; (B) cocoyam; (C) gari and (D) cassava.

The concentrations of glutathione and protein are however not the only factors that control the cohesiveness of the pounded foods. As shown in Figs 1 and 3, at the zero concentrations of glutathione and protein respectively, the cohesiveness had a value of about 0.25 kN × m^{-2}, which should be largely contributed by the carbohydrates of the food. This conclusion is in agreement with the findings of Ayernor (1976) who attributed the rheological properties of pre-gelled yam to the degree of damage suffered by the starch of yam tuber during the pre-gelling process. There is however experimental evidence (Osuji, in prep.) which shows that the rapid textural changes described as retrogradation (Coursey & Ferber, 1979) of the starch of freshly pounded yam are not affected by GSH.

The practical importance of these results is that if increased levels of glutathione are not toxic to mammals, then a simple method can now be formulated for preserving pounded cassava, cocoyam, gari and yam. Such a preservative method would help to minimize the wastage of cooked staple food in the tropics. Glutathione is however a normal cell constituent, performing many metabolic functions (Osuji, 1980b; Meister, 1981).

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Preliminary studies on the chemical and weight composition of some commercially important species of fish and shrimp caught in the Nigerian inshore waters

A. O. EMOKPAE

Summary

The paper mainly deals with the chemical composition of commercially important marine fish of Nigeria in relation to their nutritive values. The selection is restricted to those species which can readily be obtained by standard fishing techniques, and whose biological characteristics can be studied throughout the year. The paper also includes a study of the changes in the composition according to season of the year. In addition, the work was further extended to an investigation of the carcass composition (i.e. the percentage flesh, head, skin, tail, bones, liver and entrails).

Introduction

The tropical West African coastline is rich in a diversity of fish species. This is evident from the landing of a typical haul that would contain no less than fifteen families of fish (Tobor, 1978). These diverse species of fish are distributed ecologically into two faunas, or assemblages by the location of the thermocline which is between 40–60 m depth. These two faunas do not mingle (Longhurst, 1961, 1964). The first fauna consisting of predominantly grey or silvery fish of the families *Sciaenidae* (croakers), *Polynemidae* (threadfins), *Pomadasydae* (grunters), *Ariidae* (catfishes), *Cynoglossidae* (soles) and *Clupeidae* (herrings and sardines) occurs only in the water above the bottom of the thermocline and on soft muddy deposits, it is relatively uniform in specific composition from Guinea to Gabon, and shows no gross bathymetric variation in composition.

The second fauna consists of predominantly red or brown fish of the families including *Sparidae* (breams), *Lutjanidae* (snappers), *Triglidae* (gurnards), *Mullidae* (mullets) and *Priacanthidae* (Atlantic big-eye) and occurs

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both above and below the thermocline—above in the tropical surface water, it is restricted to hard sandy and corally deposits, while below in the colder South Atlantic central water, it occurs on both hard and soft deposits (Longhurst, 1964).

The soft mud deposits of the tropical West African coastline are also generally abundant in large penaeid prawns which are found either in estuaries or offshore. Most of the commoner penaeid prawns, *Penaeus duorarum* and *P. kerathurus* and the smaller *Penaeopsis miersi* appear to be restricted to the area above the thermocline offshore. The relatively small *Parapenaeopsis atlantica* appears to be the common shrimp of the estuaries and brackish water areas (Longhurst, 1964).

The Nigerian demersal trawl fisheries are based on the first fauna group of species and on the penaeid shrimps.

The fishery for fish operates 80 km to the west and about 100 km to the east of Lagos port. The shrimp fishery operates east of the Benin River to the Nigerian/Cameroun border. However the standing crop of demersal fish is relatively rather poor when compared with the areas to the west, from Ghana to Senegal and to northern and southern cold water areas of West Africa.

In his study of the general demersal trawl fishery in the inshore waters of Nigeria, Tobor (pers. comm.) gave the Lagos inshore trawler landings from 1968–77 as shown in Table 1.

The category 'miscellaneous' includes a variety of small sized species, juveniles of larger species and relatively valueless fish. Species making up the miscellaneous group include *Larimus peli, Chloroscombrus chrysurus, Sciaena mbizi, Caranx* sp., *Elops lacerta, Saurida parri, Ilisha africana, Gerres melanopterus, Cynoglossus monodi,* and small individuals of *Trichiurus lepturus* and *Drepane africana.* It is not uncommon to find few red snappers (family *Lutjanidae*) and sparids (family *Sparidae*) among miscellaneous fish because these fish migrate up and down across the thermocline. Fishermen often do not separate these fish into separate groups when few numbers are caught.

Croakers account for an average of 42.9% by weight of total average annual landings as seen from Table 1. They thus form the mainstay of the Nigerian fishery over the years.

As discussed above, Nigeria possesses a diversity of species. There is a need to determine their comparative food value and conversion into suitable fishery products. But there is paucity of information on the chemical and weight composition of Nigerian fish. In the industrial processing of fish, a thorough knowledge of their chemical composition and of their availability is of paramount importance.

Similarly information regarding the chemical composition of West African fish is limited to studies on *Ethmalosa dorsalis* from Sierra-Leone (Stevens, 1945; Watts, 1957) a brief survey of the meals from some thirty-three marine species from Sierra-Leone (Watts, 1958). *Ethmalosa fimbriata, Sardinella eba* and *Sardinella aurita* from Senegal (Mainguy & Doutre, 1958) and to three

Table 1. Lagos inshore trawler landings* 1968–77 (millions of tons)

Species/ groups	1968	1969	1970	1971	1972	1973	1974	1975	1976	1977	Average annual landing	Average annual landing (%)
Croakers	184.6	249.2	268.3	315.4	500.0	838.6	1350.7	1823.0	2349.2	3918.8	1179.8	42.9
Big eye	59.4	81.6	71.6	93.5	84.0	136.3	225.2	242.7	224.0	237.7	145.6	5.3
Soles	27.9	20.3	33.0	48.7	46.3	93.1	120.1	117.0	135.1	226.6	86.8	3.2
Cat fish	12.0	25.7	28.0	63.5	50.4	85.4	135.8	146.5	144.2	159.4	85.1	3.1
Moon fish	20.3	2.1	10.0	—	4.6	2.2	103.2	173.5	218.6	266.1	80.1	2.9
Crustaceans	38.5	26.5	24.9	31.7	39.6	66.7	75.6	82.9	83.0	130.9	60.0	2.2
Thread fins	18.3	7.3	10.9	12.5	10.2	29.5	81.5	70.8	123.0	234.2	59.8	2.2
Rays	17.9	33.9	34.0	49.0	35.5	39.3	65.9	71.5	68.3	95.9	51.1	1.8
Grunters	16.9	20.6	20.5	42.2	31.6	41.9	61.6	63.5	58.8	60.8	41.8	1.5
Sharks	1.1	1.5	7.7	30.3	26.5	41.6	67.5	76.0	72.2	73.2	39.8	1.4
Spade fish	12.6	0.5	0.6	0.2	0.5	0.1	2.1	49.4	105.4	209.7	38.1	1.4
Miscellaneous	288.6	284.9	332.8	404.4	573.6	636.8	975.6	1311.7	1883.2	2135.6	882.7	32.1
Total	698.1	754.1	842.3	1091.4	1402.8	2011.5	3264.8	4228.5	5465.0	7749.4	2750.8	100.0

* Trawl landings were supplemented by cast net, gillnet and driftnet catches of local fishermen (Tobor, pers. comm.).

demersal species—*Drepane africana, Pseudotolithus elongatus* and *Pristipoma jubelini* (Watts, 1960). In view of the lacking information in Nigeria, it would be of interest to examine those fish species of commercial importance to the Nigerian fishery.

As a prelude to determining the nutritive value of these fish and to obtaining information applicable to the development of fish processing, this paper reports a study of the seasonal variation in the chemical composition and also a determination of the weight composition of twelve species of fish and shrimp which usually form the bulk of the inshore trawl catches from the Nigerian inshore waters.

These selected species are *Pseudotolithus typus* (long croaker), *Brachydeuterus auritus* (big eye), *Cynoglossus browni* (sole), *Arius latiscutatus* (marine catfish), *Arius heudeloti* (marine catfish), *Pentanemus quinquarius* (threadfins), *Chloroscombrus chrysurus* (bumper), *Ilisha africana* (herring), *Caranx hippos* (Jack or horse mackerel), *Trichiurus lepturus* (silver or ribbon fish), *Carcharias taurus* (shark) and *Penaeus duorarum* (pink shrimp).

Materials and methods

The fish used in this survey were caught by the Research Vessels *R.V. Kiara* and *M.V. Federal Argonaut* fishing with granton trawl nets in the Nigerian inshore waters at various depths ranging from 5 to 11 fathoms. The territory fished was between 0.5–4 nautical miles off the Lagos coast.

From the landings of fish caught in a year between January and December, fifty specimens of each selected species of a particular size range as shown in Figs 1–12 were analysed weekly for 12 months. Whole cleaned and gutted fish after being finely minced and mixed together were used as samples for the different chemical analyses.

Proximate composition

Oil content was determined according to the Bligh & Dyer technique (1959). The water content was determined by drying to a constant weight in an oven, 5 g fish for 24 hr at 100°C, and the ash content by incineration in a furnace of 5 g fish at 600°C for 2 days. The protein content was determined as N×6.25 using the Kjel-Foss automatic protein nitrogen analyser (Foss 16210). All the above estimations were performed in duplicate.

Determination of weight composition

Twenty specimens (of the average weight shown in Table 2) of each selected species were used in the determination of weight composition.

In determining the weight composition the parts of the fish were separated according to its anatomy: the fish was scaled, fins and head cut off, the belly

Table 2. Weight composition of some commercially important marine fish and shrimp

Species	Average weight of fish (g)	Average weight composition (% weight of whole fish)							Total weight (%)
		Flesh (with skin on)	Head	Skeleton and other bones	Fins	Scales	Visceral organs	Roes	
Ilisha africana	69.9	56.0 (55.88–56.98)*	14.6 (12.97–16.93)	15.3 (14.65–16.06)	1.5 (1.06–1.98)	2.7 (2.01–3.08)	3.5 (2.86–4.01)	3.2 (2.92–3.78)	96.8
Trichiurus lepturus	249.5	65.4 (64.87–66.91)	12.8 (12.01–12.98)	11.5 (9.29–13.04)	2.1 (1.93–2.79)	—	3.9 (3.52–4.29)	0.6 (0.55–0.85)	96.3
Pentanemus quinquarius	46.8	58.1 (55.63–60.48)	16.9 (14.28–19.57)	8.3 (7.60–9.45)	3.9 (3.17–4.68)	—	4.0 (2.60–5.02)	1.5 (1.02–1.99)	92.7
Chloroscombrus chrysurus	84.6	55.2 (51.95–58.68)	14.3 (11.74–16.89)	14.3 (13.57–15.25)	1.4 (1.25–1.47)	1.0 (0.90–1.33)	2.3 (1.08–3.08)	2.7 (1.98–3.88)	91.2
Caranx hippos	102.3	55.1 (54.93–55.23)	20.1 (19.59–20.57)	11.9 (11.03–12.91)	2.1 (1.21–2.90)	1.0 (0.75–1.24)	4.6 (4.11–5.15)	—	94.8
Cynoglossus browni	224.9	65.19 (62.32–69.50)	11.1 (10.04–12.58)	12.8 (11.98–13.04)	0.8 (0.78–0.95)	1.8 (1.50–1.98)	0.7 (0.60–0.69)	—	92.4
Brachydeuterus auritus	92.8	41.4 (38.07–46.47)	26.6 (21.28–28.67)	9.9 (8.40–11.72)	2.6 (1.34–3.20)	5.4 (4.68–6.13)	3.3 (2.08–4.82)	3.4 (2.98–4.01)	92.6
Pseudotolithus typus	106.9	60.9 (58.04–63.36)	16.5 (15.00–17.33)	10.4 (8.59–13.07)	1.1 (0.87–1.54)	1.7 (1.15–2.53)	5.5 (4.52–6.78)	—	96.1
Arius heudeloti	202.8	49.5 (46.91–52.78)	26.6 (25.02–27.98)	4.9 (3.78–5.05)	2.2 (2.00–2.84)	—	12.9 (11.45–14.62)	—	96.1
Arius latiscutatus	136.3	47.0 (43.99–50.00)	28.9 (24.41–31.39)	6.0 (5.39–6.45)	2.1 (1.43–3.56)	—	9.9 (8.78–10.77)	—	93.9
Carcharias taurus	2500.3	58.0 (55.48–59.55)	10.9 (10.01–11.61)	5.2† (4.88–6.78)	2.3 (2.01–4.66)	7.3‡ (5.79–9.45)	4.9 (3.95–6.54)	4.6§ (3.1–5.61)	93.2
Penaeus daorarum	62.3	42.2 (40.11–44.07)	30.7 (29.46–31.46)	3.5¶ (2.12–4.95)	9.2** (8.81–9.59)	—	4.7 (3.45–5.25)	—	90.4

* Numbers in parentheses are the ranges for carcass analysis.
† Skeleton and cartilage.
‡ Skin.
§ Liver.
¶ Exoskeleton.
** Appendages and tail.

slit and the visceral organs removed and the roes, if present, were separated from the visceral organs: the headless fish was then filleted by separating the flesh from the bone. Finally the weight of each separated portion was then determined and its percentage of the whole weight of fish calculated.

Results and discussion

Figures 1–12 portray the seasonal variation in the concentration of the major chemical components of the fish. No general trend was readily discernible in compositional changes in the components. In *Ilisha africana* (Fig. 1), the fat content gave a peak in July with a corresponding low water content. On the

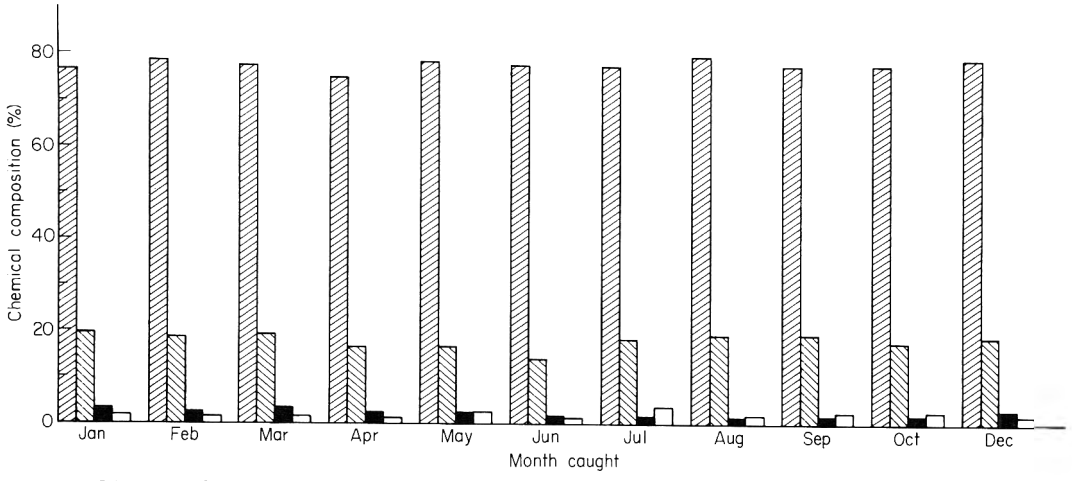

Figure 1. Seasonal variation in the average chemical composition of *Ilisha africana* (length range = 19–22 cm; weight range = 65–75 g. ▨, Water content; ▧, protein content; ■, ash content; □, fat content.

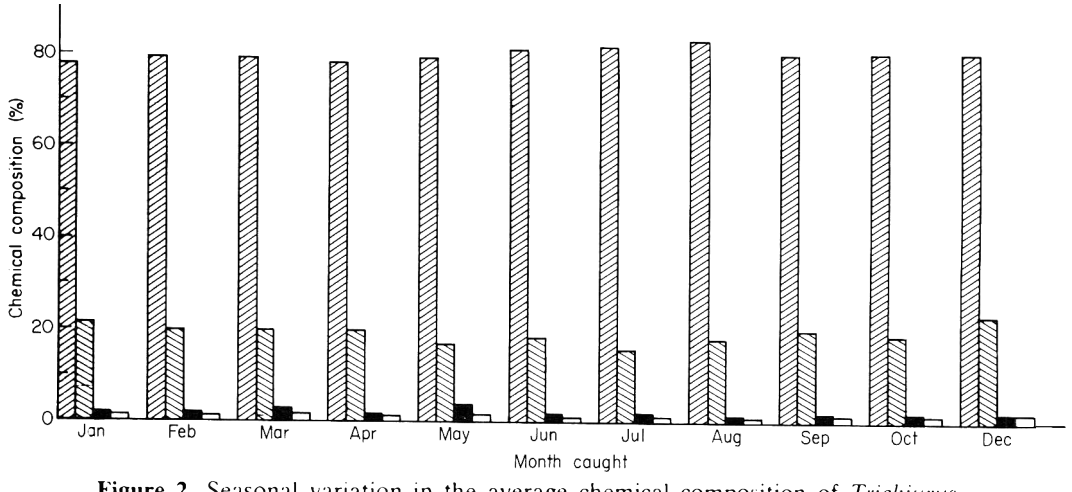

Figure 2. Seasonal variation in the average chemical composition of *Trichiurus lepturus* (length range = 40–44 cm; weight range = 240–253 g). Key as for Fig 1.

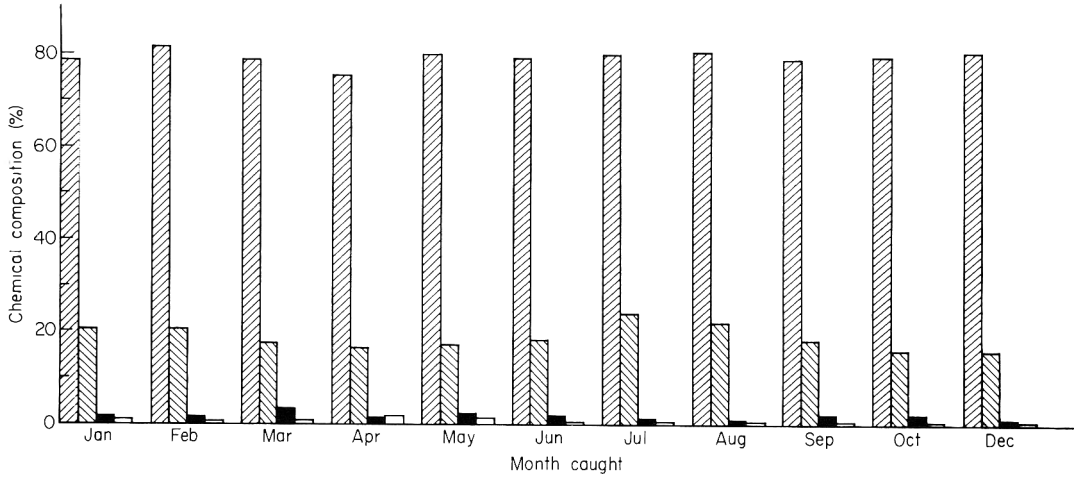

Figure 3. Seasonal variation in the average chemical composition of *Pentanemus quinquaries* (length range=12–16 cm; weight range=35–50 g). Key as for Fig. 1.

other hand *Trichiurus lepturus* had a fairly constant fat content throughout the year. However in August the fat content was very low and this was responsible for the increase noticed in its water content (Fig. 2). In Fig. 3, *Pentanemus quinquarius* showed an increase in its fat content during April and May. In Fig. 4, the fat content of *Chloroscombrus chrysurus*, increased steadily during the period August to December. *Caranx hippos* gave a peak in its fat content only in December (Fig. 5). The fat content for *Cynoglossus browni* was strikingly low and steady throughout the year (Fig. 6). *Brachydeuterus auritus* gave a peak in its fat content in August, the value decreased sharply in May and then rose towards the end of the year (Fig. 7). In *Pseudotolithus typus*, the fat content increased during August to December. However a striking

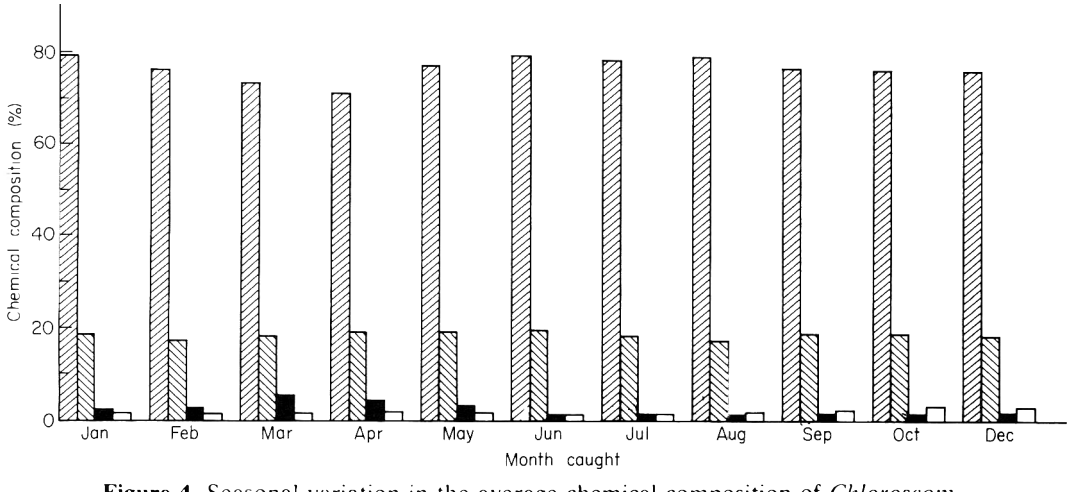

Figure 4. Seasonal variation in the average chemical composition of *Chloroscombrus chrysurus* (length range =16–20 cm; weight 80–95 g). Key as for Fig. 1.

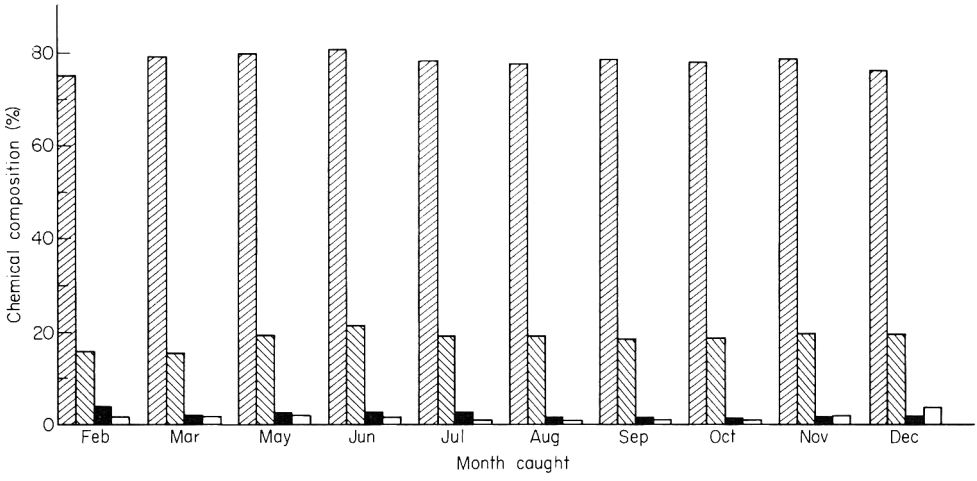

Figure 5. Seasonal variation in the average chemical composition of *Caranx hippos* (length range = 18–22 cm; weight range = 90–110 g). Key as for Fig. 1.

decrease was observed during June to July (Fig. 8). The *Arius* sp. had a high fat content in the early part of the year between January and March (Figs 9 and 10). The fat content of *Penaeus duorarum* was fairly constant throughout the year (Fig. 12).

It can be seen from Figs 1–12 that the proximate composition of the flesh of almost all the species is that of a typical lean flesh with a relatively low fat content. One main point arising from this observation is that the generally low proportion of fat in the fish may render them easily digestible. Also the low fat will augur well for the possibilities of developing a low fat protein concentrate. It appears from the determination of the proximate composition that the fat content bears an inverse relationship to the water content in almost all the

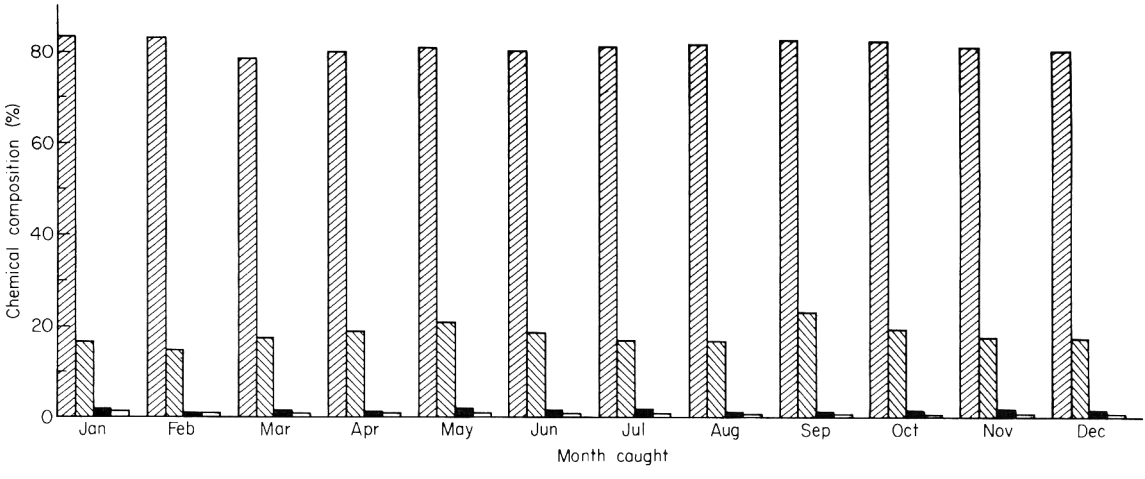

Figure 6. Seasonal variation in the average chemical composition of *Cynoglossus browni* (length range = 32–38 cm; weight range = 200–220 g). Key as for Fig. 1.

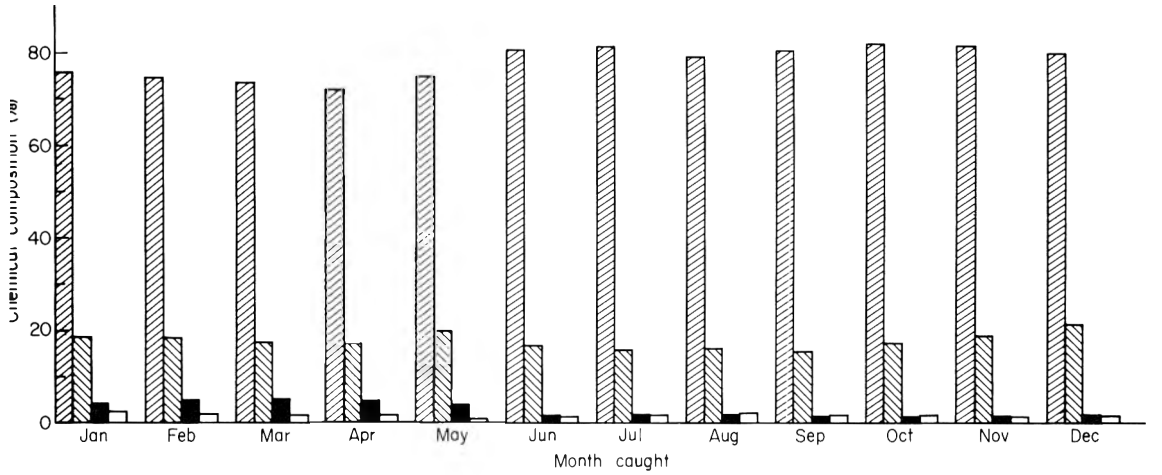

Figure 7. Seasonal variation in the average chemical composition of *Brachydeuterus auritis* (length range =15–20 cm; weight range = 90–120 g). Key as for Fig. 1.

species. This agrees well with the hypothesis of other workers (Burgess, 1965). In the statistical evaluation of the results of the mean fat, water, protein and ash contents throughout the year, it was found that a standard error of mean (s.e. mean) of 0.2; 6.4; 1.5 and 0.2 was involved in the chemical parameters respectively in *Ilisha africana*. *Trichiurus lepturus* showed a s.e. mean of 0.1; 6.7; 1.7 and 0.2 respectively in the mean values calculated for the fat, water, protein and ash contents throughout the year. In *Pentanemus quinquarius* the s.e. mean involved was 0.1; 6.6; 1.7 and 0.2 in the mean fat, water, protein and ash contents for the whole year. However in *Chloroscombrus chrysurus* the s.e. mean involved was 0.2; 6.5; 1.6 and 0.4 respectively while in *Caranx hippos*, the s.e. mean involved was 0.3; 8.8; 2.1 and 0.3 respectively in the

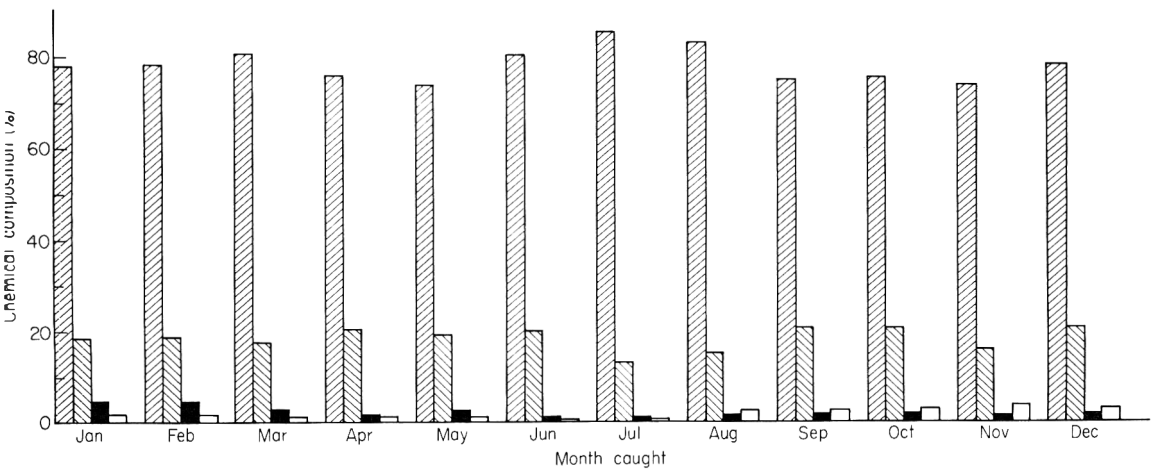

Figure 8. Seasonal variation in the average chemical composition of *Pseudotolithus typus* (length range = 30–38 cm; weight range = 100–130 g). Key as for Fig. 1.

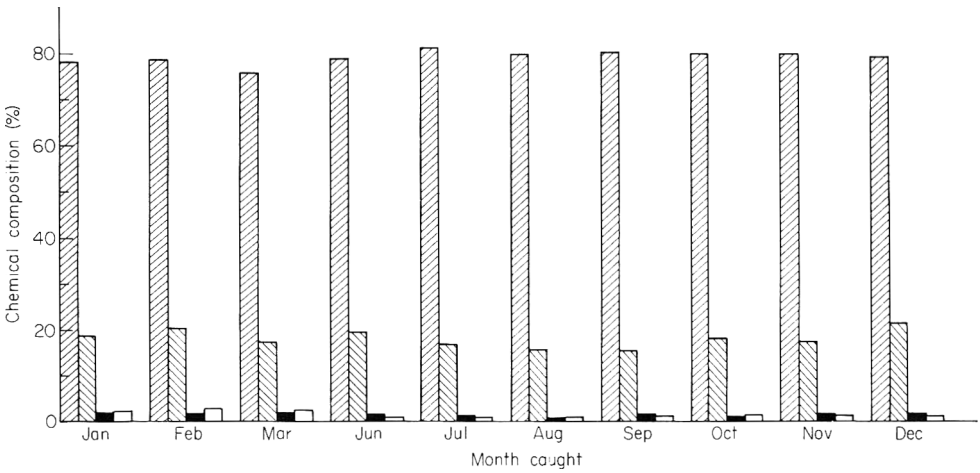

Figure 9. Seasonal variation in the average chemical composition of *Arius heudeloti* (length range = 30–35 cm; weight range = 200–230 g). Key as for Fig. 1.

four parameters. *Cynoglossus browni* exhibited an s.e. mean of 0.1; 0.4; 0.6 and 1.4 in the mean fat, water, protein and ash contents for the year. *Brachydeuterus auritus* gave an s.e. mean of 0.1; 1.0; 0.5 and 0.5 respectively in the mean values calculated for the year; for *Pseudotolithus typus*, the s.e. mean involved was 0.3; 1.0; 0.7 and 0.4 respectively, while in *Arius heudeloti* s.e. mean was 0.2; 8.9; 2.1 and 0.2 respectively. Similarly, the s.e. mean involved for Arius latiscutatus was 0.2; 8.9; 2.0 and 0.2 respectively. On the other hand, the s.e. mean involved for *Carcharias taurus* was 0.04; 0.3; 0.2 and 0.2 respectively while in *Penaeus duorarum* it was 0.1; 11.2; 2.4 and 0.4 respectively.

There was no pronounced seasonal fluctuation in the protein content of all the fish. This confirms the work of earlier workers (Clark & Almy, 1920) that

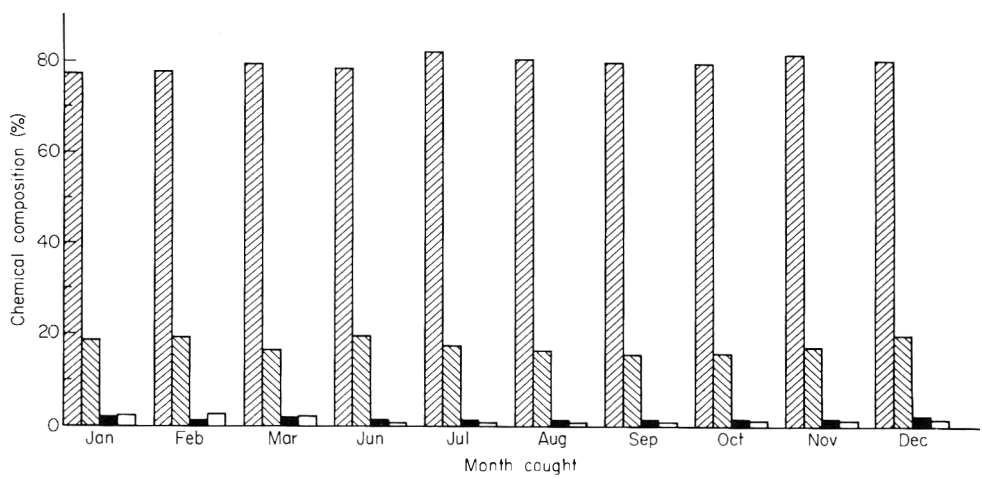

Figure 10. Seasonal variation in the average chemical composition of *Arius latiscutatus* (length range=30–35 cm; weight range =200–230 g). Key as for Fig. 1.

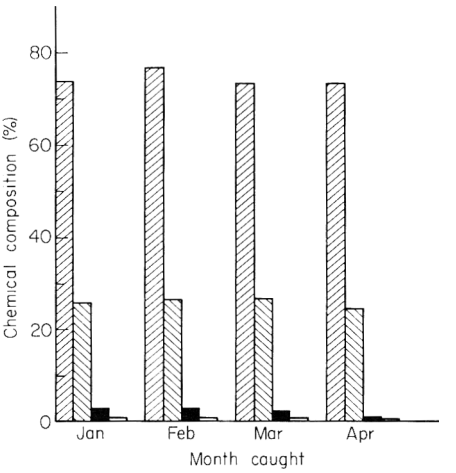

Figure 11. Seasonal variation in the average chemical composition of *Carcharias taurus* (length range = 60–70 cm; weight range = 2400–3000 g). Key as for Fig. 1.

the protein of fish changes very little with season because of the reciprocal relationship between the fat and water contents. The particularly higher protein value recorded for shark was attributable to the presence of urea in its flesh (Dill, 1924).

Thus, a significant proportion of the crude protein was urea. Some chemical peculiarities of fish can reasonably be related to their environment or way of life. It is established that the high urea concentration in the blood of marine elasmobranchs helps to preserve osmotic equilibrium and prevent the fish from being dehydrated by the sea water. The uneven distribution of herring fat may perhaps be explained in terms of buoyancy (Love, 1970).

Variations in biological conditions of fish can bring about wide scatter in their chemical composition. Factors affecting the chemical composition of fish

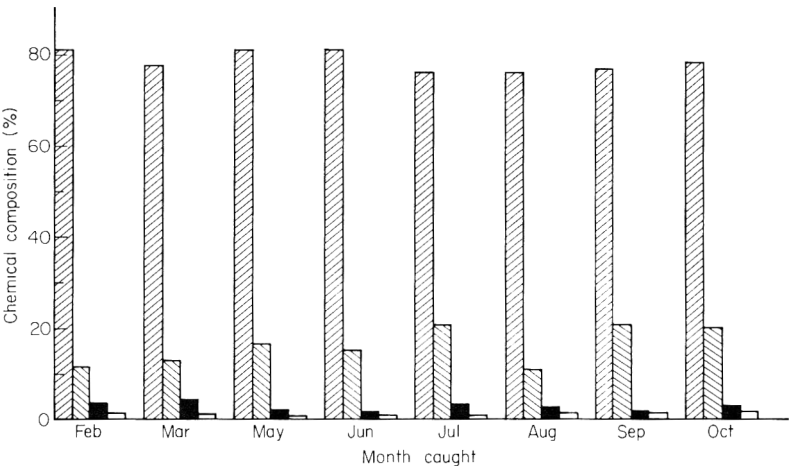

Figure 12. Seasonal variation in the average chemical composition of *Penaeus duorarum* (length range = 12–16 cm; weight range = 60–80 g). Key as for Fig. 1.

are species, age, physiological conditions (i.e. whether spawning or starving), time and the location of catch, amount and quality of food that the fish eats, and the amount of movement it makes (Zaitsev *et al.*, 1969). It has not been possible however in this paper to relate the chemical composition with all these factors.

Results of the weight composition of the fish are presented in Table 2.

It will be seen from this table that the flesh (including the skin) constitutes the bulk (41.4–65.4%) of the fish, followed by the head (10.9–30.7%). In addition the table also reveals that the edible part of the fish, i.e. the flesh (usually with the skin on) and the edible internal organs (developed gonads and sometimes the liver) constitute from 45.8–66.0% according to the different species. These values are close to values of 45–75%, given by other workers (Zaitsev *et al.*, 1969). All the other parts of the the fish including head, bones, fins, scales and viscera are generally considered inedible because they are usually not consumed by human beings. This system of dividing the parts of a fish into edible and inedible is rather loose and in practice, the so-called inedible parts may be used in part, if not wholly as food. In canning for example, unboned headless fish may be packed into tins, i.e. they may be canned with the backbone and ribs; after cooking (sterilizing) the whole of the contents are perfectly edible.

The yield (or weight composition) varies considerably with the species as can be seen from Table 2, and this is a function of the anatomy of the species involved. Fish with large heads and with large visceral relative to the muscle produce smaller flesh yield. *Trichiurus lepturus, Cynoglossus browni* and *Pseudotolithus typus* contain more flesh at 65.4; 65.2 and 60.9% respectively and therefore offer more in terms of consumption.

Such fish with much flesh, a high nutrient content and which are abundantly available (e.g. *Pseudotolithus typus*) may be suitable for the fresh fish market as well as for cold storage in order to cover seasonal fluctuations in supply. Similarly fish with a high protein content and a high percentage of head and bones, and therefore not much favoured for fresh consumption, may be diverted to fish protein concentrate production. *Ilisha africana* because of its low fat content, fairly large quantity of flesh coupled with relatively high percentage of bones and also due to its anatomy, may be suitable for canning and smoking.

The aim of the determination of weight composition of fish is to be able to estimate fish as human food and to ensure that all parts are processed in a rational way showing how much is of value as human food and how much can be used for fishmeal and other products. The bones, heads and fins of fish are mainly converted into meal for animal food because of their large proportion of nitrogenous substances and minerals (mainly calcium phosphate). But the heads of certain large fish (e.g. *Lates niloticus,* Nile perch) found in rivers and Lakes in northern part of Nigeria) which have a fairly large amount of flesh and fat, are also used for human consumption.

From the data collected, it has been possible to conclude that during the

months when there is a surge in the fat content and the fish are most liable to deterioration, greater care in the handling of the fish should be exercised. Results presented in Figs 1–12 and Table 2 leave little doubt that some of the variation found between individual fish can be ascribed to variations in their size and sexual maturity. To make a proper assessment of fish as food it is therefore necessary to know its relative content of edible materials as well as the proportion and quality of the nitrogenous compounds, fat, vitamins and mineral salts.

On the basis of this preliminary study further work needs to be carried out on the composition of the important sea fish of Nigeria. This knowledge is needed primarily to improve the methods of utilization, preservation, storage, and marketing of fish and for the formulation of new products for human consumption.

Acknowledgments

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I should like to acknowledge the considerable help I have had from Mr J. G. Tobor for giving me necessary information on landings and also for reviewing the manuscript.

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External corrosion of cans during thermal treatment

C. H. MANNHEIM, HADASSA ADAN AND NEHAMA PASSY

Summary

Factors affecting external corrosion of food cans, in contact with various metals in aqueous medium, during thermal processing were investigated. Simple electrochemical measurements of potentials and couple currents were found to be suitable for evaluation of the tendency and extent of corrosion. A stainless steel bath was found to cause external corrosion of tin cans. Several inhibitors were investigated as to their efficiency in minimizing this phenomenon. Two commercial inhibitors were found to have a limited effect, whereas sodium nitrite proved to be effective at a level of 300 p.p.m. It became apparent that increasing the anodic area (tinplate) in relation to cathodic area (stainless steel), i.e. loading equipment to capacity, was beneficial in reducing corrosion in this system. An aluminium electrode afforded protection to tin cans in a stainless steel bath at 85°C.

Introduction

External corrosion of tin cans is a complex phenomenon and its rate and extent depend on a variety of factors. Some are associated with the metal itself, its hydrogen over-voltage and surface homogeneity, and others with the formation of galvanic microcells in the heat processing metallic equipment due to non-homogeneity of its metal surface and due to contact of one metal with another. Often stainless steel is used as the material of construction in process equipment. In this case the stainless steel is more noble than the can, increasing the corrosion tendency of the food can itself.

Other factors governing the external corrosion are associated with the environment. These include food residues, rust from equipment, quality of process water or steam, corrosive glues or labels, poor handling which causes

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scratching of the can surface, improper cooling and storage conditions, i.e. temperature, relative humidity, dust or presence of corrosive fumes or deposits in warehouses (Anon, 1962; Board, 1972; Foskett, 1956; Goeting & Collenteur, 1974; Mannheim & Mager, 1972; Darre, Birk & Marsal, 1978; Sanders, 1955).

In the external corrosion reaction, iron is anodic to tin and dissolves through pores and scratches in the tin coating. Inhibition of the process may be accomplished by prevention of electron transfer from anode to cathode, interference with the cathodic reaction and the ionic conductance.

In practice this may be done by reducing the aggressive nature of the environment by removing the cathodic reactant; by erecting an electronic barrier through alloying or cathodic protection or by isolating the metal from the environment by interposing a physical barrier such as a passivation film or lacquer coating.

Another way is by adding suitable inhibition reagents to the medium which form various kinds of surface films (West, 1971). This is a common way for corrosion prevention in heat process equipment in the food industry (Nathan, 1973). Materials such as chromates, phosphates, oils and nitrites are commonly used. The nitrite mechanism of reaction is explained by its oxidative properties. It oxidizes the ferrous ions into ferric ions and the stannous ions into stannic ions (Hoar, 1958). These ions form insoluble hydroxides which are deposited on the metal and act as barrier to further dissolution. The nitrite concentration has to be optimal for corrosion prevention, otherwise corrosion may be accelerated (Conoby & Swain, 1967). Recommended concentrations vary from 300–1000 p.p.m., depending on water quality (Board, 1978; Nathan, 1973).

Cathodic protection as a method for preventing corrosion can be applied by one of two methods, *viz.* power impressed current or sacrificial anodes. A galvanic couple is formed when the sacrificial anode is attached to the protected structure. The anode must have a potential more negative than that of the protected area and the essential requirement is that it will polarize the steel to a point where it will either not corrode at all, or corrode at an acceptable rate. This anode is consumed by dissolution during protection.

In this work various factors such as material of construction of heat processing equipment and temperature on the external corrosion process of metal cans and methods of its prevention were evaluated.

Materials and methods

Potential measurements

Electrode potentials were measured versus a calomel reference electrode, via a KCl bridge, with a Honeywell, Electronic 19 recording potentiometer. The test electrodes were immersed in water in a 2-litre glass beaker for 20 min at 85°C and then measurements were taken. Metal samples tested were: tin

plate (5.6 g/m^2); pure tin; steel base of tin plate; alloy ($FeSn_2$); steel plate (used for construction of bath); stainless steel type 316.

The alloy sample was prepared by removing the tin layer from tin plate with a solution of 1% potassium iodate in 5% NaOH, by dipping it 5 min at 35–40°C. Steel base plate was obtained by dipping the tin plate into a 4% antimonyoxide solution in concentrated hydrochloric acid. Samples were cut in strips of 2×10 cm.

Couple currents

Couple currents between various metals were measured in a 2.5-l stainless steel or steel bath (representing the pasteurizing equipment) after connecting an external 100 Ω resistance. Samples were held in the baths by a special sample holder (Landau & Mannheim, 1970) exposing an area of 2.55 cm^2. Metal samples tested were tin plate and steel wire (A.R. 99.9% pure) in stainless steel and steel baths. The steel wire was used in order to obtain a ratio of 1:2000 between sample and bath.

Inhibitors

The following commercial inhibitors were tried in this work: (1) Pag-Cheled, (Ayalon, Israel), based on polyphosphates and polysilicates); (2) Inhibitor No. 610 (Ayalon, Israel) based on organic acids, amines and EDTA; and (3) Nitrite as $NaNO_2$, practical grade (Frutarom Ltd, Israel).

Aluminium sacrificial electrode

The electrode used for cathodic protection was made by Metal Gesellschaft, West Germany, and its composition was as follows: 0.1% Si; 0.1% Fe; 0.35–0.5% Zn; 0.1% Sn; 0.8% Mg; 0.1% Cu and the rest aluminium. This electrode was tested in a stainless steel (type 306) bath (45×40×11 cm).

Results and discussions

In order to measure polarity and extent of corrosion and evaluate the effect of various inhibitors, potentials and galvanic current measurements were taken.

The potentials of those metals that participate in the external corrosion process of food cans during heat treatments are given in Table 1. Results are averages of at least three measurements and reproducibility was in the range of ±5 mV.

Measurements were made against a calomel electrode in tap water at 85°C after 20 min to simulate heat treatment conditions. Stainless steel was found to be the most noble of the tested metals, the base steel used for can

Table 1. Potentials of various metals against a calomel electrode in tap water at 85°C

Metal	Potential (−mV)
Steel base of tin plate	740
Steel plate	730
Tinplate	640
Alloy ($FeSn_2$)	590
Pure tin	550
Stainless steel (316)	270

manufacturing was the most anodic metal and tin plate was in between. This would indicate that in a stainless steel bath the food can might undergo corrosion, while in a steel bath the steel will be the active metal thus protecting cathodically the tin cans.

The couple current densities of some pairs of metals, after 20 min immersion in tap water at 85°C are given in Table 2. Results are averages of at least three measurements and reproducibility was in the range of $\pm 5\ \mu A/cm^2$. The ratio of areas between the various metals, simulated conditions which may exist in a commercial heat treatment process, such as exhausting or pasteurization. The high current flowing between tinplate and stainless steel shows that the steel base of the tinplate may dissolve in a stainless steel bath, unless otherwise protected. The tinplate, after being coupled to the stainless steel for 20 min in the bath at 85°C, showed severe signs of attack as indicated by pores. When the tinplate was coupled to a steel plate, the current was negligible and no change in its appearance could be observed.

Table 2. Couple currents of some metal pairs in tap water at 85°C

Metal pair	Area ratio	
Metal 1 –Metal 2	Metal 1 : Metal 2	Current density ($\mu A/cm^2$)
Tin plate –stainless steel bath	1 : 200	30
Steel wire–stainless steel bath	1 : 2000	900
Tin plate –steel plate bath	1 : 200	Negligible
Steel wire–steel plate bath	1 : 2000	Negligible

Current density values of the tinplate–stainless couple measured at different temperatures are shown in Fig. 1. The curve obtained shows that current increases with temperature up to about 80°C and then decreases. The rise in temperature leads on the one hand to an increase in corrosion rate. On the other hand oxygen solubility decreased and its diffusion rate is increased. The overall effect is a reduced corrosion rate above 80°C (Bachvalov & Turkovskaya, 1965).

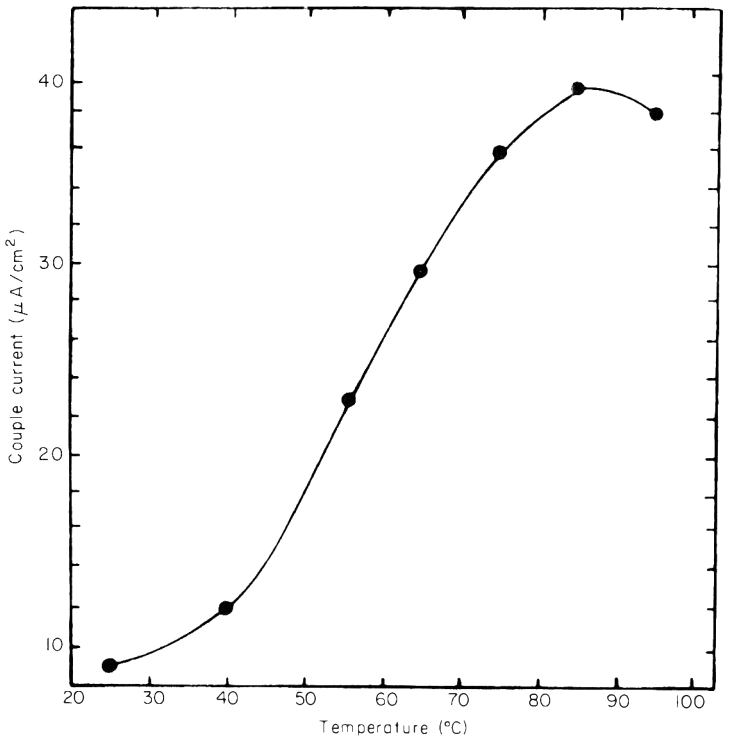

Figure 1. Couple currents of tin plate and stainless steel as a function of temperature.

Inhibitors

The effect of several commercial inhibitors at various concentrations on the current flowing between tinplate and stainless steel was evaluated. Inhibitor No. 610, which is based on organic acids, amines and EDTA reduced the current to less than half its initial value at a concentration of 300 p.p.m. Inhibitor Pag-Cheled 102, which is based on polyphosphates and polysilicates, also reduced the current to half its initial value at about 200 p.p.m.

Sodium nitrite which is often used as an inhibitor in cooling and heating water systems was also tried. Its mechanism is well known, and its effective concentration range is fairly low. Couple current density values between tin plate and stainless steel, as affected by nitrite concentration are presented in Fig. 2. The reduction in current density was about fifteen-fold at a concentration of 100 p.p.m. No further reduction in current was obtained above 150 p.p.m. and no attack on the tinplate could be observed. The limiting pH, at which nitrite concentration is effective, is above 6, otherwise HNO_2 may be formed. At a lower pH the nitrite may be oxidized to nitrate, resulting in a reduced concentration affecting the inhibition process. Nitrite disappearance in tapwater at 85°C and at pH 6.5 was evaluated as a function of processing time and its initial concentration (Table 3, Fig. 3) (AOAC,

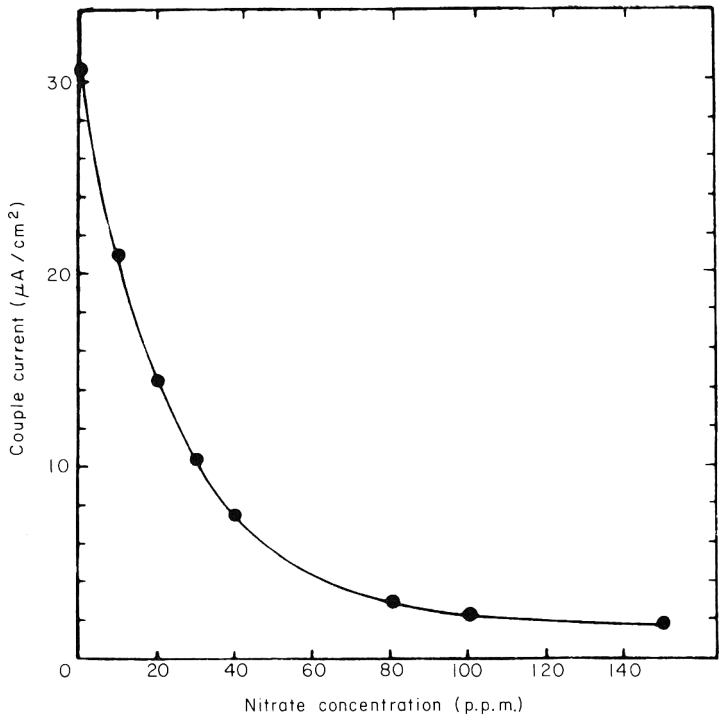

Figure 2. Couple current as a function of nitrite concentration at 85°C.

1975). There was no difference in rate of nitrite disappearance as affected by the initial concentration. Initially the rate of nitrite disappearance was fast, it slowed down with time and stabilized after less than 20 hr at 60% of the initial concentration. Therefore 300 p.p.m. is the recommended nitrite concentration to be used in this system since about 40% disappears during a working day.

Couple current measurements were made simultaneously in the above system and were found to be constant for the first 6 hr, after which a reduction

Table 3. Nitrite concentration as a function of time at 85 C (pH 6.5)

Time (min)	Concentration (ppm)			
	100	150	200	300
20	90	135	181	272
60	86	131	168	256
120	80	123	161	240
240	75	114	153	224
360	73	108	141	216
1200	62	90	118	171
Lost (%)	38	40	41	43

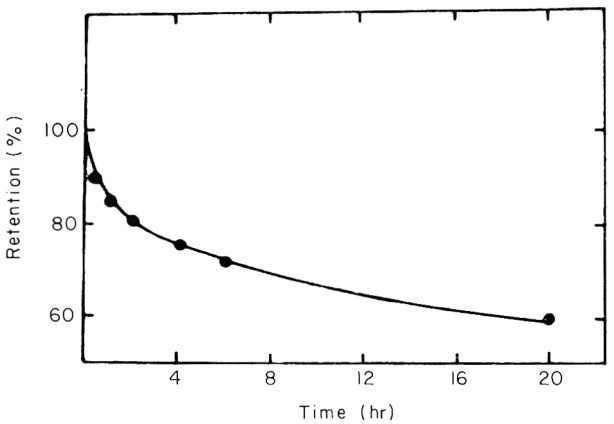

Figure 3. Retention of nitrite as a function of time at 85°C.

of 20% in current values was recorded. The tinplate kept its bright appearance during the entire period.

Processing water is often contaminated with the product. To simulate such conditions the processing water was spiked with an acid and an acidic product, like tomato juice, and the effect on couple current is demonstrated in Table 4. These results show that only when the water contains above 2% tomato juice is there a significant change in the current densities. Such contamination of process water is rather high and will seldom be encountered in industry. When the pH was adjusted with HCl the effect on the couple current seemed smaller per pH unit. This could be due to the different anions in the system.

The ratio of cathodic to anodic areas determines the couple current value, i.e. the lower the ratio, the lower will be the extent of corrosion. Couple current measurements were taken for different area ratios as obtained by changing the number of cans in a stainless steel bath (Table 5). The increase of the anodic area resulted in a decreased couple current. This means that

Table 4. The effect of contaminants on the couple current between a tin can and a stainless steel bath at 85 C

Water and acid		Tomato juice		
pH*	Current density (μA/cm^2)	Juice (%)	pH	Current density (μA/cm^2)
5.5	150	0	7.1	39
6.5	55	1	6.8	35
7.5	42	2	6.6	41
8.5	51	3	6.4	47
—	—	5	6.1	59

* pH was adjusted with HCl or NaOH.

Table 5. The effect of changing cathodic/anodic area on couple currents between cans and stainless steel at 85 C in tap water

Cathodic/anodic area	Current density (μA/cm^2)
15	0.32
7.5	0.30
5.0	0.29
3.0	0.28
2.5	0.27
1.8	0.25
1.2	0.23
0.9	0.21

loading the process equipment to its maximum capacity is beneficial in reducing the degree of corrosion.

A sacrificial anode is often used to prevent iron corrosion. The most common electrodes are: zinc, magnesium or aluminium which have a more negative potential than iron. Zinc is the cheapest metal but is not effective above 75°C, therefore an aluminium electrode was tried in a 306 stainless steel bath at 85°C. The anode potential was −1.05 V and the cathode (bath) potential was −0.70 V. The output, or the electrochemical equivalent of the aluminium sacrificial electrode, is given by the potential difference between the protecting and the protected metals divided by the anode resistance. The anode resistance can be calculated (Shreir, 1976) from the equation

$$R = \frac{P}{2L}\left[\frac{\ln (2L - 1)}{r}\right],$$

where P is resistance of water (Ω-cm), L is length of anode (cm) and r is effective average radius of anode taken after 40% of anode is used (cm). The anode resistance was found to be 3.24 A.

It is known that the efficiency of such an electrode is about 85%. Using the above described anode a current density of 80 mA/m^2 (between the electrode and bath) was obtained. From the specifications of the electrode, and from the current density measurements between aluminium anode and the bath, the number and weight of anodes needed for protection can be estimated by

$$\text{No. of electrodes} = \frac{\text{current density in system} \times \text{area of bath}}{\text{output}}.$$

Attaching the sacrificial anode to the stainless steel bath offered complete protection of cans in the bath for many hours as indicated by an unchanged appearance of the cans.

In summary, it was shown in this work that if stainless steel equipment is used for thermal processing of tin cans external corrosion of the latter may occur. Simple electrochemical measurements of potentials and couple

currents can be used to evaluate the tendency for external corrosion, and its extent. Couple current measurements can also be used to determine the effectiveness of various inhibitors and for monitoring quantities required to prevent external corrosion of cans.

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Amylolytic activity in cooked luncheon meat

R. G. BELL AND C. O. GILL

Summary

Glucose accumulated in chub packed luncheon meat cooked at 66°C. Glucose was also released from pork starch slurries, but not similar slurries prepared with beef or mutton. The amylolytic activity of pork was not inactivated by heating at 60°C, and this thermostable activity appears to account for the accumulation of glucose in pasteurized chub packed luncheon meat stored under conditions that preclude bacterial growth.

Introduction

Glucose accumulates in chub packed luncheon meat during storage (Bell & Gill, 1982). Mitrica & Granum (1979) have suggested that starch degradation in pasteurized starch containing meat products is due to the activities of *Bacillus* organisms that survive processing. However, glucose accumulation in chub packed luncheon meat has been observed when microbial growth was prevented by storage of chubs in an anoxic atmosphere (Bell & De Lacy, 1982). Therefore, glucose might be formed by chemical hydrolysis or through the action of meat enzymes on starch.

Muscle tissue is thought to have amylolytic activity because glucose accumulates in meat during the onset of rigor (Sharp, 1957; Bendall, 1973). Provided there is sufficient glycogen in the pre-rigor muscle, all meats contain small quantities of glucose when the rigor process is completed. The process of glucose accumulation has received little attention, but it is known that the rates of glucose accumulation are in the order of pork > mutton > beef while glycogen breakdown rates are in the order of pork > beef > mutton. Amylolytic activity in post-rigor meat has been examined only for pork.

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Starch hydrolysis has been reported in a minced pork starch mixture during storage at 1°C (Anon, 1959).

Loss of water binding capacity, caused by starch hydrolysis, has been reported in meat, particularly liver, starch mixtures which have been heated above the gelatinization temperature for the starch but not above 80°C at which the meat enzymes are inactivated (Cate, 1963).

Thus, the potential for amylolytic activity in post-rigor meat is uncertain, but there is an indication that such activity could survive the commercial cooking of products like chub packed luncheon meat (wet heating to 65–75°C for 1–3 hr, depending on the size of the chub). We have therefore examined the amylolytic activity of commercial chub packed luncheon meat, and of meat homogenates and meat starch slurries from the three meat species (beef, mutton and pork) used in commercial luncheon meat formulations to see if such activities could account for the accumulation of glucose in starch-containing pasteurized meat products.

Materials and methods

Meat homogenates and meat starch slurries

To obtain meat samples with little or no microbial contamination, portions of deep muscle tissue (75 g) were removed with sterile instruments from meat cuts previously swabbed with alcohol (Borton, Webb & Bratzler, 1968). Each meat sample was placed in a sterile blender bowl and homogenized with 75 ml sterile 3% (w/v) soluble starch in 0.2 M phosphate buffer, pH 7.0. The slurry temperature was maintained below 5°C during blending. A homogenate of each meat with phosphate buffer alone was similarly prepared.

Portions (2 ml) of meat starch slurries, meat homogenates and starch solution were distributed in sterile universal bottles, and samples of each were incubated at 37, 60 and 95°C, all ±0.1°C. A solution of antibiotics (0.1 ml) was added to the 37°C samples to give final concentrations of vancomycin, 10 μg/ml; polymixin B, 2.5 iu/ml and trimethoprin lactate 5 μg/ml.

Five bottles from each temperature series were removed for glucose analysis at hourly intervals between 0–6 hr, and also at 24 hr for the 37°C series. In addition, a single bottle from each series was removed at 0, 3 and 6 hr (and at 24 hr for the 37°C series) for microbiological examination.

Chub packed luncheon meat

Twelve raw 250-g luncheon meat chubs were placed in a water bath set at 66 ± 1°C. Pairs of chubs were removed after 0, 1, 1.5, 3.5, 5.5, and 9.5 hr and placed in iced water for 30 min. Core and surface samples, 5 g for glucose determination and 10 g for microbiological examination, were then taken by means of the cork borer technique of Bell & Gill (1982).

Glucose determination

The glucose content of neutralized perchloric acid extracts of the test materials (Gill, 1976) was determined with glucose oxidase–peroxidase blood sugar reagents (Boehringer-Mannheim, West Germany).

Microbiological examination

Homogenates of luncheon meat samples (1:10 dilution) were prepared in 0.1% (w/v) peptone supplemented with 0.2% (v/v) tergitol as described previously (Bell & Gill, 1982). Homogenates of the meat starch slurries and meat homogenates were prepared by shaking the sample with peptone:tergitol diluent in a universal bottle containing glass beads. Serial dilutions of the homogenates were prepared in 0.1% (w/v) peptone and three spread plates of appropriate dilutions prepared on plate count agar (Difco, U.S.A.). The aerobic plate count was determined by counting the colonies that developed after 72 hr incubation at 25°C.

Results

Meat homogenates and meat starch slurries

Of the test and control systems examined, only those that contained both starch and pork accumulated glucose. At 37°C there was a lag of 4 hr before

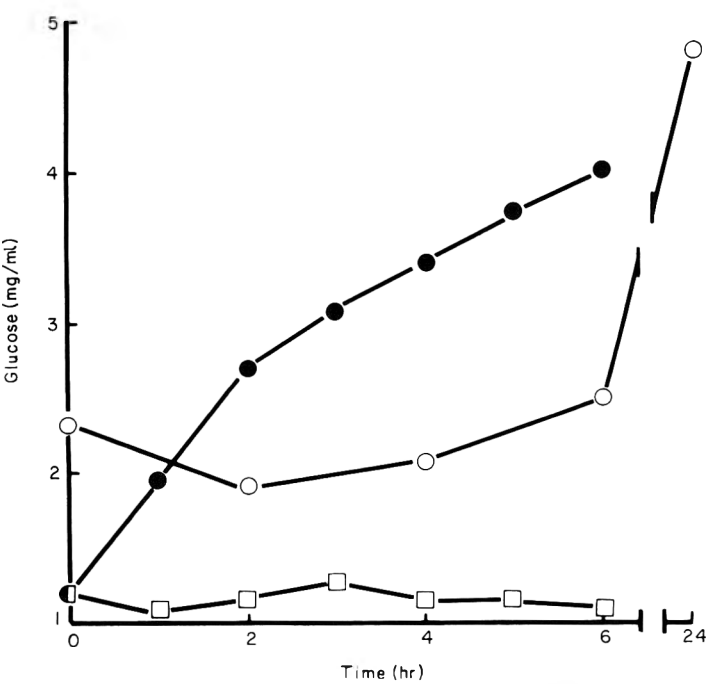

Figure 1. Accumulation of glucose in pork-starch slurries incubated at 37°C (○), 60°C (●) and 95°C (□).

glucose concentration increased; at 60°C glucose concentration increased immediately, and at 95°C glucose did not accumulate. The initial rate of glucose accumulation at 60°C declined after 2 hr, after which it remained steady for the remaining 4 hr (Fig. 1). No bacteria were detected in any of the homogenates or slurries after incubation.

Chub packed luncheon meat

Chubs, which were cooking at 66°C, accumulated glucose immediately. The rate of accumulation fell with time and had almost ceased after 9.5 hr by which time 5.1 mg glucose/g were present (Fig. 2). Cooking beyond the 1.5 hr that is normal for 250 g chubs produced a gradual darkening of the product and release of gelatin. The aerobic plate counts of the raw luncheon meat emulsion 1.11×10^6/g was reduced to 5.4×10^3/g and 1.7×10^4/g at the surface and core respectively after 1.5 hr cooking and further reduced to 3.4×10^2/g and 8.0×10^2 after 7.5 hr.

Discussion

Clearly, pork meat contains thermostable amylolytic enzymes, the activity of which would account for the accumulation of glucose observed in chub packed

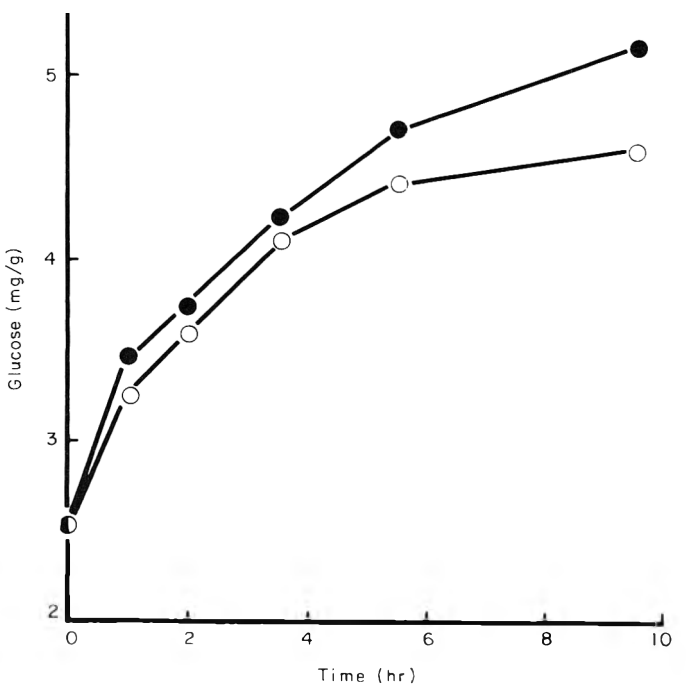

Figure 2. Accumulation of glucose at the surface (○) and the core (●) of luncheon meat chubs during cooking at 66°C.

luncheon meat. The delayed release of glucose in pork starch slurries incubated at 37°C suggests that some change must occur in the meat slurry before amylolytic activity is expressed. This change apparently is rapidly completed at 60°C allowing glucose accumulation to proceed without obvious delay. The amylolytic activity apparently does not survive heating at 95°C, so could not mediate glucose release in canned processed meats heated to sterilization temperatures. However the persistence of amylolytic activity can be expected in pasteurized products since the enzyme system is not rapidly inactivated until the temperature exceeds 80°C (Cate, 1963). The observation of such residual activity in commercially cooked luncheon meat chubs (Bell & De Lacy, 1982) confirms this supposition.

Glucose release in commercial meat products would be expected to occur only in formulations containing both pork and starch filler. This release would be detected as an increase in glucose content only if the rate of release exceeded the rate of consumption by a developing microflora. Thus an increase in glucose content is likely to occur only in pasteurized products when the heat treatment is mild enough not to destroy the amylolytic activity but severe enough to significantly reduce bacterial numbers. The phenomenon of glucose accumulation has been observed in pork liver pâté stored at 10°C (unpubl. data) as well as in chub packed luncheon meat (Bell & Gill, 1982; Bell & De Lacy, 1982).

Conclusions

The glucose release phenomenon observed in starch-containing luncheon meat is associated with thermostable amylolytic activity present in pork that remains active after the cooking afforded commercial chub packed luncheon meat. Glucose will accumulate in starch-containing pasteurized processed meats when the rate of glucose release by residual amylolytic activity exceeds the rate of glucose consumption by a developing spoilage microflora.

Acknowledgments

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Determination of a thermal process schedule for guava (*Psidium guajava* Linn.)

N. NATH AND S. RANGANNA*

Summary

A thermal process schedule for guava canned in syrup has been evolved on the basis of inactivation of pectinesterase (PE) which was found to be more heat resistant than peroxidase. The values for thermal inactivation and thermal resistance of PE in syrup homogenate containing guava pulp and sugar syrup in the ratio of 11:6 and total soluble solids content of 20% at pH 4.0 were $F_{205.1}^{29.22} = 1.0$ and $D_{205.1}^{29.81} = 0.592$ respectively. The F value of 1 was equivalent to 1.69 D. In commercial canning, a 2.5 D process is recommended which ensures a microbiologically safe and organoleptically acceptable product.

Process time was calculated both by graphical and formula methods. Calculation by the formula method using slopes of heating and cooling curves plotted on semilog paper by least squares analysis, and f_h/U: g tables for actual values of j_c gave process times very close to those obtained by the graphical method. The process times required at different initial and processing temperatures are given.

To minimize viscosity increase in the covering syrup during storage, firming of guava in 1% calcium chloride solutions for 30 min prior to canning is suggested. In addition, use of covering syrup containing 0.125% ascorbic acid with or without citric acid (0.06%) yields a product of good colour, texture and flavour.

Introduction

Guava, a native of tropical America and grown in almost all parts of India is a delicious fruit with sweet aroma and refreshingly acidic flavour. The ascorbic acid content of guava (212 mg/100 g) is higher than that of fresh orange juice (68 mg/100 g) and is a good source of iron, calcium and phosphorus (Swaminathan, 1974).

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Occasional development of pink discoloration in canned guava caused by leucocyanidin and leucodelphinidin present in the fruit has been a serious concern to canners (Ranganna, Setty & Nagaraja, 1966; Setty, Nagaraja & Ranganna, 1968). The discoloration is mainly caused by the polymerization of leucoanthocyanins which increases with time and temperature of processing and/or storage (Ranganna, 1974). Organic acids naturally present or added enhance the extent of discoloration. Canning in syrup containing 0.06% citric acid and 0.125% ascorbic acid either prevented or minimized the extent of discoloration (Ranganna *et al.*, 1966). In conjunction with this treatment, the thermal process given should be minimum. Based on peroxidase inactivation ($F_{187.0}^{10.5} = 1.0$ at pH 4.2), the thermal process required for 401 × 411 cans was found to be 8 min at 212°F (Ranganna, 1974). The time found was sufficient to inactivate a strain of yeast isolated from under-processed cans of guava pulp. The processing time commercially followed for 401 × 411 cans is 25–30 min in boiling water. In addition to pink discoloration, another draw-back in the canned product is considerable increase in the viscosity of the covering syrup during storage.

Guava contains ascorbic acid oxidase, polyphenol oxidase, peroxidase and pectinesterase (PE) (Jimenez, 1947; Rodriguez, Agarwal & Shaha, 1971; Rieckehoff & Rios, 1956; Shastri & Shastri, 1975). Graces (1963) found the inactivation time of peroxidase to be minimum (7.5 min at 149°F) and of PE to be maximum (5 min at 208.4°F). Investigations carried out to evolve the minimum safe thermal process required in the canning of guava, and the effect of calcium firming of guava on the viscosity of the covering syrup are reported in this paper.

Materials and methods

Firm ripe Allahabad variety of guava grown locally were used in these studies. The fruits were lye-peeled by dipping in 2.5% boiling sodium hydroxide solution, washed in water, and dipped in 0.2% citric acid solution.

Studies on thermal inactivation of enzymes

Preparation of syrup homogenate. The peeled fruits were cored, blended in a Waring blender and strained. The pulp was quick frozen in a plate freezer at −40°F and stored at 0°F.

Syrup homogenate was prepared by blending the pulp with sugar syrup in the ratio 11:6 so as to get a final Brix of 20°, similar to the cut-out Brix of the canned product. The pH was adjusted to 4.0.

Thermal inactivation and decimal reduction times of pectinesterase. Activity, thermal inactivation time (TIT) and decimal reduction time (*D* value) of PE and TIT of peroxidase were determined according to the procedures described earlier (Nath & Ranganna, 1977a, b, c). The TIT and thermal

resistance curves were plotted on semilog paper by applying the analysis of least squares (Hurwicz & Tischer, 1956).

Heat penetration studies

The peeled fruits were cut into halves or quarters. cored, and filled into 401 × 411 (A 2½) cans, covered with 45° Brix sucrose syrup (300 g) at room temperature (referred to as cold-filled cans) or with hot syrup and exhausted (referred to as hot-filled cans), and sealed. The canned product was processed in boiling water (207°F, the temperature at which water boils at Mysore situated at an altitude of 750 m) or at 215°F in steam, and cooled in water. The slowest heating point in the cans was found to be at about one-tenth of the can height from the bottom.

Heat penetration rate into the cans was measured using Ecklund's non-projecting, plug-in, needle type thermocouples and a manually operated Leeds and Northrup potentiometer. The temperature during heating and cooling were recorded every 2 min. Six cans were used for each run, and three runs were done for each processing condition.

Plotting of heat penetration data

The temperatures corresponding to various times were plotted on rectangular coordinate graph paper for calculation of process time by the graphical method (National Canners Association, 1968). To calculate by the formula method, heat penetration data were plotted on a semilog paper (National Canners Association, 1968). The line through the points were drawn in three different ways: (1) the line of eye fit, and (2) a line joining the initial can temperature and a point within 1°F of the final can temperature (Gillespy, 1962) and the line of statistical fit by applying the analysis of least squares. There was no come-up time for the water bath at 207°F. It varied from 0.75 to 1.0 min at 215°F. Hence, in drawing the line, no allowance was made for the heating lag, and j_h was assumed to be 1.0.

The cooling curves were drawn similarly.

Calculation of process time

Process time was calculated by graphical (Bigelow *et al.*, 1920) and formula (Ball, 1923) methods. The following three procedures were made use of in the calculation of the process time by the formula method.

Procedure A. In this procedure using Ball's formula $B_B = f_h (\log j_l - \log g)$, f_h from the curve drawn by eye-fit was used. The value of g corresponding to the actual j_c (lag of the cooling curve) was found from $f_h/U{:}g$ tables of Purohit & Stumbo (1972).

Procedure B. Same as 'A' except that f_h and j_c found from heating curve drawn according to the procedure suggested by Gillespy (1962) were used in the calculation.

Procedure C. Same as 'A' except that f_h from the heat penetration curve drawn by applying the analysis of least squares was used in the calculation.

The $f_h/U{:}g$ tables of Ball & Olson (1957) could not be used in these calculations because the z value of 29.20 of PE was outside its range.

There was no time lag between placing of the cans in the boiling water bath and the bath again attaining the boiling temperature. However, when the processing was done in steam in retort, an allowance of 42% of the come-up time was given to calculate the pseudo-initial temperature (Ball & Olson, 1957).

Tests for the microbiological safety of process evolved

The hot-filled cans without added acid (pH 3.95) were processed for 0, 2, 4, 6, 8 and 10 min (calculated time being 10.7 min) at 207°F, and cooled. The cans were incubated at 86, 98.6 and 122°F, and examined microbiologically (National Canners Association, 1968).

Bacillus coagulans and *Clostridium pasteurianum* which have been reported to cause spoilage in canned acid foods were made use of for any likely spoilage by these organisms. Lyophilized cultures of these organisms were purchased from the American Type Culture Collection. The spore suspension of *B. coagulans* was prepared according to the procedure of NCA (1968) and of *Cl. pasteurianum* according to the procedure of Stumbo (1973) using screw-capped tubes and bottles for obligate anaerobic organisms. To check whether guava supported the growth of these organisms or not, pulp and syrup homogenate adjusted to pH 4.0, 4.5, 5.0 and 5.5 were sterilized at 240°F for 20 min, inoculated with the spores of *B. coagulans* and *Cl. pasteurianum*, and incubated at optimum temperature for 15 days. Since the pulp supported the growth of *Cl. pasteurianum* but not of *B. coagulans*, thermal resistance of the former was first ensured by determining the thermal death time (TDT) in tomato juice serum (pH 4.5), and then in guava pulp and drained syrup from the canned product. Thermal death time was determined by the tube method of Bigelow & Esty (1920). Correction required for the come-up time in the determination of TDT was ascertained by the same procedure as that used for TIT of the enzymes (Nath & Ranganna, 1977b, c).

Inoculated pack studies. Hot-filled cans of guava (pH 4.0) were inoculated with 5 million spores of *B. coagulans* or *Cl.pasteurianum*, exhausted to a centre temperature of 160°F and processed for 5, 8 and 11 min at 207°F. Uninoculated cans were processed alongside as control. The cans were incubated at 82.4–86°F, and subjected to microbiological examination.

Calcium firming of guava. To prevent the covering syrup from becoming viscous, three different procedures of calcium treatment were investigated: (1) incubating the prepared halves initially in hot water at 140°F for 15 min to lower the degree of esterification of the pectin naturally present by activating the PE (Hoogzand & Doesburg, 1961), and dipping thereafter in 1% calcium chloride solution for 30 min to render the pectin insoluble; (2) immersing in

1% calcium chloride solution for 30 min and washing; and (3) adding 0.371% calcium chloride to the covering syrup. The hot-filled cans were exhausted, sealed and processed for 10 min at 207°F. Untreated cans were processed alongside as control.

Examination of the canned product

Cut-out examination of the canned product, estimation of ascorbic acid content by the indophenol-xylene extraction procedure, pectin content in the drained syrup as alcohol precipitate, methoxyl content of the pectin, and measurement of reflectance colour using Bausch and Lomb Spectronic 20 colorimeter were carried out as given in the *Manual of Analysis of Fruit and Vegetable Products* (Ranganna, 1977). Apparent molecular weight of the pectin was determined by the procedure of Smit & Bryant (1967); colour difference (ΔE) by the Adam's chromatic value diagram method (Mackinney & Little, 1962); and viscosity by using Brookfield synchroelectric viscometer (Model LVT). Texture was measured in a shear apparatus developed by Voisey & Hansen (1967) using a single plate, and expressed as the maximum force (g) required to shear a piece. An average of ten readings was taken as the texture of the fruit. Sensory evaluation was carried out by a panel of ten members and ranking test.

Results and discussion

Acidity, pH, TSS and activity of peroxidase and PE in the edible portion of fresh fruit varied considerably (Table 1). As the tendency for pink discoloration increased with acidity (Ranganna, 1974) and as the pH of the fruit varied between 3.8 and 4.15 (Table 1), thermal inactivation studies and

Table 1. Total soluble solids, acidity, pH and enzyme activity in guava and in the preparation used for thermal inactivation studies

Particulars	Edible portion of guava*	Syrup homogenate used for inactivation studies†
TSS (%)	10–13.5	20.0
Acidity as anhydrous citric acid (%)	0.224–0.450	0.205–0.256
pH	3.8–4.15	4.0
Pectinesterase activity (PE. u × 10^4 ml)	7.8–19.9	3.36–8.60
Peroxidase activity (k_1/g)	0.234–0.367	0.211–0.223

Prepared by *hand-peeling, and by †lye-peeling, and blending with sucrose syrup.

process evaluation were carried out at pH 4.0. Analytical data of the syrup homogenate used in the TIT studies are given in Table 1.

F, D and z values of pectinesterase and peroxidase

The uncorrected TIT of PE was $F_{214}^{38.04} = 1.0$, and of peroxidase was $F_{185.5}^{18.38} = 1.0$. The mean values for the come-up time in the determination of TIT of PE and of peroxidase were 5.84 and 2.48 min respectively of which 48.38 and 39.9% had lethal effect. The come-up time was less in the case of peroxidase because of the use of tubes of smaller diameter (Nath & Ranganna, 1977a, b). The correction required to be subtracted from the heating lag during the come-up time in the determination of TIT of PE and peroxidase was 3.01 and 1.45 min respectively.

The TIT curves of PE and peroxidase are shown in Fig. 1. The TIT value of peroxidase was $F_{180.5}^{14.9} = 1.0$ at pH 4.0 (Table 2) as against a value of $F_{187}^{10.5} = 1.0$ at pH 4.2 reported earlier in guava pulp (Ranganna, 1974). The TIT of PE was $F_{205}^{29.22} = 1.0$. The heat inactivation data for PE as 5 min at 208.4°F given by Garces (1963) is considerably higher than that found in the present study. Whether the data given by the author are actual TIT or the time required for

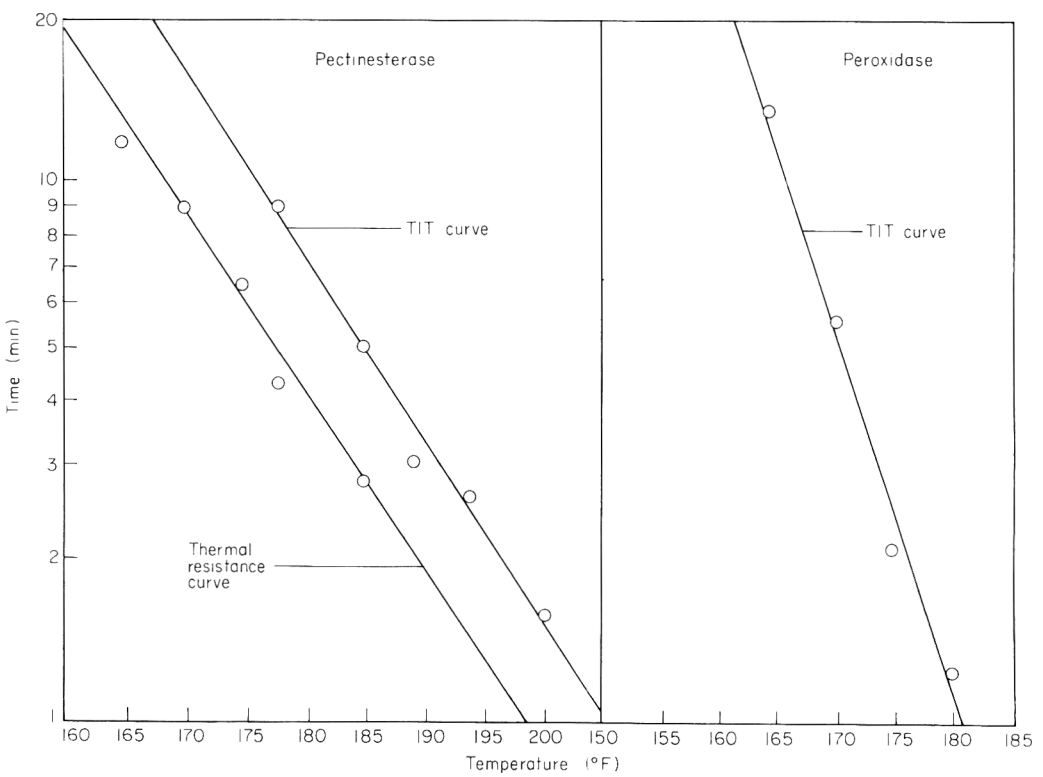

Figure 1. TIT and thermal resistance curves of PE and peroxidase in guava syrup homogenate at pH 4.0.

Table 2. *F*, *D* and *z* values of pectinesterase and peroxidase

Enzyme	TSS (%)	pH	*F* value from TIT curve	*D* value from thermal resistance curve	Decimal reductions (*F*/*D*)
Pectinesterase	20.0	4.0	$F_{205.0}^{29.22}=1.0$ ($r=-0.9886$)	$D_{205.0}^{29.81}=0.592$ ($r=-0.9841$)	1.69
Peroxidase	20.0	4.0	$F_{180.4}^{14.97}=1.0$ ($r=-0.9947$)		

inactivation under pasteurization condition is not clear. In the present study, TIT of PE was found to be more than that of peroxidase. Hence, the process time was evolved using the TIT of PE actually found (Table 2).

The thermal resistance curve is shown in Fig. 1, and the *D* and *z* values in Table 2. The *F* value of 1 min at 205°F was equivalent to 1.69 decimal reductions. The TIT and thermal resistance curves, drawn by applying the analysis of least squares, showed a high degree of correlation (Table 2).

Heat penetration studies

Since guava is a firm fruit, process evaluation was carried out at 215° in addition to 207°F. Heat penetration rate into A2½ cans containing guava in the form of quarters (f_h = 33.6 min) was faster as compared to halves (f_h = 38.0 min). Hence, subsequent studies were carried out with guava halves only.

Process calculation by graphical method. The heat penetration curves for cold- and hot-filled cans of guava halves processed at 207°F and the corresponding inactivation rate curves are shown in Fig. 2. The graphical

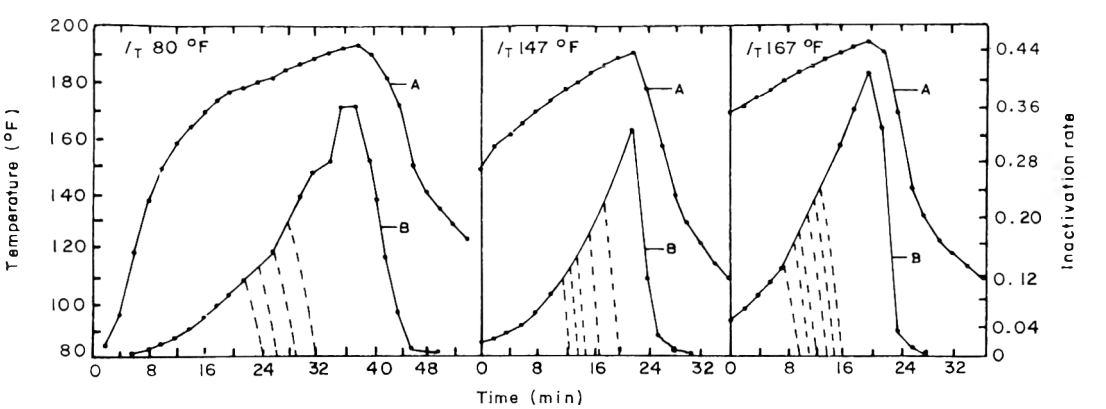

Figure 2. Heat penetration curves (A) for guava halves canned in 401 × 411 (A2½) cans processed at 207°F, and the corresponding inactivation rate curves (B).

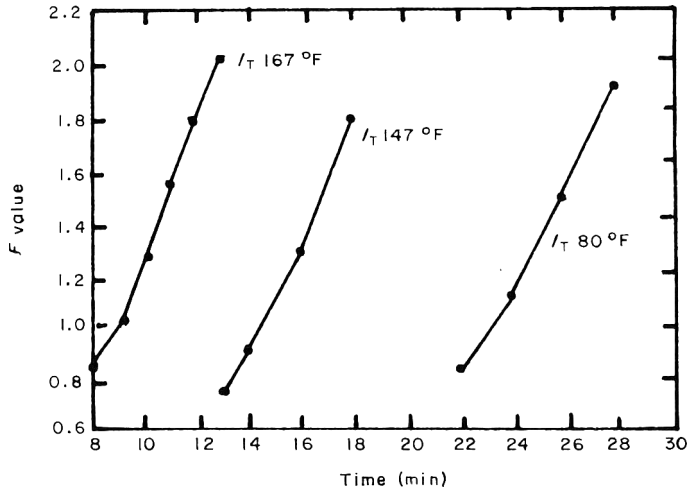

Figure 3. *F* value *versus* process time plot corresponding to Fig. 2.

interpolation curves to find the process times corresponding to the desired *F* values are shown in Fig. 3. The process times calculated are given in Table 4.

F value of PE was equal to 1.69 *D* (Table 2). Since the PE activity in guava varies considerably (Table 1), to ensure complete inactivation under commercial processing conditions, thermal process equivalent to a *D* value higher than 1.69 is advisable. The process time equivalent to 1.69 *D* for cold-filled cans was 23.2 min at 207°F (Table 3). The process times corresponding to 2.0 and 2.5 *D* were 24.4 and 26.1 min respectively. The processing times at higher initial temperature to achieve different *D* values were still less (Table 3). Hence, in the commercial processing, a 2.5 *D* process instead of a 1.69 *D* process is recommended. In addition to complete inactivation, process variables such as piece size, fruit maturity, etc. which might be encountered would also be taken care of. Similar studies were carried out at the processing temperature of 215°F, the calculated process times for a 2.5 *D* process were lower than at 207°F (Table 4).

Table 3. Process time calculated by the graphical method for different *F* values

$F_{205.0}^{29.22}$ value for process calculations	Process equivalence	Process time in min at 207 F for initial temperature of		
		80 F	147 F	167 F
1.00	1.69 *D*	23.2	14.4	8.8
1.18	2.00 *D*	24.4	15.4	9.6
1.48	2.50 *D*	26.1	16.8	10.7
1.78	3.00 *D*	27.4	18.0	11.8

Table 4. Comparison of the process times calculated by the formula method by different procedures and graphical method to achieve $F_{205.0}^{29.22} = 1.48$ (2.5 *D* process) at pH 4.0 for guava halves canned in 401 × 411 (A 2½) cans

		Formula method						
			Process parameters					Graphical method
P_T (°F)	I_T (°F)	Procedure	f_h (min)	j_h	f_c* (min)	j_c	Process time (min)	process time (min)
207	80	A	38.00	1.00	28.00	1.48	23.96	26.1†
		B	38.00	1.00	28.00	1.48	23.96	
		C	39.70	0.92	30.67	1.34	25.12†	
	147	A	40.00	1.00	22.80	1.00	15.47	16.8†
		B	40.00	1.00	22.80	1.00	15.47	
		C	39.79	1.00	22.46	1.00	15.39†	
	167	A	42.80	1.00	19.60	1.18	7.08	10.7†
		B	42.80	1.00	19.60	1.18	7.08	
		C	40.55	1.05	23.96	1.04	8.97†	
215	85	A	23.20	1.00	18.00	1.19	14.57	15.8†
		B	23.20	1.00	18.00	1.19	14.57	
		C	21.62	1.04	19.53	1.14	14.39†	
	152	A	19.60	1.27	13.60	1.59	8.40	10.8†
		B	21.60	1.00	13.60	1.59	6.00	
		C	18.38	1.34	14.50	1.43	8.94†	
	165	A	21.60	0.96	11.60	1.16	5.65	8.4†
		B	24.00	1.00	11.60	1.16	5.14	
		C	21.84	0.96	11.91	1.08	5.91†	

* f_c values have not been used in process calculations.

† Figures are the recommended process times calculated by the formula and the graphical methods.

Correlation coefficient (r) for heating (f_h) and cooling (f_c) curves varied from −0.9839 to −0.9988.

Process calculation by formula method

The graphical method of process calculation is an accurate method as no assumptions about the nature of heating or cooling curves are involved. The only possible source of error is in the manual graphical integration of lethality. However, this method can be used only when the processing conditions such as can size, and initial and processing temperatures are identical to the conditions used in the heat penetration studies. The formula method overcomes these disadvantages.

Lenz & Lund (1977) have compared newer methods of process calculation with the well established graphical or the formula methods. But the comparative data of the process time evolved by the graphical and the formula methods are scarce. Moreover, these data are limited to low acid conduction heating foods wherein the process requirements are high.

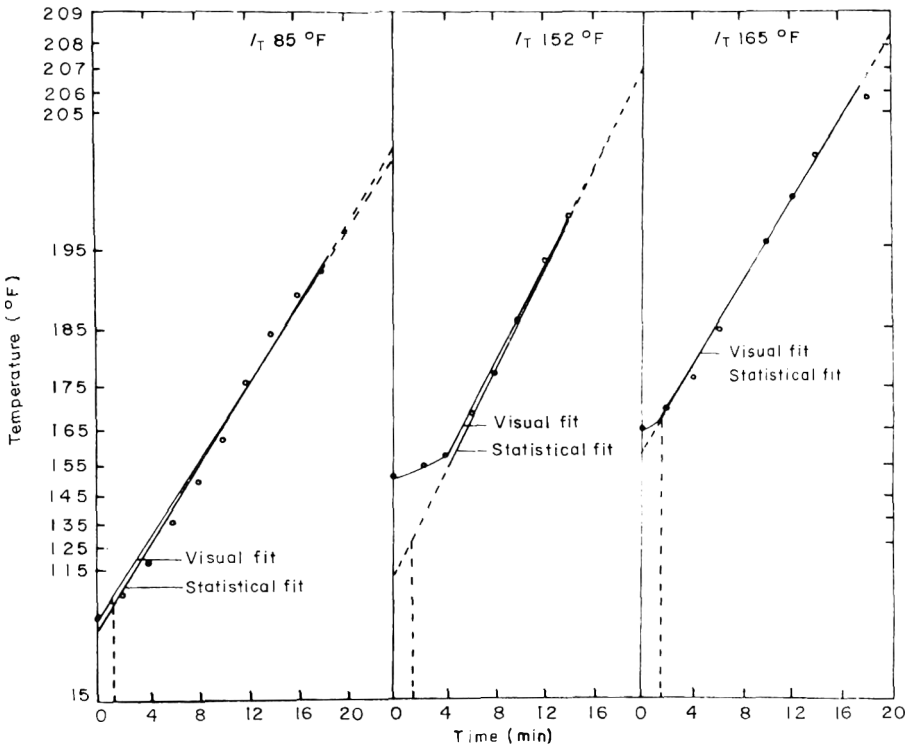

Figure 4. Semilog plot of heating curves for guava halves processed at 215°F (x-axis scale represents only the portion required to find f_h and j_h).

The semilog plots of heating and cooling curves for cans processed at 215°F are shown in Figs 4 and 5. Similar curves were drawn for the cans processed at 207°F.

In the calculation of the process time by the formula method (Ball & Olson, 1957), Ball assumed f_h to be equal to f_c and the log of cooling curve (j_c) to be 1.41. Irrespective of the method of drawing the heating and cooling curves, f_c was always lower than f_h, and j_c was not equal to 1.41 (Table 4). Moreover, the tables of Ball & Olson (1957) could not be used as the z value of 29.22 of PE was beyond the limits of the tables. The relationship, $f_h/U{:}g$, is virtually independent of process parameters but is greatly dependent upon the j of the cooling curve (j_c) (Stumbo, 1973). Hence, $f_h/U{:}g$ tables corresponding to actual j_c developed by Purohit & Stumbo (1972) were used in the calculations. Irrespective of the procedure (A, B or C) used, the process times (B_B) calculated were lower than the values found by the graphical method (Table 4). Of the three procedures, the process times calculated by Procedure C using f_h and f_c found by statistical fit were most of the times nearest to the values found by the graphical method.

Microbiological safety of process evolved

Hot-filled (167°F) cans of guava without acidification (pH 3.95) processed for 6, 8 or 10 min (the last one being the calculated time) at 207°F remained

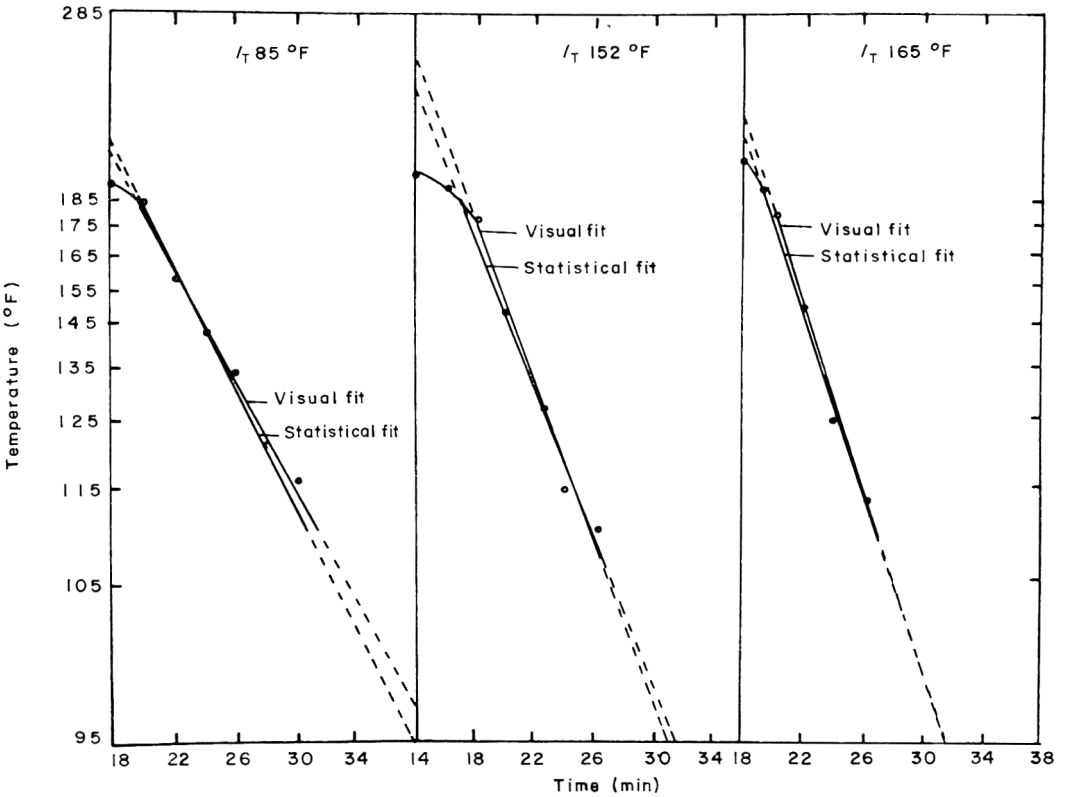

Figure 5. Semilog plot of cooling curves for guava halves processed at 215°F and cooled in water at 85°F (x-axis scale represents only the portion required to find f_c and j_c).

normal, while those processed up to 4 min bulged. Microflora in the latter consisted of non-spore forming gram-positive and gram-negative rods and cocci, and yeasts. Hot-filled cans of guava inoculated with these organisms and processed for 6, 8 and 10 min at 207°F remained normal on incubation, and no microbial growth was observed on subculturing.

Of the test organisms *B. coagulans* and *Cl. pasteurianum* used, guava pulp and syrup homogenate supported the growth of the latter at pH 5.0 and 5.5 but not at pH 4.0 and 4.5; the former did not grow. The TDT of *B. coagulans* and *Cl. pasteurianum* in tomato serum at pH 4.5 were $F_{250}^{14.4} = 0.15$ and $F_{200}^{13.6} = 2.48$ respectively. These values are slightly lower than the values reported (National Canners Association, 1968). The TDT of *Cl. pasteurianum* determined in drained syrup from canned guava was $F_{201}^{12.2} = 1.0$ and in pulp $F_{203}^{14.27} = 1.0$ at pH 4.0 (Fig. 6). These values are lower than the TIT ($F_{205}^{29.22} = 1.0$) of PE at pH 4.0. Hence, process time evolved on the basis of PE inactivation is adequate enough to prevent any rare chance of spoilage by *Cl. pasteurianum*. Inoculated packs using spores of *B. coagulans* and *Cl. pasteurianum* remained normal after incubation, and subculturing studies revealed no microbial growth indicating that the organisms had been inactivated during processing.

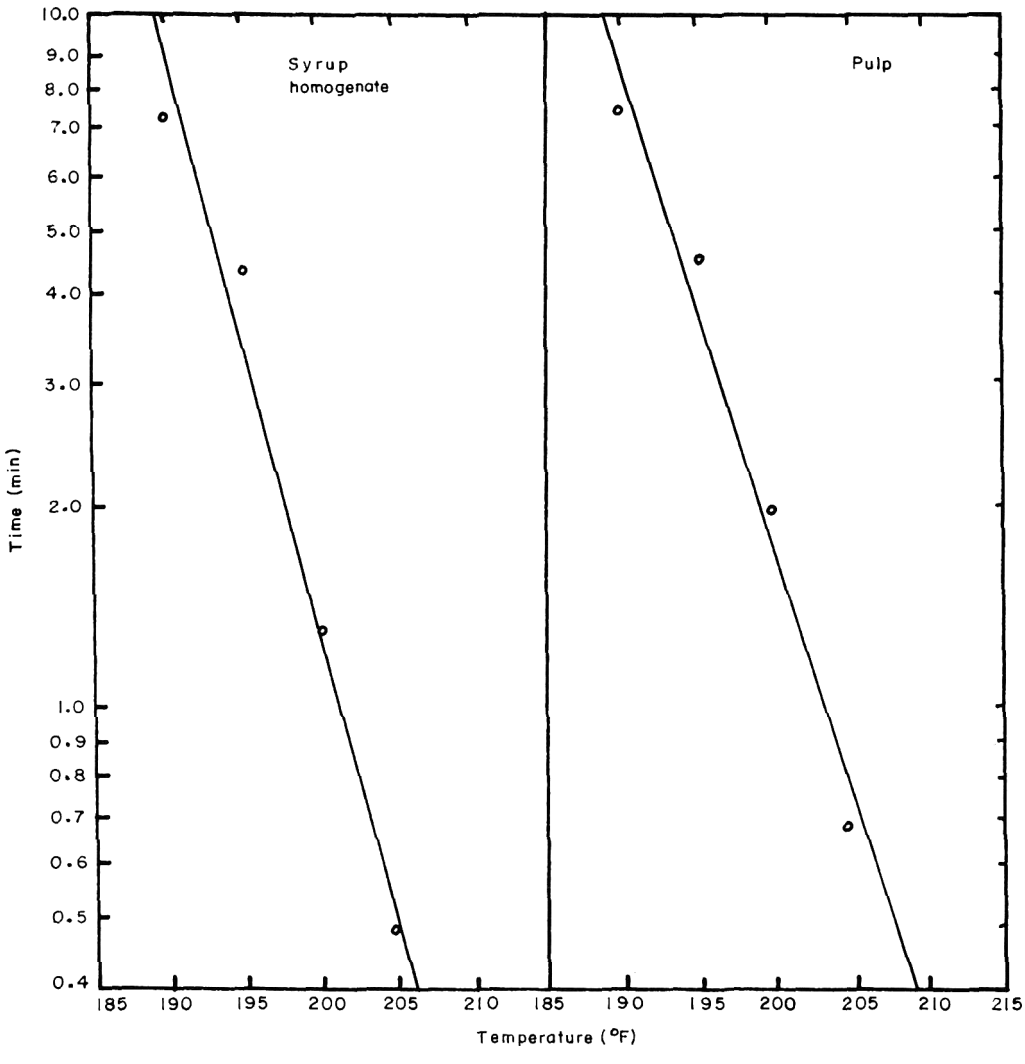

Figure 6. TDT curves of *Cl. pasteurianum* in guava preparations at pH 4.0.

These results confirm that the thermal process evolved on the basis of PE inactivation renders the canned product microbiologically safe.

Quality of the canned product

When the thermal process given was equivalent to 2.5 *D*, no residual PE or peroxidase activity was found in the canned product either immediately after processing or during storage up to 12 months. Vacuum in the cans decreased slightly during storage. Drained weight ranged from 61 to 66%, cut-out Brix from 20 to 21%, and pH from 3.9 to 4.0 (Table 5).

Initial reflectance colour data of the canned product indicated that the samples processed with added citric acid and ascorbic acid at 207°F for 10 min

Table 5. Results of the cut-out examination of hot-filled cans of guava processed at 207°F

Processing conditions									
Ascorbic acid and citric acid	Process time (min)	Storage period (month)	Vacuum (in Hg)	Drained weight (%)	TSS (%)	Acidity (%)	pH	Viscosity of syrup (cP)	Ascorbic acid (mg/100 g)
+	10	0	18	64.42	21	0.248	4.00	6.5	64.50
−	10		17	64.84	21	0.248	4.00	6.5	29.50
+	25		18	61.21	21	0.248	3.90	9.0	—
+	10	4	14	61.40	21	0.244	3.90	115.0	59.61
−	10		14	64.70	21	0.244	3.90	117.5	13.60
+	25		17	62.13	21	0.244	3.90	142.5	57.57
+	10	8	10	62.86	20	0.239	3.90	150.0	22.00
−	10		15	60.00	20	0.239	3.95	136.0	12.91
+	25		11	62.86	20	0.239	3.90	146.0	17.23
+	10	12	10	65.71	20	0.197	3.95	192.0	20.94
−	10		16	64.12	20	0.197	3.90	237.0	10.05
+	25		17	65.71	20	0.197	3.92	250.0	—

were slightly more bright ($Y\%$) than that processed for 25 min or the samples processed without the canned sample containing no added additives for 10 min at 207°F (Table 6). After storage for 8 months, the brightness ($Y\%$) decreased slightly, the dominant wavelength shifted towards the yellow region, and the colour difference (ΔE) was more. The extent of the changes followed the same pattern as observed with variations in the treatment.

Canned fruit retained the shape and texture even without calcium treatment which made them more firm. The maximum force required to shear the control samples was 309.4 g, as compared to 424.4 g in the treated samples.

Table 6. Reflectance colour data of guava canned in syrup, processed at 207°F and stored at room temperature

Processing conditions							
Treatment*	Process time (min)	Storage period (month)	Reflectance colour data				
			x	y	Y (%)	D (nm)	ΔE†
CA and AA added	10	0	0.3523	0.3679	44.38	575.0	—
CA and AA added	25		0.3591	0.3680	42.21	576.1	—
No CA and AA added	10		0.3322	0.3450	41.87	574.0	—
CA and AA added	10	8	0.3652	0.3690	39.52	578.8	4.503
CA and AA added	25		0.3659	0.3692	37.14	578.8	5.764
No CA and AA added	10		0.3654	0.3652	37.96	579.6	6.064

* 0.06% citric acid (CA) and 0.125% ascorbic acid (AA) was added to the covering syrup.

† Calculated using the sample containing AA and CA, and processed for 10 min as reference.

Table 7. Effect of calcium firming of guava halves on pH, viscosity and pectin content of covering syrup drained from guava cans filled hot and processed for 10 min at 207°F

Treatment	pH	Viscosity† (cP)	Percentage of pectin† (wt/vol)
Plain syrup (45° Brix)	—	4.5	—
Control*	3.82	23.5	0.2555
(1) Dipped in hot water (140°F) for 15 min and then in 1% $CaCl_2$ solution for 30 min	3.68	9.0	0.1519
(2) Dipped in 1% $CaCl_2$ solution for 30 min	3.68	9.0	0.1610
(3) 0.371% $CaCl_2$ added to the covering syrup	3.64	11.5	0.2010

* Fruit pieces were not treated with $CaCl_2$.

† Determined after 3 days of storage at room temperature.

The viscosity of the covering syrup from cans processed for 10 and 25 min at 207°F was initially 6.5 and 9.0 cP respectively, and increased to about 237 and 250 cP respectively at the end of 12 months of storage. Viscosity increase is caused by the leaching of the pectin from the fruit into the syrup (Table 7). The pectin recovered from the syrup was a high methoxyl (7.35%) high molecular weight (209 482) pectin. Treatment with calcium reduced the viscosity of the syrup. Of the three different procedures investigated, dipping in 1% calcium chloride solution for 30 min either with or without incubating in hot water at 140°F was found to be the best. Viscosity in the treated sample was 20 as against 58 cP in the untreated samples after 4 months of storage (Table 8).

Calcium treatment reduced the pH from 3.82 to 3.68 (Table 7), and does not affect heat penetration.

Ascorbic acid content in the control and treated samples of the canned product decreased considerably during storage at room temperature (Table 5).

Table 8. Effect of calcium firming on viscosity of covering syrup*

	Viscosity at 90°F (cP)	
Storage time (day)	Control	$CaCl_2$ treated†
1	6.5	6.0
15	25.0	8.5
60	29.5	11.5
120	58.0	20.0

* Cans hot-filled and processed at 207°F.

† Guava halves dipped in 1% calcium chloride solution prior to canning.

Sensory evaluation showed the prepared fruit immersed initially in water at 140°F for 30 min and then dipped in calcium chloride solution to be superior with respect to texture, the untreated sample to be superior with respect to flavour, and the sample soaked in calcium chloride solution without preliminary incubation in water at 140°F to be superior with respect to overall quality.

Recommendations for commercial processing

Dipping of prepared halves or quarters of guava in 1% calcium chloride solution reduces the viscosity of the covering syrup. Canning in syrup containing 0.125% ascorbic acid and with or without 0.06% citric acid, and a thermal process equivalent to $F^{29.22} = 1.48$ based on PE inactivation yields an organoleptically acceptable and microbiologically safe canned product. Thermal process schedules required to achieve this at different initial temperatures and processing temperatures of 207 and 215°F are tabulated.

Acknowledgments

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Heat transfer characteristics and process requirements of hot-filled guava pulp

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Summary

In the in-can processing of canned fruit pulp in batches, the pulp is filled hot into cans, sealed, processed and cooled. The hold-up time between sealing and processing may vary from 10 to 30 min when the temperature of pulp decreases in the peripheral regions. During processing and cooling, temperature changes in the peripheral regions are rapid but not so in regions near geometric centre. In guava pulp filled into cans at 170°F (76.9°C) or above, the desired F value is achieved at the geometric centre even without processing but not so at other regions. The point of minimum lethality shifts from the geometric centre towards the periphery with an increase in the filling temperature and a decrease in the hold-up time. Based on these findings, a thermal process schedule has been evolved for canned guava pulp with different filling temperatures and a hold-up time of 10 and 30 min.

Introduction

In the conventional method of canning of fruit pulp in small canneries, the pulp is heated in a heat exchanger or steam-jacketed kettle, filled hot into cans, sealed and processed in boiling water in batch type retorts. The capacity of sealers used may vary from ten to twenty cans per min and of retorts 180 to 350 A 2½ (401 × 411) cans. Hence, the hold-up time between sealing and processing of a batch of cans may vary between 10 to 30 min when the temperature near the periphery and in the surrounding regions drops.

In the hot fill-hold-cool procedure recommended by the National Canners Association (NCA), fruit pulps below pH 4.0 are to be heated at 190°F (878°C) for 1 min and between pH 4.0 and 4.2 at 205°F (95°C) for 30 sec, filled into cans at temperature not less than 185°F (85°C) and the sealed cans

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are to be processed at least for 2 min in steam or water at 190°F (87.8°C) before cooling (Ranganna, 1977). The above procedure is subject to the condition that (1) the hold-up between filling and sealing is not more than 1 min, (2) the processing is continuous, and (3) the can surface temperature is not less than 160°F (71.1°C) at the start of processing. Since the batch processing does not meet these conditions, the process for guava pulp under conditions similar to those prevailing in the canneries was investigated.

Materials and methods

Fully ripe guava of the Allahabad variety purchased from the local market were used in this study.

Heat penetration studies

The fruits were lye-peeled by dipping in 2.5% boiling sodium hydroxide solution for 60–70 sec, washed in water and dipped in 0.2% citric acid solution. The peeled fruits were cut into pieces and, after the seeds had been removed, pulped in a pulper. The pulp was heated to the required temperature in a steam-jacketed kettle, filled into the cans, sealed and processed. The filling temperature ranged from 160°F (71.1°C) to 190°F (87.8°C). The cans were held in air for 10 min or 30 min, thereafter processed in boiling water (207°F [97°C] in Mysore) and cooled in water.

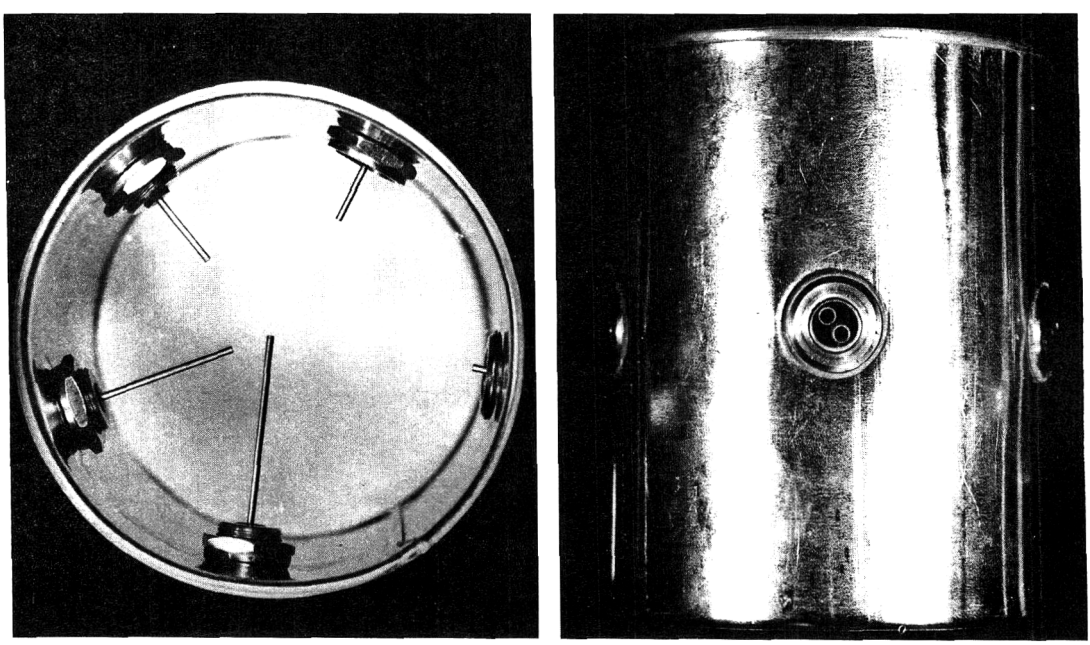

Figure 1. Thermocouple positions in a (401× 411 A 2½) can used for determining the rate of heat penetration into canned guava pulp.

Table 1. Thermocouple positions and their distance from the inner surface of the can

Thermocouple no.	Position	Distance from periphery of can (cm)
1	1	0.5
2	2	1.7
3	3	2.3
4	4	3.7
5	5	5.1

Preliminary studies carried out by positioning the thermocouples at different heights along the central axis of the can indicated that the heat transfer was by conduction and the cold point was at the geometric centre. In subsequent studies, five thermocouples were fixed on the circumference of the body wall at the geometric centre. Each thermocouple increased progressively in length from the body wall, the fifth reaching the geometric centre of the can (Fig. 1). The distance of each thermocouple from the body wall is given in Table 1. The term 'position' is used under results and discussion to denote the different thermocouples. Thus, Position 1 refers to Thermocouple No. 1 which was 0.5 cm away from the inner surface of the can body wall.

The temperature of the canned product at various positions was measured at 1-min intervals during hold-up time, processing and cooling.

Calculation of process time

The process time was calculated by the improved graphical method (Bigelow *et al.*, 1920).

Inoculated pack studies

Guava pulp was heated to 180°F (82.8°C), filled into (401 × 411 A 2½) cans, inoculated with about 5 million spores of *Bacillus coagulans* or *Clostridium pasteurianum*, sealed, kept inverted in the air for 10 min, processed for 4, 6 and 8 min at 207°F (97.2°C) in boiling water and cooled.

The cans inoculated with *Cl. pasteurianum* were incubated at 86°F (30°C) and those with *B. coagulans* at 113°F (45°C) for 7 days, and at room temperature for 30 days. The incubated cans were examined for any external sign of spoilage. At the end of the incubation period, they were subcultured to detect the presence of any dormant bacteria. Uninoculated cans were also processed and incubated alongside as control.

Results and discussion

Viscosity of guava pulp ranged from 32 000 to 96 000 cP. Heat transfer was by conduction.

In batch processing of canned pulp, during the hold up time between sealing and processing, temperature of the canned product decreased. Consequently, the temperature between the can sealed in the beginning and at the end of a batch varies. To simulate these conditions, sealed cans of hot pulp were held in air for 10 and 30 min before processing, and temperature changes observed at 1-min intervals. At Positions 1, 2 and 3, the temperature decrease was considerable; at 4 and 5, temperature remained almost constant up to 10 min hold up time and decreased thereafter only very slightly (Table 2).

In the cans filled at 175°F (79.4°C) and held for 10 min, the temperature during processing increased at Positions 1, 2 and 3, and began to decrease soon after cooling started (Fig. 2). On the contrary, temperature decreased at Positions 4 and 5 during processing; during cooling, temperature increased at these positions initially before it began to drop. The period of temperature rise during cooling is referred to, hereafter, as the period of residual heating.

Table 2. Temperature drop at different thermocouple positions during the hold-up period

Filling temp. (°F)	Hold-up time (min)	Temperature at the end of hold-up time (°F)				
		1*	2	3	4	5
165	1	145	160	163	165	165
	5	138	157	162	165	165
	10	135	154	161	165	165
	20	131	149	156	161	162
	30	128	144	150	157	159
175	1	150	171	172	174	175
	5	145	168	172	174	175
	10	138	162	168	173	173
	20	134	156	163	168	170
	30	130	149	157	161	164
180	2	146	171	176	178	179
	5	147	167	175	178	179
	10	147	162	172	177	179
	20	140	154	166	168	175
	30	135	149	161	169	171
185	1	143	174	177	182	184
	5	147	172	176	182	184
	10	144	169	173	181	183
	20	140	165	167	176	178
	30	139	159	162	171	174
190	1	150	176	184	188	189
	5	156	174	183	188	189
	10	154	170	181	187	188
	20	148	164	174	181	184
	30	142	159	168	174	177

* Thermocouple position.

Process calculation

Thermal inactivation time (TIT) of pectin esterase (PE) in guava syrup homogenate at pH 4.0 ($F_{205}^{29.22} = 1.0$) was higher than that of peroxidase ($F_{180.5}^{14.97} = 1.0$) or thermal death time of *Cl. pasteurianum* ($F_{203}^{14.27} = 1.0$) in pulp (Nath & Ranganna, 1983). In musk melon, orange, papaya and mango, TIT of the enzyme was higher in syrup homogenate than in pulp (Nath & Ranganna, 1977a, b, c, 1980, 1981). Hence, TIT of PE in guava syrup homogenate at pH 4.0 was used as the basis for evolving the process time of pulp. The *F* value was equivalent to 1.69 D.

The inactivation rate curves at different thermocouple positions are shown in Fig. 2. To calculate the process time, curves parallel to the cooling portion of the inactivation rate curve are required to be drawn at different intervals. The period of residual heating at Positions 4 and 5 was 9 and 11 min respectively, and the temperature rise at these positions was by 3 and 5°F respectively. In drawing the parallel curves, to give allowance for the residual heating period, extent of temperature increase and pattern of heating were assumed to be constant (Fig. 2). Positions 5 and 4 were positions of minimum lethality when the filling temperature was 162 and 165°F (72.2 and 73.9°C) respectively, and the hold-up time was 30 min (Table 3). At other filling

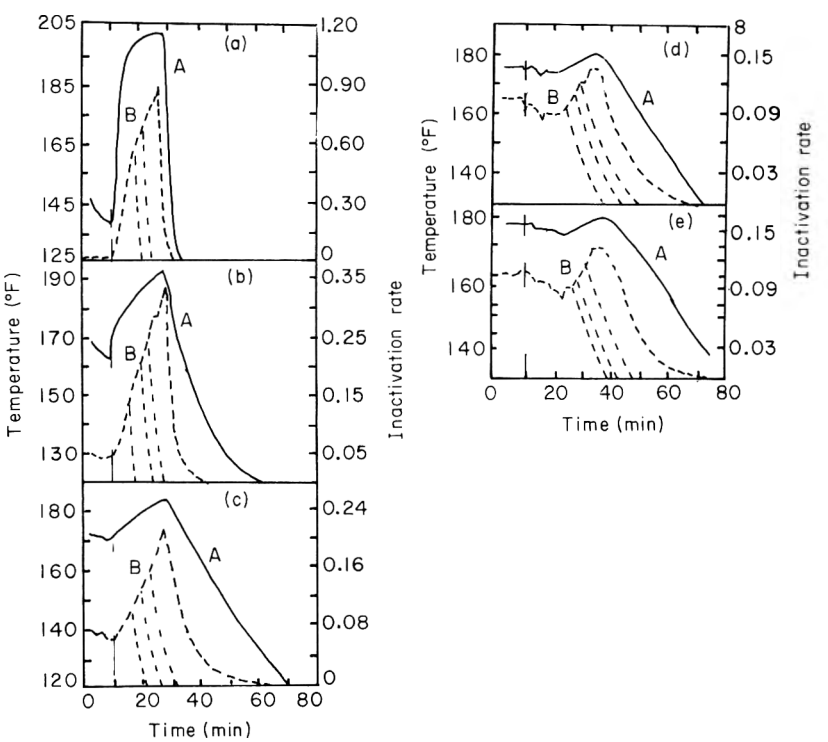

Figure 2. Heat penetration curve and the corresponding inactivation rate curve at different thermocouple positions in guava pulp. Cans filled at 175°F, held in air for 10 min, processed at 207°F for 17 min and cooled.

Table 3. Relationship between filling temperature, hold-up time and actual initial temperature at the point of minimum lethality, and the process time*

	Hold-up time of 10 min			Hold-up time of 30 min		
Filling temp. (°F)	Position of minimum lethality	Actual initial temp. (°F)	Process time (min)	Position of minimum lethality	Actual initial temp. (°F)	Process time (min)
162	3	154	18.4	5	155	21.4
165	3	161	13.8	4	157	21.8
173	2	168	9.8	3	158	16.4
175	2	162	7.9	3	157	16.0
180	—	174	6.1	3	162	13.9
190†	—	—	—	3	168	10.1

* Process times for pulp filled at 185°F and hold-up times of 7, 28 and 36 min were 6, 9.7 and 13 min, respectively.

† Process times for hold-up times of 7 and 28 min were 4.7 and 10 min, respectively.

temperatures and at hold-up time of 10 or 30 min, Position 2 or 3 formed the point of minimum lethality. Temperature rise and fall at these positions were rapid during heating and cooling. Hence, the process time calculated was not affected. *F* values at Positions 4 and 5 shown in Fig.4, corresponding to the desired *F* value of 1 at the point of minimum lethality might vary. In these calculations, lethality contributed during the hold-up time was not considered in the process calculations. It was regarded as a safety factor to account for the processing variables.

The graphical interpolation (*F* value *versus* process time) curves to find the process time equivalent to the desired *F* value are shown in Fig. 3. At Positions 4 and 5, pulp remains at higher temperatures during the entire

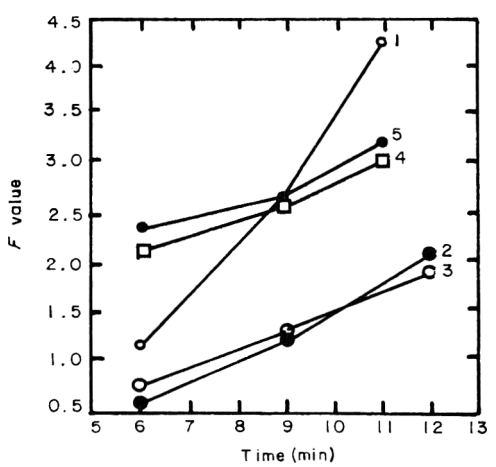

Figure 3. *F* value *versus* process time plot corresponding to Fig. 2.

period of holding, processing and initial period of cooling (Fig. 2). When the initial temperature is 175°F (79.4°C), to achieve $F_{205}^{29.22} = 1.0$, no processing is required if either of them is considered as the point of minimum lethality. At Positions 2 and 3, 7.9 and 7.4 min respectively are required. Hence, Position 2, which requires a slightly higher process time, is the position of minimum lethality. If a higher F value is desired to be achieved, length of processing required would be more, and the position of minimum lethality would shift towards the geometric centre. At the end of the processing time of 7.9 min equivalent to F value of 1.0 at Position 2, F values achieved at Positions 1, 3, 4 and 5 were 2.08, 1.07, 2.38 and 2.53 respectively. The plot of these F values against thermocouple position, represented as the distance of its tip from the wall of the can, is shown in Fig. 4 which shows that the Position 2 is the point of minimum lethality. By the time, the pulp achieved the desired lethality at this point, all other points would have achieved considerably more lethality.

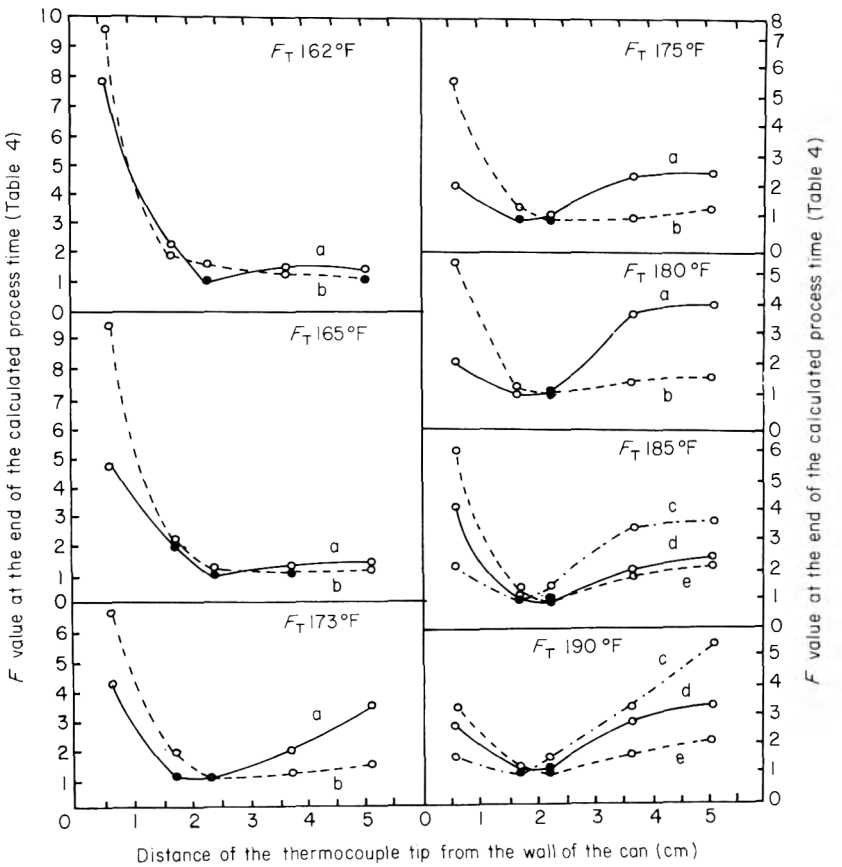

Figure 4. F value achieved at different thermocouple positions when the F value at the point of minimum lethality is 1.0 (Note: see Table 3 for particulars of processing). a, b, c, and e represent hold-up time of 10, 30, 28 and 36 min respectively. ●, Represent position of minimum lethality.

The observations were similar even when the hold-up period was 30 min except that the point of minimum lethality was at Position 3 instead of 2.

Similar studies were carried out at different filling temperatures and holding times. Actual initial temperature at the end of the hold-up time, position of minimum lethality and the process time required to achieve $F_{205}^{29.22} = 1.0$ at the position of minimum lethality are given in Table 3 and Fig. 5. The F values attained at other positions when the F value at the point of minimum lethality was 1 are given in Fig. 4.

Figure 4 shows that the geometric centre (i.e. Position 5), which is the point of slowest heating is not always the position of minimum lethality. The position of minimum lethality shifted from the geometric centre towards the periphery with an increase in the filling temperature and a decrease in the hold-up time. Position 5 was 5.1 cm while 2 and 3 were 1.7 and 2.3 cm respectively from the periphery. Hence, heat transfer near Positions 2 and 3 was considerably faster as compared to Position 5. Consequently, the process

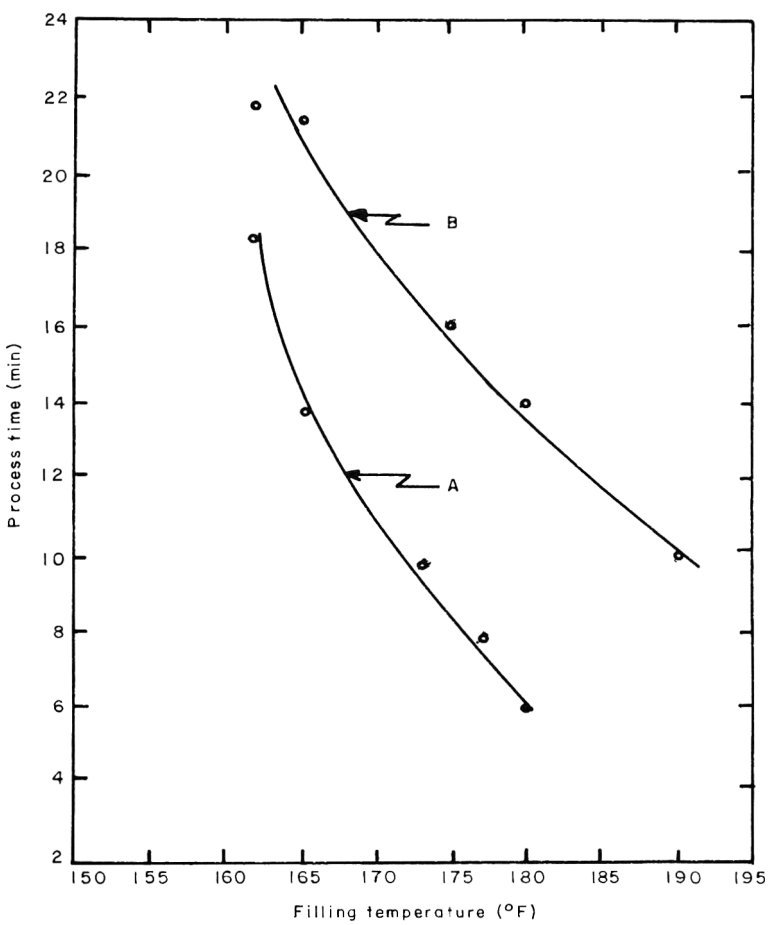

Figure 5. Plot of process time *versus* filling temperature for (401 × 411 A 2½) cans at 207°F. A, 10 min hold-up time; B, 30 min hold-up time.

time required reduced with an increase in the filling temperature and a decrease in the hold-up time.

Because the cans were processed after holding for 10 or 30 min, filling temperatures did not represent the actual initial temperatures (Table 3). Irrespective of filling temperature and hold-up time, processing time should have been the same when the actual initial temperatures were similar. It was so only when the points of minimum lethality were also the same but not when they were different (Table 3).

Generally, in products which heat by conduction, the geometric centre is the 'cold point' to evolve the process time. Under conditions where there is a time lag between sealing and processing, the temperature of the material in the outer regions decreases rapidly. These regions do not achieve the desired F value if the process time is calculated assuming the geometric centre as the point of minimum lethality. The point lies between the geometric centre and the periphery of the can. The actual position depends upon the filling temperature and the hold-up time. With an increase in the filling temperature and reduction in the hold-up time, the 'cold-point' shifts from the geometric centre towards the can periphery. Even in the case of some low acid foods, the cold point is reported to be at positions other than the geometric centre. Powers *et al.* (1962) applied the extreme value method and other statistical procedures to heat penetration data and found that the point of minimum lethality was slightly away from the geometric centre. Hurwicz, Gardner & Tischer (1956) observed during heat processing of beef that the 'cold-spot' was a doughnut-shaped zone.

Microbiological safety of process evolved

Guava pulp after heating to 180°F (82.22°C) was mixed with the spores of *B. coagulans* or *Cl. pasteurianum*, filled into cans, sealed, inverted, held for 10 min in air and processed for 4, 6 or 8 min in boiling water as against the calculated process time of 7.9 min. The cans remained normal on incubation indicating that the thermal process evolved rendered the canned product microbiologically safe.

The process time so evolved is much less than the process time of 40 min at 185°F (80°C) for 301 × 309 cans recommended by Jain & Borkar (1968) and commercial processing conditions.

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Aqueous processing of lupin seed

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Summary

Processing of two species of lupin seed by an aqueous extraction process has been explored and the resulting products have been examined. It has been shown that aqueous processing is an effective method for the separation of oil, protein and alkaloids of lupin seeds.

Introduction

Although lupins have been exploited by man since ancient times their use as food has been restricted by the high content of bitter, poisonous alkaloids. Breeders have succeeded in isolating low alkaloid varieties which contain between 0.01–0.05% alkaloids, as compared to 1–2% for bitter seeds (Aguilera & Trier, 1978). One such variety, released by the Georgia Costal Plain Experiment Station, U.S.A., is Tifwhite 78, a white lupin that combines the attributes of seed-shatter resistance, soft seed, sweetness (low alkaloid content) and adequate winter hardiness (Miller, 1980).

Lupin comprises a substantial range of species that vary widely in oil and protein content (Hudson, Fleetwood & Zand-Moghaddam, 1976). Characteristics of *Lupinus albus* spp. include a protein content of about 40% and oil contents which range from 9 to 13%. Commercial recovery of oil by solvent extraction is not economical at these low levels, but sweet lupins will require conversion into shelf-stable defatted ingredients before broad application in foods becomes possible. Bitter *Lupinus mutabilis* spp., on the other hand, have oil and protein contents similar to soybeans, the drawback in their utilization being the high levels of water soluble, poisonous alkaloids present in the seeds.

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Methods reported previously for production of refined lupin products used defatted flour as raw material (Pompei & Lucisano, 1976). The aqueous extraction process, developed at the Food Protein R&D Center, Texas A&M University, utilizes water for simultaneous recovery of oil and protein. The process consists of an aqueous extraction of comminuted seed, followed by centrifugal separation of the slurry into oil, solids and an aqueous phase which contains most of the protein and water soluble components. Aqueous extraction has been applied to coconuts (Hagenmaier, Cater & Mattil, 1978), peanuts (Rhee *et al.*, 1977), sunflower (Hagenmaier, 1974), cottonseed (Rhee, unpublished data), and soybeans (Mattil, Rhee & Cater, 1979).

This paper reports findings on yields and products compositions of lupin seed processed by aqueous extraction on a pilot plant scale.

Materials and methods

Seeds

Sweet lupin seeds (*Lupinus albus* var Tifwhite) were obtained from Georgia Costal Plain Experiment Station, U.S.A. Bitter seeds of the species *Lupinus mutabilis* were grown in Peru.

Processing of sweet seeds

Whole seeds were cracked and dehulled in a Carver dehuller and separator. The clean cotyledon fraction was ground in a 250 CW Alpine Contraplex pin mill so that over 90% of the particles passed through a 100 mesh screen. Three runs were made using 22.7 kg ground cotyledons, and tap water as extraction medium in a 1:15 (w/w) flour:solvent ratio. Extraction proceeded for 40 min at 65°C. Extractions A and C were conducted at pH 8.0, while extraction B was performed at pH 9.0. A two-phase Westfalia centrifuge was used to separate residue from extracts in Runs A and B, while a Sharples centrifugal decanter was utilized in Run C. Separation of aqueous phase and oil emulsion was accomplished in the Westfalia centrifuge with a three-phase separating bowl system which also permitted discharge of residual solids. Protein precipitation from the de-oiled extract was accomplished at pH 4.5 and 32°C, except for Run C, where a pH of 4.4 was attained. Protein curd and whey were separated in the Westfalia centrifuge. The resulting curd was neutralized to pH 6.8 with NaOH, and spray dried at an air inlet temperature of 193°C and air outlet temperature of 90°C.

Processing of bitter seeds

Bitter lupins were cracked by corrugated rolls, and hulls removed by air aspiration. Cotyledons were divided into two lots of 20.5 kg each. Those for Run D were ground as described previously, while those for Run E were

flaked to ten to twenty thousands of an inch. Further processing was similar to that of the sweet seeds except that the residue was re-extracted at pH 8.2 with 45.5 kg water, and the protein curd was washed twice with acidified water (pH 4.5) to remove residual alkaloids. A summary of processing conditions is presented in Table 1.

Table 1. Summary of processing conditions

	Run code				
Factor	A	B	C	D	E
Type of seed	Sweet	Sweet	Sweet	Bitter	Bitter
Raw material preparation	Grinding	Grinding	Grinding	Flaking	Grinding
pH of extraction	8.0	9.0	8.0	8.0	8.0
Separation equipment	Westfalia	Westfalia	Sharples	Westfalia	Westfalia
Re-extraction of residue	No	No	No	Yes	Yes
pH of protein precipitation	4.5	4.5	4.4	4.5	4.4
Washing of curd	No	No	No	Yes	Yes

Analytical procedures

Moisture, oil, ash, crude fibre and nitrogen solubility index were determined according to official AOCS methods (AOCS. 1978). Oil content of liquid samples was determined by acid hydrolysis (AOCS, 1978). Nitrogen solubility was estimated by AOCS method Ba 11-65. Amino acid analyses were performed according to Moore, Speckman & Stein (1958). Total alkaloids in the full-fat flour and protein concentrates from sweet lupin were determined by acid-base titration with *p*-toluene sulphonic acid and expressed as lupanine (Ruiz, 1977). In the case of bitter lupin, alkaloids were analysed by gas chromatography as described by Von Baer & Feldheim (1982). Total alkaloids were calculated from the concentrations of individual alkaloids

Results and discussion

Figure 1 presents the flow diagram for aqueous processing of lupins. Cracking and dehulling were effectively achieved since the light hulls loosened easily from the cotyledons, and were separated by air aspiration. Proximate compositions of lupinseeds, hulls, and full fat flours are presented in Table 2. Full fat flour from *L. albus* contained 11.0% oil and 39.4% protein, while that from *L. mutabilis* had 19.1% oil and 41.4% protein. Alkaloid content of the bitter variety was 2.0%, 100-fold greater than that of sweet seeds. Fibre content in flours was low due to efficient removal of hulls.

Distribution of oil and protein in selected processing streams for sweet lupin is presented in Fig. 2. Recovery of protein in the extract ranged from 69% in

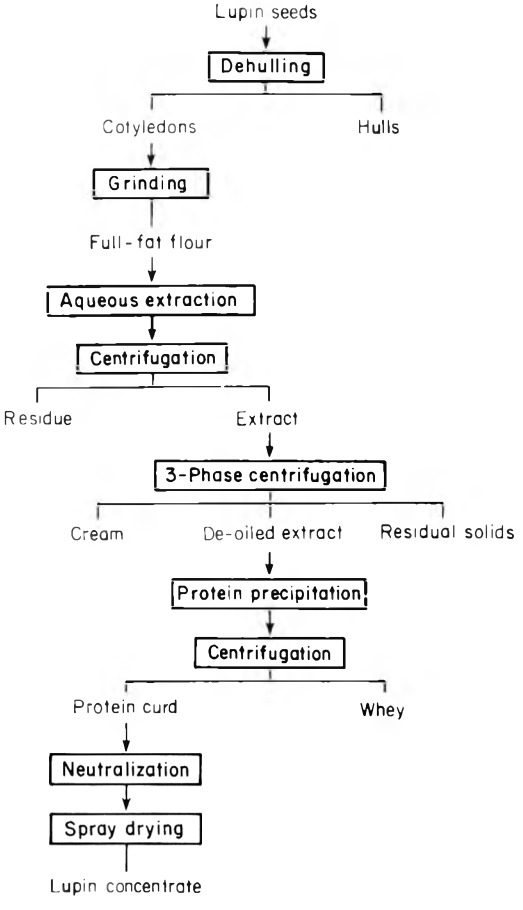

Figure 1. Flow diagram for aqueous processing of lupines.

Table 2. Analyses of lupin seed and products

Sample	Moisture (%)	Oil (%)	Protein* (%)	Crude fibre (%)	Ash (%)	Alkaloids (%)
Lupinus albus						
Whole seed	8.4	11.2	38.0	4.7	4.1	
Hulls	7.6	2.6	2.4	56.0	2.1	
Full-fat flour†	7.1	11.0	39.4	2.1	4.5	0.02
Concentrate A	5.3	5.0	79.4	0.08	4.3	<0.01
Concentrate B	7.3	6.1	71.1	0.07	5.5	<0.01
Concentrate C	5.1	3.7	72.0	0.11	4.1	<0.01
Lupinus mutabilis						
Full-fat flour	7.8	19.1	41.4	1.7	3.6	2.0
Concentrate D	6.0	3.6	77.5	0.07	3.8	0.002
Concentrate C	4.1	9.9	73.5	0.07	5.0	0.003

* Calculated as N × 6.25.

† Composite of samples from the three runs.

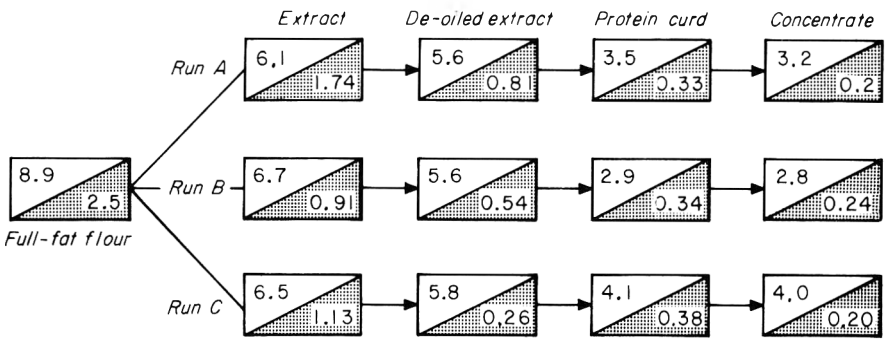

Figure 2. Oil and protein balance for sweet lupines (figures in kg).

Run A to 75% in Run B. Oil extraction was highest in Run A, where almost 70% of the oil present in the seed was recovered, and lowest in Run B, with only 36% recovery. Apparently, a higher extraction pH significantly improves protein recovery but impairs oil extraction.

Between 84–91% of the solubilized protein was recovered in the deoiled extract after three-phase centrifugation. Although 71% of the solubilized protein was precipitated at pH 4.4, lower yields were achieved at pH 4.5. According to laboratory scale experiments, between 80–85% of the extracted protein should have precipitated at the isoelectric point.

Almost one-fifth of the original weight of flour was recovered as a high protein powder. Lupin protein concentrate obtained in Runs A, B and C had 79.4, 71.1 and 72.0% protein and 5.0, 6.1 and 3.7% oil, respectively. These results compare well with those reported by Mattil *et al.* (1979) for soybeans. Amino acid analyses revealed that concentrates had lysine contents ranging from 3.64–3.75 g/16 g of nitrogen. Methionine, the limiting amino acid in lupin seed together with cysteine, varied from 0.62–0.69 g/16 g N. Functionally, sweet lupin concentrate powders had over 70% nitrogen solubility at pH 6 and above, and a pleasant taste and cream-white colour.

In the case of bitter seeds, several improvements in processing were introduced. Yields of oil and protein for different fractions are reported in Fig. 3. Recoveries of oil and protein in the combined extract were 51 and 81% for Run D, and 50 and 82% for Run E respectively. Approximately, one-fifth additional protein and about one-third to one-fourth additional oil were obtained after re-extracting the spent residue with water, thus, confirming results reported by Rhee *et al.* (1973). Upon three-phase centrifugation, 78% of the extracted protein for Run D, and 83% of that extracted in Run E, were recovered in the de-oiled extract. The efficiency of oil separation was significantly improved by flaking. The de-oiled extract of flaked cotyledons contained only 7.5% of the extracted oil, while 80% was in the cream. It appears that fine grinding produces small oil droplets which form an oil-in-water emulsion stabilized by protein, that is later recovered with the de-oiled extract. Flaking does not reduce extraction yield and also does not

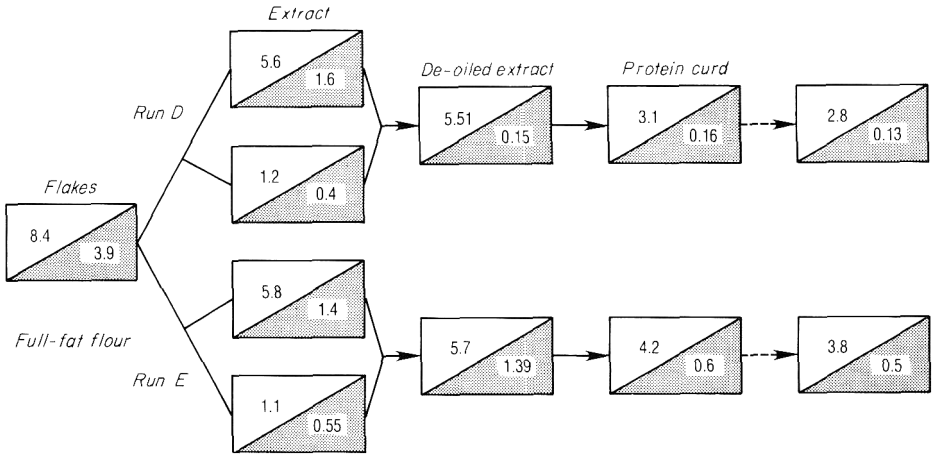

Figure 3. Oil and protein balance for bitter lupines (figures in kg).

promote emulsification, probably because it is less disruptive to the microstructure. The use of higher speeds and larger centrifuge bowls, which provide for longer residence times, may further increase the yield of free oil while reducing residual oil in the concentrates.

pH 4.4 was more effective in precipitating the protein of bitter lupins, as in the case of sweet lupin. About 74% of the extracted protein was recovered at this lower pH, compared to 60% at pH 4.5. Washing significantly reduced bitterness in the curd, without affecting the final yield of product. Lupin concentrates in Runs D and E contained 77.5 and 73.5% protein, and 3.6 and 9.9% oil respectively. Powders were bland and whitish, with no bitterness present.

As shown in Table 2 the alkaloid content in concentrates made from sweet seeds was below the limit of detection of the method (0.01%). Concentrates from bitter seeds contained 0.002–0.003% alkaloids, representing a 1000-fold reduction of the original content of seeds.

Aqueous processing of oilseeds also generates the residue from protein extraction, a nutritious by-product. This residue, representing about one-fifth of the original solids present in the cotyledons, contains less than 20% of the protein and less than 50% of oil originally present in lupins. It might be useful as a wet adjunct, or as a flash-dried product for animal feeding.

Conclusion

Aqueous processing has been shown to be an effective method in separating oil, protein and alkaloids in lupin. Main advantages of the process are: (1) The use of water as solvent; (2) concentration of protein and removal of oil are effected in a single step; and (3) alkaloids are reduced to very low levels by simple washings. Further work needs to be done in improving the yields of protein concentrates and oil fraction.

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Occurrence of *Aspergillus* spp. and aflatoxins in commercial mushroom compost

JANET S. MacFALL, G. A. BEAN* AND K. L. DEAHL†

Summary

One hundred and thirty-three isolates of *Aspergillus* spp. were collected from commercial mushroom houses. Aflatoxins were detected in three samples of compost. Seventy-three per cent of the isolates produced aflatoxins in liquid media. Aflatoxins were produced on sterilized and pasteurized compost plus the components of compost including rye grain, corn cobs and commercial spawn; 24°C was a more favourable temperature for aflatoxins than 14°C. Aflatoxin production varied with isolate, substrate and temperature and ranged from no aflatoxins to all four aflatoxins B_1, B_2, G_1 and G_2.

Introduction

Aspergillus flavus and *A. parasiticus* can be found on nearly all types of decaying plant materials throughout the world and the aflatoxins they produce have been detected in a wide range of food commodities (Hesseltine *et al.*, 1966; Nesbitt *et al.*, 1962; Stoloff, 1977). Isolates of *Aspergillus* spp., including *A. flavus* have been collected by several workers from samples of compost used in commercial mushroom cultivation (Beach, 1937; Fergus, 1978; Fordyce, 1970).

Mushroom compost supports a dynamic and competitive microbial population of fungi, bacteria and actinomycetes. Due to this mixed population, aflatoxin production in compost would not be expected since workers have observed that if an aflatoxigenic *Aspergillus* spp. is growing in a mixed culture with other micro-organisms, aflatoxin production will not occur due to competitive pressures from the other organisms present (Christensen,

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Mirocha & Meronuch, 1977). However, in a preliminary survey of mushroom compost from commercial mushroom houses *Aspergillus* spp. were frequently observed growing in isolated pockets of compost as well as on spawn grains. In an early study, Mattoni *et al.* (1972) added aflatoxin contaminated peanut meal and cotton seed meal to mushroom compost prior to pasturization. However, they were unable to detect aflatoxins either in the compost or mushrooms at the first cropping period. They suggested that aflatoxins are broken down during mushroom production either by chemical ammoniation or microbial inactivation.

The present study was to determine if aflatoxins can occur in compost used for mushroom production and thus become potential health hazards both to workers contacting the compost and to consumers purchasing contaminated mushrooms.

Materials and methods

Isolation of aflatoxin production by Aspergillus *spp.*

A total of thirty commercial mushroom houses in which *Aspergillus* spp. growth on mushroom compost was observed, were sampled. Samples of compost were placed in Petri dishes containing 2% water agar and sterile rye grains. Plates were sealed with Parafilm and shaken, allowing the grains to become coated with *Aspergillus* spp. spores. Within 3 to 4 days, the *Aspergillus* spp. were transferred to potato dextrose agar plates. Isolates were maintained on PDA slants at 24°C in the dark.

Aflatoxin production was determined by transferring each isolate into two 50 ml flasks containing a semi-synthetic liquid sucrose media (SMKY) (Marsh, Simpson & Truckcess, 1975). Still cultures were incubated at 23°C for 10 days, and autoclaved for 5 min. The media was filtered through cheesecloths and the filtrate extracted twice with 25 ml chloroform. The extracts were flash evaporated to dryness, re-dissolved in 2 ml chloroform, and 30 μl sample spotted onto thin layer chromatographic plate coated with 250 μm silica gel 60 without fluorescent indicator (EM Laboratories). The plates were then developed in chloroform, acetone (9:1 v/v) until the ascending development distance was 10 cm from the point of spotting.

Aflatoxin extraction from compost samples

A modified version of Velasco's clean-up procedure was used (Velasco, 1970). A sample of compost weighing 50 g dry weight was placed in a beaker to which 300 ml of acetone–water (85:15 v/v) was added. The beaker was covered tightly with aluminium foil and shaken for at least 1 hr. The sample was then filtered and 180 ml collected. Fifty millilitres of distilled water were placed in another 600 ml beaker, plus 10 ml of 10% ferric chloride and 15 ml of 4.83% sodium hydroxide. Next, 25 ml of a buffer solution was added which

contained 27.5 g of sodium acetate, 12 ml of glacial acetic acid plus distilled water to make 1 litre. The sample filtrate was added to the gel, stirred for at least 3 min, and filtered. One hundred and sixty millilitres of this filtrate were collected, placed in a separatory funnel, and extracted twice with 25 ml portions of chloroform. If poor separation occurred after vigorous shaking, an additional 50 ml distilled water and 10 ml chloroform were added to each flask. The chloroform fraction was drained into a boiling flask and flash evaporated to dryness. The flask was washed twice with about 4 ml chloroform each time, and the wash placed in a 1 cm diameter glass column packed first with glass wool, then 2 g sand, and about 8 g alumina, Brockman activity I, 80–200 mesh and finally 2 g sand. The column was then eluted with 10 ml chloroform which was flash evaporated, the aflatoxins re-dissolved in chloroform, and then transferred to glass vials. The samples at that point exhibited a yellow pigmentation of varying intensity. Samples were then spotted on pre-coated TLC plates. Thirty-microlitre aliquots of each sample were spotted 1 cm above the bottom of the plates and 5 ng samples of B_1 were spotted in adjacent spots. These were chromatographed as previously described. Confirmation of aflatoxins was done by lightly spraying plates with a solution of 15% sulphuric acid (Przybylski, 1975). Although this method did allow extraction and confirmation of aflatoxins, it did not allow for aflatoxin quantitation due to interfering compost components of which co-chromatographed with aflatoxin B_1.

Aflatoxin production in compost

Samples of compost were taken from houses of three commercial mushroom growers in the Kennett Square, Pennsylvania region. A total of nine samples of compost were collected, including spawn grains. Each of the samples exhibited *Aspergillus* spp. growth both on the spawn grains and the compost straw material. The samples were dried at room temperature, weighed and extracted for aflatoxins. Dry weights ranged from 8 to 52 g dry weight. Extracts were examined for aflatoxins using thin layer chromatography as described in the previous section. An experiment was performed to test the suitability of several compositing materials to support growth and aflatoxin production by *Aspergillus* spp. at temperatures frequently used for mushroom production. An isolate of *A. flavus*, Asp-30, which produced B_1, B_2, G_1 and G_2 in SMKY media was inoculated onto nine different substrates including compost components. There were five replicate flasks containing 20 g/l flask of each substrate except with compost; 5 g (dry wt) was used. Substrates included rye grains, Spawn Mate (a commercially prepared delayed release nutrient supplement), corn cobs, two spawns of different ages, sterile compost, and pasteurized compost as used for muchroom cultivation. Spawn is rye grain or millet which is inoculated with *Agaricus* spp. which is used as the source of mushroom inoculum in the compost. Samples of pasteurized compost were also inoculated with sterile, cooked rye grains lightly coated

with *Aspergillus* spp. spores and after incubation the grains were removed prior to aflatoxin extraction. The temperatures of incubation were 24°C, the temperature of mushroom colonization of compost, and 14°C, the temperature at which commercial mushroom beds are maintained to induce mushroom sporophore formation. After 10 days, the treatments were autoclaved for 10 min; samples were extracted for aflatoxin as previously described, except the sample weights were less than 50 g and the amounts of solvents used and filtrates collected were reduced proportionately. Also, all treatments except compost and the semi-synthetic liquid sucrose media (SMKY) were first ground with the extraction solvent in a Waring Blender and filtered. The compost and liquid media treatments were extracted with solvents and then filtered.

Experiments were also done to determine whether *Aspergillus* spp. can grow and produce aflatoxin in the mushroom beds simultaneously with *Agaricus* spp. colonization. The *Aspergillus* isolate used was Asp-22 which had been obtained from commercial mushroom compost and produced aflatoxin B_1 in SMKY media, and on sterile compost at room temperature. Five different treatments were used and they were as follows: compost + spawn; compost + spawn + *Aspergillus* spp.; compost + spawn + Spawn Mate; compost + spawn + Spawn Mate + *Aspergillus* spp.; compost + *Aspergillus* spp. The compost used was pasteurized prior to the addition of spawn and *Aspergillus* spp. Two 1 cm^2 blocks cut from cultures of the *Aspergillus* spp. growing on a PDA Petri dish, were mixed with the various treatments in plastic bags. After thorough mixing, 250 g wet weight of each treatment were placed in plastic quart containers. There were seven containers used for each treatment, or a total of thirty-five containers. After 20 days of incubation at 24°C, the compost was dried at 80–90°C overnight and analysed for aflatoxins as previously described. Samples of at least 50 g from each container were analysed.

In the following experiment a second isolate was used. Mushroom Research Project culture Asp-30 was isolated from compost and produces all four aflatoxins in SMKY media at room temperature. Three spawn isolates were compared, Spawn A and Spawn B, an off-white and brown strain of *Agaricus brunnescens* which are commercially grown for compost colonization at 24°C, and Spawn C, a strain of *A. bitorquis* which is a commercial variety that is cultured at a constant temperature of around 30°C. The spawn isolates were grown on sterilized millet. Prior to inoculation, a spore suspension of *Aspergillus* spp. containing 0.5 mg spores/ml in distilled water was made and 2 ml of this suspension was added to each flask containing the three spawn isolates. After a thorough mixing in a plastic bag, 250 g wet weight of the mixture were placed in 1 l containers. There were ten containers for each of the various treatments, or a total of 60 l containers. The containers were incubated at 30°C and at 5 day intervals the contents from two containers for each treatment were collected, dried overnight at 90°C and extracted for aflatoxins as described previously. The entire contents for each treatment,

each 50 g dry weight, were extracted and tested for aflatoxins. Based on a compost dry weight of about 100 g per container, this gave four 50 g samples for each treatment at each sampling date.

Results and discussion

Aspergillus *spp. occurrence and aflatoxin production*

A total of 133 *Aspergillus* spp. isolates was collected from a variety of locations in the mushroom houses. The sources of these isolates and their aflatoxin producing ability is summarized in Table 1. Isolates of *Aspergillus* spp. were readily isolated from compost, compost constituents, mushroom

Table 1. Aflatoxigenic and non-aflatoxigenic *Aspergillus* isolates collected from sources in commercial mushroom houses

Source	Total isolates (*n*)	Aflatoxigenic (*n*)	Toxins produced	Isolates (*n*)
Spawn	5	1	$B_1G_1G_2$	1
Corn	4	4	B_1G_2	2
			B_1	1
			$B_1B_2G_1G_2$	1
Compost	91	64	B_1B_2	17
			$B_1B_2G_1G_2$	15
			B_1G_1	13
			B_1	12
			$B_1B_2G_1$	3
			$B_1B_2G_2$	2
			$B_1G_1G_2$	1
			G_1	1
Compost and spawn	11	11	B_1B_2	6
			$B_1B_2G_1G_2$	3
			$B_1B_2G_1$	1
			$B_1B_2G_2$	1
Corn on cob	5	4	B_1B_2	3
			B_1G_1	1
Corn on cob and commercial additive	4	1	B_1B_2	1
Commercial additive	5	4	B_1B_2	2
			B_1G_1	1
			$B_1B_2G_1G_2$	1
Air sampler	6	6	$B_1B_2G_1G_2$	2
			B_1B_2	2
			B_1G_1	1
			$B_1G_2G_1$	1
			$B_1B_2G_2$	1
Others	2	2	B_1B_2	2
Total	133	97		

beds and air from within a mushroom house. Toxigenic and non-toxigenic strains can both be isolated from the same location as well, however, the types of aflatoxin each strain produced varied greatly. Seventy-three per cent of the isolates were aflatoxigenic when grown in liquid media. These data indicate that both aflatoxigenic and non-aflatoxigenic strains of *Aspergillus* spp. may be encountered in routine mushroom cultivation, as a component of the compost microflora. Aflatoxins were detected in three samples of the compost taken from two commercial mushroom houses. One sample contained aflatoxins B_1 and G_1. A second grower had aflatoxin B_1 in one sample from one house and B_2 in a second sample from another house. Therefore aflatoxigenic *Aspergillus* spp. are not only able to grow but also to produce aflatoxins in the compost of commercial mushroom houses.

Aflatoxin production on compost components

To determine what effect the temperatures and substrates used in mushroom production have on aflatoxin production, eight substrates were examined at two temperatures (Table 2). Two *Aspergillus* spp. isolates were chosen for these tests. Asp-30 is an isolate of *A. parasiticus* obtained from a commercial mushroom house where aflatoxins were detected in the compost, whereas Asp-75 is an isolate of *A. flavus,* collected from a commercial mushroom production facility, where no aflatoxins were detected in compost samples.

As seen in Table 2, aflatoxin production occurred at 24°C, however 14°C was unfavourable for aflatoxin production. This temperature difference is in agreement with that observed by others (Christensen, Mirocha & Meronuch, 1977). At 24°C, Asp-30 produced detectable amounts of aflatoxin in all of the mushroom compost components tested with the exception of corn cobs and the commercial spawn designated Spawn 1. Spawn 1 was grown on millet and

Table 2. Aflatoxin production by *Aspergillus* spp. isolates on varying substrates and temperatures

	14 C		24 C	
Substrate	Asp-30	Asp-75	Asp-30	Asp-74
SMKY media	—	—	$B_1B_2G_1G_2$	$B_1B_2G_1$
Rye grain	B_1B_2	—	$B_1B_2G_1G_2$	$B_1B_2G_1$
Spawn mate	—	—	$B_1B_2G_1G_2$	—
Corn cob	—	—	—	—
Sterile compost	—	—	$B_1B_2G_1G_2$	—
Pasteurized compost	—	—	B_1B_2	—
Spawn 1	NT	—	—	—
Spawn 2	NT	—	B_1	—
Spawn 3	NT	—	$B_1B_2G_1G_2$	—

NT, not tested.

was considered to be at its most vigorous and competitive stage when inoculated with Asp-30 or Asp-75. Spawns 2 and 3 were grown on rye grains and were obtained from the same spawn maker. Spawn 2 was also fresh spawn, and a vigorous culture. Spawn 3, however, was not inoculated with the *Aspergillus* spp. until the spawn was several weeks old and approaching the end of its shelf life. Asp-75 isolate produced detectable aflatoxins only at 24°C and only on the SMKY media and sterile rye grains.

Aflatoxin production in compost

The isolate of *A. flavus* designated Asp-22 was observed as growing abundantly in containers co-inoculated with spawn. No growth was observed when Asp-22 was not co-inoculated with spawn, which supports previous observations in commercial mushroom houses that *Aspergillus* spp. first colonizes a spawn grain, then grows into the surrounding compost. This isolate also produced aflatoxins on sterile compost, indicating that compost is nutritionally suitable for toxin production. However, no aflatoxins were detected in any of the treatments of this experiment, using pasteurized compost indicating that competitive pressures from the compost microflora were such that aflatoxin synthesis was not possible or else produced aflatoxins were being rapidly broken down.

The isolate of *A. parasiticus*, Asp-30, when co-inoculated onto compost and Spawn C, grew well and produced detectable amounts of aflatoxins B_1 and G_1 in all treatments as summarized in Table 3. Spawn C, the isolate of *A. bitorquis*, is potentially an important commercial species in that as energy cost rises, more growers may be cultivating this mushroom to avoid the necessity of operating air conditioning units. However, since 30°C is the ideal temperature for *A. bitorquis*, theoretically aflatoxins may be produced throughout the mushroom cultivation process and the risk of aflatoxin contamination would be greatly increased.

These studies indicate that the presence of aflatoxin-producing strains of *Aspergillus* spp. in mushroom compost does not necessarily mean that aflatoxins will be detected in the compost although they are probably being

Table 3. The growth and aflatoxin production by *A. parasiticus* in compost at 30°C

	Sample interval			
Treatment	5 days	10 days	15 days	20 days
Spawn A + Asp-30	—	B_1G_1	B_1	—
Spawn A + spawn mate + Asp-30	B_1	—	B_1G_1	B_1G_1
Spawn B + Asp-30	—	B_1	—	B_1G_1
Spawn B + spawn mate + Asp-30	—	B_1G_1	B_1G_1	B_1G_1
Spawn C + Asp-30	—	B_1G_1	B_1G_1	B_1G_1
Spawn C + spawn mate + Asp-30	—	B_1G_1	B_1	B_1G_1

produced. One isolate, Asp-75, was unable to produce aflatoxins on any of the tested compost components, including sterile compost, although it did produce aflatoxins in liquid media. In contrast, isolate Asp-22 produced aflatoxin B_1 on sterile compost, but not on pasteurized compost which is more microbally active with competitive microorganisms and less likely to support growth and aflatoxin production. A third isolate, Asp-30, produced all four aflatoxins, B_1, B_2, G_1 and G_2 in SMKY media and on sterile compost but when grown on pasteurized compost, only aflatoxins B_1 and B_2 were detected. Interestingly this same isolate was obtained from aflatoxin-contaminated compost. When the same isolate was added with and without a nutrient supplement into larger samples of compost, only B_1 and G_1 were detected. These differences in aflatoxin production reflect both different responses by *A. flavus* and *A. parasiticus* strains to different competitive pressures in different samples of compost as well as inherent differences between the two species. It must also be pointed out that mushroom compost is chemically and microbiologically an extremely heterogeneous material, in which compounds and microorganisms present differ with time and composition and this could influence aflatoxin production by *Aspergillus* spp. isolates that are probably present in mushroom compost as part of the natural microflora. Thus the strains of *Aspergillus* spp. may or may not produce aflatoxins in the compost depending on the nutritional and competitive suitability of that particular sample of compost and the strains of other microorganisms that may be present.

The higher temperatures used in mushroom cultivation does support *A. flavus* and *A. parasiticus* growth and aflatoxin production. This is of particular importance in the production of *A. bitorquis*, a commercially cultivated mushroom often grown during the summer months because it does not require a temperature reduction for fruiting to occur. The temperatures remain between 24–30°C throughout production, possibly allowing aflatoxin production to occur during the entire cultivation process. It was from a house in which *A. bitorquis* was being grown that a compost sample containing B_1 and G_1 was taken. In contrast the lower temperature used to induce sporophore formation of *A. brunnescens*, does inhibit toxin production which may be a convenient and effective means of reducing the possibilities of aflatoxin contamination whenever this mushroom species is grown commercially.

To conclude, aflatoxin producing strains of *Aspergillus* can be detected in commercial mushroom houses. Although aflatoxigenic strains of *Aspergillus* appear to be part of the mushroom compost microflora the intense microbial activity that occurs in compost greatly reduces the risk of aflatoxin contamination either by preventing the synthesis of aflatoxins or by decomposing aflatoxins that are produced. Although our studies agree with the finding of Mattoni *et al.* (1972) that aflatoxins in mushroom compost are very likely to be broken down during mushroom production, as a precaution mushroom producers should avoid using mushroom components such as corn especially if they are known to be aflatoxin-contaminated.

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Moisture desorption isotherms of rough rice

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Summary

Desorptions isotherms of rough rice have been determined at 40, 50, 60 and 70°C and water activities (a_w) from 0.036 to 0.823. An attempt is made to describe the experimental equilibrium moisture content data using mathematical relationships available in the literature. A statistical analysis was performed to evaluate the goodness of fit of the different equations. From this analysis it was concluded that Henderson's equation permits to correlate satisfactorily the experimental data for the whole range of water activity investigated. Finally, a simple empirical equation was postulated to take into account the effect of temperature on water sorption isotherms of rough rice grain.

Introduction

The knowledge of the sorption isotherms of cereal grains is of practical and theoretical interest. Sorption isotherms of such products constitute an essential part of the theory of drying (King, 1968) and provide useful information in the design of drying equipments. Particularly, in the case of rough rice grain it has been found that information related to the sorption characteristics of this material is very limited.

Coleman & Fellows (1925) were the first to investigate the hygroscopic equilibria of rough rice, measuring it at 25°C. Lately, Karon & Adams (1949) determined hygroscopic equilibria of rough rice by placing the samples in desiccators with different salt solutions, stored in a room at 25°C. Other studies have been reported on rough rice covering different ranges of temperature and relative humidities. Juliano (1964) measured the equilibrium

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moisture content of waxy and non-waxy varieties of rough rice at 27.5 and 32.5°C and relative humidities between 44 and 96.5%. On the other hand, the hysteresis in the hygroscopic equilibria of rough rice at 25°C was analysed by Breese (1955).

Henderson (1969) used a dynamic method for the equilibrium determination of rice, which consisted of recirculating air in a closed system at 23°C. Given the relatively short times required for equilibration, no problem of mould growth was observed. Desorption isotherms of rough rice were determined by Zuritz *et al.* (1979) at 10, 20, 25, 30 and 40°C and relative humidities from 11.2 to 92.5%. An empirical equation was proposed by the authors to correlate the equilibrium data taking into account the influence of temperature. However, from the point of view of the drying process, the range of temperatures investigated by these authors is too limited to apply to drying operations of this material. The present study was conducted to supplement published data of desorption isotherms of rough rice, covering a range of temperature between 40 and 70°C, for which there is no information in the literature. Experimental data were analysed using mathematical relationships available in the literature for physical sorption phenomenon. An attempt was also made for relating equilibrium moisture content, water activity and temperature by means of an empirical equation.

Materials and methods

A local Argentine variety (Itapé) of medium rough rice grain was used in our experiments. The grain was harvested in 1982 (April) and field-dried to a moisture content of 13% (dry basis), approximately. Prior to use in the experiments, they were conditioned for 1 month to a relative humidity of 95% by placing the grains in thin layers on trays. The samples were stored during this period in a freezer at 4°C to equilibrate; the final moisture content of the grains was 23% (dry basis). This value was taken as the initial moisture content for the determination of the desorption isotherms. Mould growth was not observed during the storage.

Triplicate samples, about 2 g each, were taken from the trays and placed in vacuum desiccators with saturated salt solutions of a known relative humidity. The different salts used in this work allowed for a range of water activities from 0.036 to 0.823 to be tested. The desiccators were placed in an oven at constant temperature (±0.5°C); four temperatures were used in this work: 40, 50, 60 and 70°C. During the storage in the oven the samples lost water until they reached the equilibrium moisture content. Equilibrium was judged to have been attained when four consecutive weight measurements gave the same reading.

The moisture content of the equilibrated samples was determined by placing the samples in a vacuum oven, for 96 hr at 70°C, over magnesium perchlorate as desiccant. It was found that the interval of 48 hr recommended

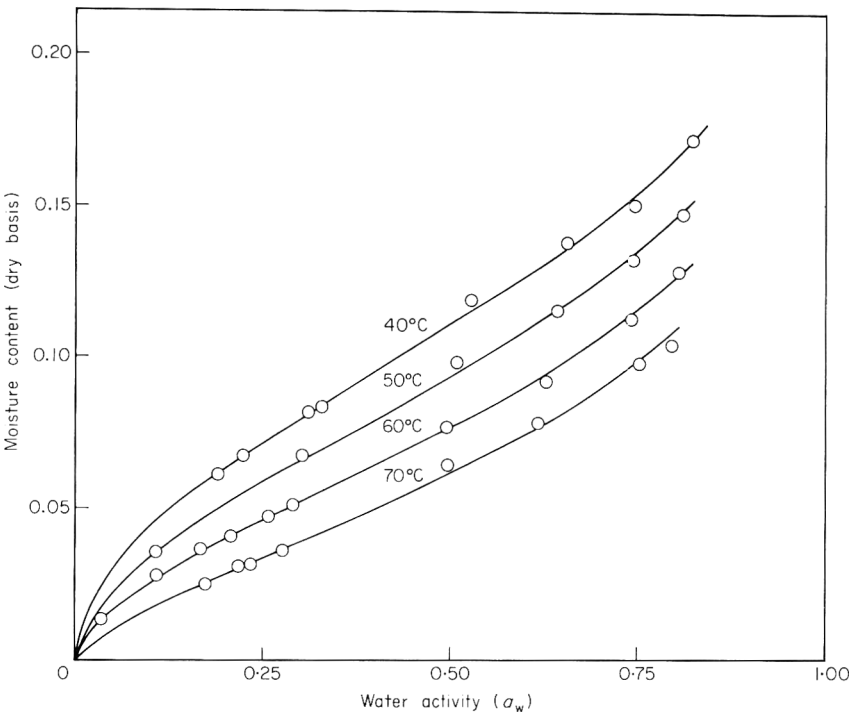

Figure 1. Desorption isotherms of rough rice at different temperatures. ○, Experimental; —— Henderson's equation.

by Iglesias, Chirife & Lombardi (1975) for this purpose, was not sufficient in the case of rice grain.

Results and discussion

The desorption isotherms of rice grain at different temperatures are shown in Fig. 1. It can be seen that for the range of water activity investigated, the effect of temperature on water sorption is in agreement with the fact generally observed that the quantity of adsorbed water decreased as the temperature is increased, at a given relative humidity.

According with the B.E.T. classification, the rough rice isotherm is of type II. This 'sigmoid' shape was found for different cereal grains (Becker & Sallans, 1956; Chen & Clayton, 1971; Gustafson & Hall, 1974). The fitting of the experimental data was conducted using mathematical relationships available in the literature. The equations tested were the following (in all cases a_w refers to water activity and M to moisture content, dry basis):

Oswin equation (1946)

$$M = A\left(\frac{a_w}{1 - a_w}\right)^n \tag{1}$$

Halsey equation (1948)

$$a_w = \exp\left(-\frac{A}{M^b}\right) \tag{2}$$

Henderson equation (1952)

$$1 - a_w = \exp(-KM^n) \tag{3}$$

The parameters of the isotherm Equations (1), (2) and (3) were calculated using a linear regression program; the equations were previously linearized. The results are given in Table 1 for the different temperatures investigated. In order to evaluate the goodness of fit of Equations (1), (2) and (3) as applied to the experimental data, a statistical analysis was performed. The results of this analysis are reported in Table 2; it is worth noticing that the standard deviation (s.d.) of the regression analysis was calculated with the experimental and predicted values of equilibrium moisture content as:

$$\text{s.d.} = \left(\sum_1^N \frac{(M_i - PV_i)^2}{(N-1)}\right)^{1/2} \tag{4}$$

Table 1. Values of the parameters of sorption isotherms for rough rice at 40, 50, 60 and 70°

		Oswin's eqn		Halsey's eqn		Henderson's eqn	
T(°C)	Range of a_w	A	n	A	b	K	n
40	0.190–0.823	0.1055	0.3442	0.0064	2.044	65.694	2.061
50	0.111–0.812	0.0881	0.3957	0.0118	1.628	61.580	1.889
60	0.036–0.802	0.0720	0.4669	0.0242	1.241	56.309	1.709
70	0.175–0.795	0.0573	0.4978	0.0127	1.371	40.846	1.457

Table 2. Statistical values obtained by application of Equations (1), (2) and (3)

T(°C)	Equation no.	s.d. (%)	F test	Probability level	E(%)
40	(1)	0.450	492	0.0005	2.6
	(2)	0.902	118	0.0005	5.3
	(3)	0.189	2816	0.0005	1.8
50	(1)	0.690	138	0.0005	5.8
	(2)	1.452	28	0.01	10.5
	(3)	0.210	1541	0.0005	1.7
60	(1)	0.519	420	0.0005	6.9
	(2)	1.680	33	0.0005	18.0
	(3)	0.178	3620	0.0005	2.3
70	(1)	0.507	230	0.0005	4.8
	(2)	0.868	74	0.0005	8.1
	(3)	0.344	504	0.0005	2.6

where M_i = experimental value; PV_i = predicted value and N = number of determinations. However, from a practical point of view, the mean relative percentage deviation modulus, E, defined as:

$$E = \frac{100}{N}\left(\sum_{i=1}^{N}\frac{|M_i - PV_i|}{M_i}\right) \tag{5}$$

permits to have a direct visualization of the fitting abilities of the different models which have been proposed. This criterium was used by Boquet, Chirife & Iglesias (1978) to test different mathematical relationships to a large variety of food products. These values are also included in Table 2. It can be seen from this table that the best equation for correlating the experimental data of rough rice, in the range of the water activities investigated, is that of Henderson, although Oswin's equation gives a reasonably good representation of experimental data. Similar conclusions were obtained by Boquet *et al.* (1978) who applied two-parameter equations for describing water sorption isotherms of different cereal grains (corn, wheat, sorghum and cooked rice). The comparison of the experimental data with Henderson's equation, for the various temperatures investigated, are shown in Fig. 1.

In order to consider the dependency of the desorption isotherm with temperature, the following empirical equation was postulated:

$$T = C_1(a_w)\exp\left(-C_2(a_w)\,M\right) \tag{6}$$

where T=temperature in °K, $C_1(a_w)$ and $C_2(a_w)$ are empirical parameters which are depending on water activity. Assuming that C_1 and C_2 depend on water activity in a rather simple way, i.e. a linear and a power form for C_1 and C_2 respectively, this dependency was found by linear regression analysis, being

$$C_1(a_w) = 60.801722\,a_w + 355.485292 \tag{7}$$

and

$$C_2(a_w) = 1.4601599\,a_w^{-0.3568276}. \tag{8}$$

Equation (6) was plotted in a linear form as $\ln C_1/T$ *versus* C_2M, together with the experimental data obtained in this work and the results are shown in Fig. 2. It can be seen that the experimental data are satisfactorily correlated by means of Equation (6); the s.d. was calculated being s.d. = 1.22%. It must be pointed out that the range of validity of Equations (7) and (8), in terms of water activity is: $0.036 \leq a_w \leq 0.823$.

In Fig. 2 were also included the equilibrium moisture content data (measured from the desorption branch of the sorption isotherm of rough rice) reported by Zuritz *et al.* (1979), in the range of temperatures from 10 to 40°C. This was done in order to extend the range of application of Equation (6); it was found that the isotherms determined by these authors at 20, 30 and 40°C are well correlated by Equation (6); the s.d. in this case was less than 1.5%

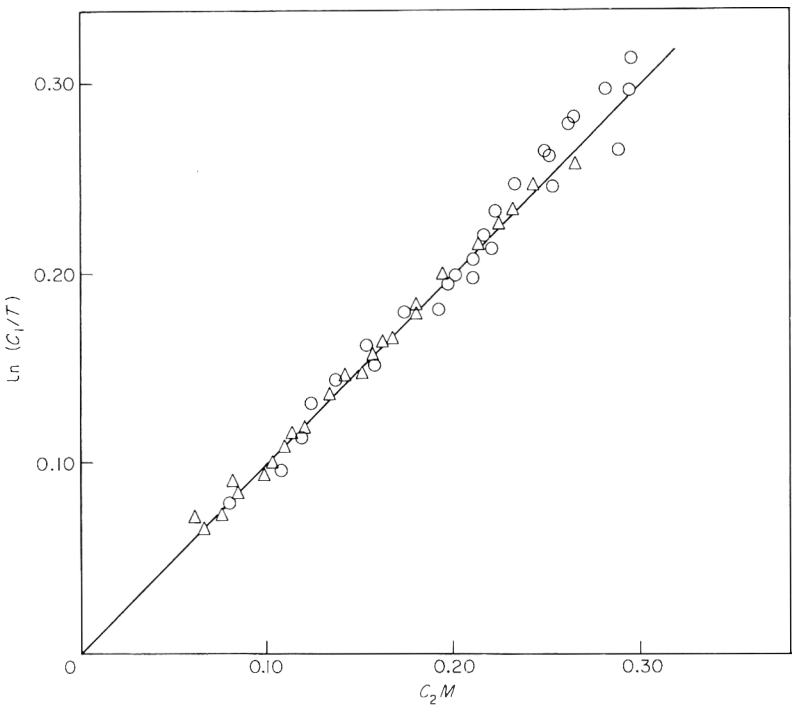

Figure 2. Relationship among temperature, equilibrium moisture content and water activity. △, This work; ○, Zurite *et al.* (1979); ——, Equation (6).

(on average) for the three temperatures. However, for the isotherm at 10°C the experimental data begin to deviate from the empirical correlation in a significant way (s.d. = 5%).

Conclusions

It was found that the best equation for correlating the equilibrium moisture content for rough rice, in the range of water activity 0.036–0.823 is that of Henderson. Oswin's equation also gives a reasonably good representation of the isotherms. It is worth noting that this result is quite surprising considering that this equation has not been used very frequently in the literature, in particular for describing the sorption characteristics of cereal grains.

Although Halsey's equations was developed on a theoretical basis as a criticism of the B.E.T. equation, it was found here that it does not perform satisfactorily for the experimental equilibrium moisture content of rough rice. Similar results were found by Fish (1958) for starch gel and by Boquet *et al.* (1978) for starchy foods.

On the other hand, a simple empirical equation,

$$T = C_1(a_w) \exp(-C_2(a_w) M),$$

which correlates temperature, water activity and moisture content, may be used to predict accurately the effect of temperature on water sorption isotherms of rough rice, practically from 10 to 70°C. It must be pointed out that the only merit of this equation is its simplicity. This simplicity comes from the smaller number of parameters required for its evaluation, in comparison with some four parameter equations available in the literature (Day & Nelson, 1965; Chen & Clayton, 1971).

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Quality characteristics of physically refined soyabean oil: effects of pre-treatment and processing time and temperature

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Summary

Conventional alkali refining of edible oils is being replaced progressively by physical refining, which offers improved yields, reduced processing times and few by-product problems. The variables involved in physical refining—pre-treatment, processing time and processing temperature—have been studied on a laboratory scale for the refining of soyabean oil, and related to the quality characteristics of the refined products. The best results are obtained with phosphoric acid degummed, partially bleached oil processed at up to 250°C for not more than 2 hr. Higher temperatures and longer times lead to quality defects such as loss of stability, increased viscosity, darkening and chemical changes reflected in reduction of iodine value and increase in free fatty acids.

Introduction

The object of refining edible oils is to remove unacceptable materials with the least possible effect on desirable components and with the least possible loss of oil. Conventional alkali refining essentially involves four successive operations, degumming, neutralization, bleaching and deodorization. It is unsuited to continuous handling and is increasingly becoming uneconomic. There is a trend internationally towards the replacement of alkali refining by physical or steam refining, in which the three last stages of the former are combined into a single operation (Sullivan, 1974, 1976; Braae, 1976; Carr, 1976, 1978; Young, 1978).

Physical refining offers important practical advantages. Oil yield is increased by up to 3% (Young, 1978). Since soapstock is no longer a product,

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fatty acid recovery is more direct and effluent problems are minimized. Capital investment costs are reduced and the time-scale involved in the refining sequence is much less.

The question arises—how does the quality of a physically refined oil compare with that of the same oil refined by the classical alkali neutralization process? Quality in this context means those features that would be considered important by the oil refiner. It could be extended to include subtle chemical features having no obvious bearing on colour, flavour or stability, but the present study is not immediately concerned with such aspects.

Since at the present time the most important oil in world trade is soyabean oil, this oil was chosen as the most appropriate for the present study, as well as because physical refining is being increasingly applied to it.

Materials and methods

Crude soyabean oil, partially degummed by means of one water wash, was provided by Unimills, U.K., and physically refined, using various temperatures and times.

Experimental

Degumming. The apparatus used for degumming consisted of a 5 l water jacketed reaction vessel with a flange joint. One of the quick-fit adaptors of the flange joint was fitted with a variable speed glass stirrer. The degummed oil was removed from a stop cock, fitted at the base of the apparatus.

Degumming was carried out using two methods. In the first, crude soyabean oil was gently heated to 65–70°C with continuous stirring, under a nitrogen atmosphere. Then 2% of hot deionized water was added, and stirring was continued for about 30 min. The oil was then cooled to 50°C and centrifuged at 16 000 rev/min for 30 min.

In the second, degumming was carried out by adding 0.6% of 8% aqueous phosphoric acid dropwise to the oil at 80°C under nitrogen, and stirring was continued for about 30 min. The oil was then cooled and centrifuged at 16 000 rev/min for 30 min. The degummed soyabean oil was washed with distilled water (5–10% w/v of the weight of the oil), until the washings were neutral to litmus. Some of the phosphoric acid degummed oils were vacuum dried to remove moisture. The drying stage was done by heating the oil under 2–3 mmHg vacuum to 90°C for 30 min.

Bleaching. Bleaching was carried out with samples of dried phosphoric acid degummed oil. The oil was heated under nitrogen to 80°C with stirring, then 2% of a standard bleaching earth (Laporte Industries Ltd) was added and the temperature raised to 105–110°C and maintained for 30 min, stirring under a nitrogen blanket being maintained throughout. The oil was then cooled to

60°C and filtered under vacuum through a Buchner funnel with a Whatman No. 1 filter paper, using Celite powder to aid filtration.

Physical refining. The equipment used for physical refining consisted of a 5 l quick-fit flask, steam generator, splash head and two cold traps for pump protection. The design of the splash head was such as to limit oil entry to the cold traps. A mantle with dual heating controls was used for heating the oil. Three small holes in the steam tube provided a uniform flow of steam through the heated oil. The first trap was immersed in an ice and salt mixture and the second in liquid nitrogen. The pressure in the apparatus was measured by a Macleod gauge. The equipment was easily dismantled for cleaning purposes.

In the physical refining trials the water degummed, phosphoric acid degummed, and phosphoric acid-degummed + bleached oils were heated under 0.5–1.0 mmHg vacuum. When the oil reached the desired temperature, de-aerated steam was passed in and volatile materials were carried over and collected in the trap. At the end of the refining period, the oil was allowed to cool to about 100°C and the vacuum released with nitrogen. 0.1% of 10% citric acid w/v solution was added. The oil was then held at 100°C under vacuum for a further 20 min to remove water.

At the end of the holding period, the vacuum was released with nitrogen and the oil cooled to 60°C. Up to 7% steam relative to the weight of oil was used for physical refining. The process was carried out at 240–300°C for a variety of times.

Analytical methods. Chemical and physical tests were carried out on the crude and physically refined soyabean oils, *viz.* free fatty acids (FFA) (BSI, 1976a), peroxide value (PV) (BSI, 1976b), iodine value (IV) (BSI, 1976c), anisidine value (AnV) (IUPAC, 1979) induction periods using FIRA-Astell-(IP) (Meara & Weir, 1976), colour determination by Lovibond (BSI, 1976d), phosphorus content (AOCS, 1973), trace metals (AOCS, 1979), viscosity using the Haake Roto Visko instrument, and refractive index at 22°C using an Abbé type refractometer.

Storage tests. In order to predict the long term flavour stability of the physically refined soyabean oils, a storage test was carried out at 60°C. The conditions were similar to those of the Schaal oven test (Pardun & Kroll, 1970). Fifty grams of phosphoric acid-degummed and bleached oil physically refined at 240 and 260°C for 2 hr, and freshly deodorized commercially refined soyabean oil (Loders and Nucoline Ltd) were stored at 60 ± 2°C in 100 cm^3 clean beakers covered with watch glasses.

At intervals of 1 day, FFA, PV, AnV and OV (=2 PV + AnV) were determined for each sample.

Results and discussion

It seems from Table 1 that the levels of phosphorus and trace metal of phosphoric acid degummed soyabean oil, physically refined at 240°C for 2 hr

Table 1. Phosphorus and trace metal contents of crude and physically refined soyabean oils

	Phosphorus (mg/kg)	Trace metals (mg/kg)	
		Iron	Copper
Crude oil	140	3.3	0.08
Water degummed oil, physically refined at 240 C for 2 hr	55	0.90	0.08
Phosphoric acid degummed oil, physically refined at 240 C for 2 hr	23	0.50	0.05
Phosphoric acid degummed and bleached oil, physically refined at 240 C for 2 hr	20	0.35	0.05
Normally alkali refined and deodorized oil, from the data given by Pryde (1980)	1–15	0.1–0.3	0.02–0.06

are lower than those of water-degummed physically refined oil under similar conditions. This is probably due to the action of the phosphoric acid in reacting with trace metal compounds, and also with the calcium and magnesium salts of phosphatidic acids, so modifying these compounds as to facilitate their removal (Swern, 1964; Hvolby, 1971; Gutfinger & Letan, 1978; List *et al.*, 1978a; List, Mounts & Henkin, 1978b). However, the level of phosphorus, and especially iron, in phosphoric acid degummed and bleached physically refined soyabean oil at 240°C for 2 hr are lower than that of phosphoric degummed physically refined oil under similar conditions, as a result of the bleaching stage (Evans *et al.*, 1951, 1952; List, Mount & Henkin, 1978c).

The levels of phosphorus and trace metals determined in the phosphoric acid-degummed and bleached and then physically refined oil are roughly in line with the normal range of good quality finished oils (Pryde, 1980). As it is the pre-treatment stage rather than steam de-acidification which removes bulk of trace metals and phospholipids, the effectiveness of this step is of vital importance in physical refining. Failure to remove phospholipids has been reported to cause subsequent darkening during the steam refining process (Kock, 1980), whilst the residual trace metals will remain in the oil and catalyse oxidative deterioration (List *et al.*, 1978a, 1978b). It is known (Evans *et al.*, 1974) that oils from damaged soyabeans contain increased FFA, high iron and non-hydratable phospholipids. In fact, the quality of oil extracted from soyabeans is characterized by analysis of iron, FFA, PV, phospholipid content, colour, etc. The poor quality of a commercial sample of caustic refined and deodorized soyabean oil (Evans *et al.*, 1974) has been attributed

to both high iron (0.7 mg/kg) and phosphorus (59 mg/kg) contents. The new crushing and extraction techniques (Kock, 1980) are claimed to reduce the initial levels of phosphatides considerably in water degummed crude oils (less than 0.05%, which is about 17 mg/kg of phosphorus). It is likely that physical refining of such oils would bring about significant improvements in the quality of finished oils.

Table 2 shows that the free fatty acid levels of soyabean oil physically refined at 240–260°C for 2 hr are less than 0.1%. These are considered acceptable by the refiners. However, when the temperature employed exceeds 260°C, and for longer times, especially at 300°C for 2 hr, the FFA levels increase above their original value in the crude oil. This is evidently due to the partial hydrolysis of the triglycerides, which might occur at such temperatures.

Even when physical refining was carried out for short times, such as ½ hr at 300°C, the FFA increased. On the other hand 240°C for ½ hr was not sufficient to reduce the FFA to a low level.

Physical refining at 240–260°C for 2 hr was sufficient to reduce the PV to very low levels. However, at higher temperatures, such as 300°C, the PV increased considerably presumably due to oxidation. It can also be seen that the stability (induction period) was reduced sharply when physical refining was carried out at very high temperatures, 280–300°C and for longer times. This is probably due mainly to reduction in the levels of tocopherols (Jawad *et al.*, to be published). However, it must be noted that when physical refining

Table 2. General chemical and physical characteristics of phosphoric acid degummed and bleached physically refined soyabean oils

Oil treatment	Time (hr)	FFA (% oleic acid)	PV (mEq/kg)	IV	Induction period at 100 C (hr)	Viscosity (Poise) at 22 C	Refractive index at 22 C	Colours in 1 in cell		
								R	Y	B
None (crude oil)		0.56	4.30	134	9.0	0.46	1.4731	4.7	46.0	0.0
240 C	2	0.10	0.1	133	8.2	0.57	1.4736	0.9	3.1	0.4
250 C		0.09	0.0	132	7.8	0.57*	1.4736*	0.9	3.1	0.4
260 C		0.09	0.0	130	7.6	0.57	1.4738	0.9	2.6	0.4
280 C		0.60	0.8	126	6.0	0.76	1.4744	1.2	3.2	0.4
300 C		1.20	1.6	115	1.5	1.03	1.4750	1.4	4.1	0.4
280 C	½	0.2	0.1	132	7.8	0.59	1.4735	1.5	4.5	0.4
	1	0.2	0.3	129	6.6	0.65	1.4738	1.7	5.3	0.4
	2	0.6	0.8	126	6.0	0.76	1.4744	1.2	3.2	0.4
	3	1.1	1.8	118	2.0	0.96	1.4748	1.2	3.8	0.4
240 C	½	0.4	3.9	134	8.6	0.59	1.4731	2.5	9.2	0.4
280°C		0.2	0.1	132	7.8	0.59	1.4735	1.5	4.5	0.4
300°C		0.3	2.2	130	7.2	0.63	1.4741	1.4	3.7	0.5

* Normally alkali refined and deodorized soyabean oil (Pryde, 1980) has a viscosity at 25°C of 0.5 Poise and a refractive index of $n_D^{25} = 1.4728$.

was carried out at high temperatures (280–300°C) for a *short* time (½ hr) the induction periods were not reduced appreciably.

Iodine values fell progressively with increased temperatures of physical refining, the reduction being very significant at high temperatures, such as 300°C. This indicates a loss of double bonds, due probably to degradation or polymerization of some of the fatty acids. Refractive index and viscosity data for oils physically refined and deodorized at 240–260°C are comparable with those of Pryde (1980).

Both the viscosity and refractive index increase considerably as temperatures of physical refining rise. These increases are an indication of the extent of polymerization of the physically refined oil, but cannot be quantitatively related to it, since other factors such as triglyceride rearrangement, conjugation and *trans*-acid formation could also have a significant effect. The colours of the phosphoric acid-degummed, bleached and physically refined soyabean oil at temperatures 240–260°C for 2 hr were acceptable. However, at high temperatures such as 280 and 300°C for longer times, the oil assumes a darker colour. This deterioration in colour of oil physically refined at high temperatures, could be due to the oxidation of colourless compounds or the

Table 3. Storage tests on soyabean oils at 60 C

Oil treatment	Storage (days)	FFA (% oleic acid)	PV (mEq/kg)	AnV	OV
Commercially refined*	Fresh	0.08	0.05	1.9	2.0
Phosphoric acid degummed + bleached physically refined at 240 C for 2 hr		0.1	0.05	2.5	2.6
Phosphoric acid degummed + bleached, physically refined at 260 C for 2 hr		0.09	0.05	2.0	2.1
Commercially refined	1	0.14	0.45	18.6	19.5
240°C 2 hr		0.17	0.30	21.4	22.0
260 C 2 hr		0.21	0.40	29.6	30.4
Commercially refined*	2	0.20	5.60	34.3	45.5
240°C 2 hr		0.18	3.23	45.7	52.2
260 C 2 hr		0.25	3.60	57.2	64.4
Commercially refined	3	0.25	8.83	59.8	77.5
240°C 2 hr		0.24	5.91	70.3	82.1
260°C 2 hr		0.29	6.30	79.3	91.9
Commercially refined	4	0.29	12.8	88.3	114
240°C 2 hr		0.31	9.92	93.4	113
260°C 2 hr		0.35	10.3	103	123
Commercially refined	7	0.82	28.6	142	200
240°C 2 hr		0.61	23.3	163	210
260°C 2 hr		0.75	25.2	199	249

* By alkali, and deodorized.

existing colour pigments becoming fixed by high temperatures (Rich, 1964). Cowan (1976) reported that the chlorophyll pigments tend to be stabilized at elevated temperatures and are harder to remove.

Table 3 shows that fresh physically refined soyabean oils have similar FFA, PV, AnV and OV's to those of commercially refined alkali and deodorized soyabean oil. As the overall quality of the phosphoric acid-degummed, bleached and then physically refined oils at 240 and 160°C for 2 hr was better than that of water-degummed (or phosphoric acid-degummed) and physically refined oil, these oils were used in the comparison storage study. The commercial sample of soyabean oil was fresh, caustic refined and deodorized. This was employed because in the opinion of some refiners the stability of caustic refined and deodorized soyabean oil is marginally better than that of the oil produced by steam refining. This, of course will depend upon the initial quality of crude oil subjected to refining. All samples showed a similar rate of rise in FFA, especially after 7 days accelerated storage. PV, AnV and OV also increased with storage time, the rate of increase of formation of peroxides being higher for the commercially refined oil. On the other hand, oil physically refined at 240°C, and especially at 260°C, for 2 hr showed higher increases in AnV. Painty flavours developed in all three oils after 4 days' storage at 60°C, becoming intense after 7 days' storage.

Conclusions

Physical refining is considered a practical alternative to alkali refining, providing that the pre-treatment of the oil before physical refining reduces the phospholipids and trace metals to sufficiently low levels. Phosphoric acid-degumming and partial bleaching appears to be the most effective method of pre-treatment.

Both temperature and residence time have very important effects on the quality of the oil. Soyabean oil physically refined at temperatures such as 240, 250 and even 260°C for 2 hr has very low levels of FFA and good oxidative stability when compared with alkali refined soyabean oil.

However, when the temperature exceeds 260°C, with a processing time of 2 hr, chemical and physical damage is suffered by the oil. This involves reduction in oxidative stability, increase in FFA and darkening of the oil. These changes are accompanied by increases in viscosity and refractive index parameters, which could be used as the easiest way of identifying batches of oil which have been damaged by temperature overshoot.

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Acceptability of a canned pâté product based on some Gulf of California shrimp by-catch fish

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Summary

A canned pâté product has been developed using deboned minces prepared from five fish species commonly found in shrimp by-catch from the Gulf of California. This product was made to simulate similar products that are marketed in Mexico but which are based on meat. De-boned minces prepared from fish which had been eviscerated and cleaned in two different ways were used as the basic raw material for pâté formulations. Acceptability trials were held in which panelists were asked to score for various organoleptic characters. These data were statistically evaluated and showed that the pâtés were all acceptable. However, some major differences in quality existed particularly with respect to colour for those pâtés prepared from certain fish species. The effect on pâté quality of using different formulations and methods of evisceration of fish was minimal.

Introduction

It has been estimated that on a global basis at least 3–4 millions tonnes of shrimp by-catch is annually discarded at sea during commercial shrimping operations (Meinke, 1974; FAO, 1975; Allsopp, 1976). The development of appropriate technologies for the utilization of this material together with sufficient economic incentives for the commercial shrimp fishermen are of prime importance if the potential human food use of this presently wasted resource is to be realized. The fact that in many cases shrimping operations are conducted in the coastal waters of countries in which the availability of inexpensive food is often insufficient to meet the needs of the population

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serves to underline the important nutritional contribution that by-catch could make. The amino acid composition and overall protein content of the flesh of by-catch fish is as high as that of the more popular food fish. Fish, in general, is a very perishable commodity particularly at ambient tropical temperatures. Indeed, because of the small size of the fish, shrimp by-catch may be especially prone to spoilage. Work has been conducted, therefore, in various countries aimed at developing means of preserving under-utilized by-catch fish. In several countries fish minces obtained using meat and bone separation techniques have been incorporated into low cost semi-traditional foodstuffs, thus making them available to lower income consumers. These have included the development of dried salted fish cakes in Mexico (Del Valle *et al.*, 1973; Young *et al.*, 1979) and Malawi (Poulter & Disney, 1977), dehydrated curried fish minces and fish pickles in India (Chakrabarty *et al.*, 1972; Chandrashekar *et al.*, 1978), fish cheese in Denmark (Herborg & Johansen, 1976) and fish crackers in Ghana (Okraku-Offei, 1974). In Mexico the food distribution system, particularly of frozen and canned products, is well established. This has meant that the consumer, even in areas distant from the coast, are now more aware of the existence of a variety of fish products though their acceptability may be limited by traditional food beliefs as well as cost. The established existence in Mexico of canning technology and experience stimulated the development of canned products based on the deboned minces obtained from some Gulf of California shrimp by-catch fish species (Poulter, 1982). These products were developed to simulate other canned products which are available to the Mexican consumer but which are based on meat. The method of manufacture of these newly developed products would not necessitate any modification of existing cannery designs or equipment and it has been shown that this, in conjunction with the low cost of fish as raw material compared with its meat equivalent, results in substantially reduced production costs (Young & Marter, 1981). The present work deals with the development and acceptability testing of a canned pâté type product. These pâtés, which were formulated using different types of fat, were prepared from several of the more common shrimp by-catch fish species which had been eviscerated and cleaned in two different ways.

Materials and methods

Raw materials

Shrimp by-catch fish were obtained fresh from commercial shrimping vessels working in the Gulf of California during the latter half of the 1980–81 season. Five species (or groups of species) which are to be found with relatively high frequency in the by-catch were chosen (Young & Romero, 1979). The species used were mojarras (*Eucinostomus* spp.), orangemouth corvina (*Cynoscion xanthulus*), Gulf croaker (*Micropogonias altipinnis*), bronze striped grunt (*Orthopristis reddingi*) and cabaicuchos (*Diplectrum*

spp.). Deboned minces were prepared from these materials using a Paoli deboning system (Model 19-529) after manual evisceration (ME) or acetic acid aided evisceration (AAAE) of the fish. The AAAE technique has been developed as an efficient and rapid method of cleaning small and irregularly shaped by-catch fish at low cost (Poulter & Treviño, 1982). Fish to be cleaned with acetic acid solution were knobbed with a sharp knife. Carcasses were then chopped laterally into roughly 3 cm pieces and added to a 4% (v/v) aqueous solution of acetic acid (fish : solution, 1:1). The mixture was stirred continuously for about 1 hr at temperatures of between 27 and 33°C, then strained and rinsed in two separate volumes of ice cooled water. The acid solution in which the fish are soaked causes the visceras and black peritoneums lining the body cavities to disintegrate and dissolve to a large extent and skin and scales can be easily rubbed off. Deboned minces prepared from fish treated in this manner have similar quality characteristics to those prepared from conventionally hand eviscerated fish (Poulter & Treviño, 1982). An additional raw material which was also included in this study was deboned mince which had been prepared from frames remaining after commercial filleting of lenguado (flatfish—*Citharichthys* spp.). This material was studied since it can constitute a waste disposal problem and yet it is known that mince of high quality may be recovered from it using appropriate equipment. The frames were prepared by cutting away kidney tissue and then washing in ice cooled water. The material was deboned using a Baader 694 deboner with 0.5 cm drum holes. The characteristics of this deboned mince were: recovery of deboned mince from cleaned and washed frames entering the deboner—45.8%; proximate composition of minces, total crude protein (N × 6.25)—15.62%; fat—2.25%; moisture—82.89%; ash—0.94%; percentage of total nitrogen extracted by water—26.55%, by 5% NaCl—44.16%, by 10% TCA (Non-Protein Nitrogen)—2.60%; bone and scale content—0.74% (dry weight basis).

Pâté preparation

The deboned minces were used in formulations for a canned pâté type product (Table 1). The products were prepared by first intimately mixing the trisodium polyphosphate (TPP) solution with the raw deboned minces so as to give a TPP content of 0.5% (w/w). The minces were then fried lightly in half the quantity of mixed fat until the texture started to harden and then cooled. The cooked minces were then placed in a Hobart bowl chopper and comminuted first with the remaining quantity of fat until no longer apparent and then with the other ingredients in the descending order given in Table 1. The mixes were then filled into 211 × 300 sized lacquered cans and heated in boiling water baths until their core temperatures reached 70°C. Cans were immediately sealed using a Rooney semi-automatic can seamer (No. 701) and then processed in a static upright retort for 60 min at 245°F/15 psi, as previously determined by thermocouple heating curve measurements. The

Table 1. Formulations developed for pâtés based on shrimp by-catch fish deboned minces

Ingredients (%)	Formula 1	Formula 2
Paoli deboned minces	67.0	67.0
Trisodium polyphosphate solution (0.15 g/ml)	2.2	2.2
Fat		
Butter	10.5	5.3
Margarine	10.5	5.3
Hydrogenated soya oil	—	10.5
Ground toasted bread	8.0	8.0
Garlic salt	1.0	1.0
Ground black pepper	0.7	0.7
Lime juice	0.7	0.7

sterilized cans were cooled rapidly, cleaned, labelled and stored for at least 20 days prior to their evaluation in taste panel trials.

Sensory evaluation

Taste panel trials were conducted using panelists drawn from the staff of the Instituto Tecnológico y de Estudios Superiores de Monterrey (ITESM). Four or five samples were presented together to individual panelists, at room temperature, daily for 8 consecutive days. Samples were presented in a random order each day and then assigned an identifying letter in the order A, B, C, D or E. Sensory evaluation was duplicated for each fish species, except lenguado and mojarra samples, into two separate series of panel sessions. The first compared samples of one specie across methods of evisceration and formulation (Table 2), whilst in the other the methods of evisceration and formulation were compared across species (Table 3). Assessors were asked to compare and contrast the samples presented to them on each occasion and then give a score from 1 to 5 for the qualities of colour, hardness, particle feel and flavour. An example of the questionnaire is given in Fig. 1. Finally, assessors were asked which sample they most liked and which they least liked and to score these from 1 to 10. Overall acceptability was then calculated by summing all the best liked sample scores and the least liked sample scores for each of the products and dividing these totals by the number of panelists who scored each of the samples. This then gives a value out of 10 in which a score of 1 indicated the product to be not at all acceptable and a score of 10 that the product was excellent.

Results and discussion

The data from each of the individual taste panels were statistically evaluated using the method of analysis of variance by the randomized complete block

Table 2. Mean scores for pâtés prepared by two formulations following evisceration by two methods compared within species (number of panelists = 15)

Sample Product type	A ME 1*	B ME 2	C AAAE 1†	D AAAE 2
Orangemouth corvina				
Colour	1.47‡BCD	2.47AC	3.87ABD	2.87AC
Hardness	1.87BCD	2.80AC	3.20AB	3.40A
Particle feel	2.87	2.53	2.93	3.47
Flavour	3.13	2.80	3.47	3.33
Acceptability	6.80	6.50	6.78	8.25
Gulf croaker				
Colour	2.53D	2.33CD	3.07E	3.27AB
Hardness	2.73	2.67	2.86	2.80
Particle feel	3.20	2.87	3.13	3.27
Flavour	3.73B	2.53A	3.20	3.13
Acceptability	7.54	5.55	7.00	6.33
Cabaicuchos				
Colour	3.53BCD	2.33A	1.93A	2.40A
Hardness	2.67	2.20	2.27	2.53
Particle feel	2.87	2.73	2.73	2.73
Flavour	2.73BC	3.33AD	3.40AD	2.67BC
Acceptability	6.83	5.86	6.00	6.20
Bronze striped grunt				
Colour	3.67	3.00	3.30	3.40
Hardness	2.40	2.33	2.87	2.67
Particle feel	2.73	2.53	3.31	3.00
Flavour	3.13	3.40D	2.80	2.33B
Acceptability	5.50	5.22	6.77	5.00

* ME—manual evisceration.

† AAAE—acetic acid aided evisceration.

‡ A significant difference exists at the 1% level between that sample and those samples indicated by the letters following.

design (Amerine, Pangborn & Roessler, 1965). This allowed for the identification of a significant effect indicating that at least two of the means being compared are significantly different. If this effect was found then data were further subjected to Duncan's sequential multiple range test which allowed identification of those means which were significantly different from one another (Amerine *et al.*, 1965). Only those means which were found to be significantly different at the 1% level have been indicated in the results presented in Tables 2 and 3. The data for acceptability given in these two tables were not subjected to an analysis of variance.

Effects of evisceration methods

From a previous study (Poulter & Treviño, 1982), it was found that the acetic acid used to aid in the evisceration and cleaning of fish also tended to

Table 3. Mean scores for pâtés prepared by two formulations following evisceration by two methods compared across species (number of panelists = 15)

Sample Fish species	A Orangemouth corvina	B Gulf croaker	C Cabaicuchos	D Bronze striped grunt	E Lenguado or mojarras
ME 1*					
Colour	2.40†BCD	3.13A	3.67AE	3.67AE	2.93CD
Hardness	2.33C	2.33C	2.93ABE	2.67E	1.93CD
Particle feel	2.60	2.87	2.53	3.00	3.20
Flavour	3.60	2.80D	3.33	3.93BE	2.87D
Acceptability	7.17	5.50	5.75	7.33	6.33
ME 2					
Colour	2.53E	2.07E	2.87E	3.00E	4.07ABCD
Hardness	2.73B	1.60ACDE	2.73B	2.40B	3.13B
Particle feel	3.20	3.07	2.93	2.87	3.67
Flavour	3.73	3.07	3.13	3.60	2.73
Acceptability	8.83	5.67	5.00	8.67	7.00
AAAE 1‡					
Colour	2.60	2.60	2.33	3.27	2.73
Hardness	3.00D	2.60	2.73	2.07A	2.33
Particle feel	3.60	3.07	2.73	3.20	2.80
Flavour	3.13	2.87E	3.13	2.53E	4.07BD
Acceptability	6.67	6.40	7.00	8.00	8.60
AAAE 2					
Colour	2.80BDE	3.67ACE	2.07BD	3.80ACE	1.97ABD
Hardness	3.13E	2.47D	2.37D	3.60BCE	2.00AD
Particle feel	3.53C	3.07	2.47AD	3.47C	3.07
Flavour	3.54	3.20	3.13	2.40E	3.93D
Acceptability	8.50	6.13	5.80	6.50	8.45

* ME—manual evisceration.

† A significant difference exists at the 1% level between that sample and those samples indicated by the following.

‡ AAAE—acetic acid aided evisceration.

lighten the colour of those deboned minces derived from grey fleshed fish species, e.g. bronze striped grunt. However, the colour of the pâtés prepared from bronze striped grunt which had been manually or acid eviscerated were not significantly different (Table 2). Only three samples of pâté prepared from other fish species showed significant differences for this character. In two of these cases the ME pâtés were of a lighter colour (orangemouth corvina and Gulf croaker) whereas the third sample (cabaicuchos) had a darker colour than the corresponding AAAE pâtés. In all other samples mean colour scores were similar and non-significant (average score 2.91) indicating that the method of evisceration had little effect on this character. It should be noted here that lighter coloured pâtés (i.e. those with a lower colour score) are not necessarily more acceptable to panelists since the pâtés available in Mexico,

Name _____ Panel No. _____

You are presented with samples of pâté prepared from fish. Please compare, evaluate and taste each sample and give a score of between 1 and 5 in each box for the characteristics of colour, hardness, particle feel and flavour.
You are then asked to give a score for the one sample you most liked and the one sample you least liked of between 1 and 10, where a score of 1 indicates the sample to be not at all acceptable and 10 that the sample is excellent.

SAMPLE	A	B	C	D	E
COLOUR Light Dark 1 2 3 4 5					
HARDNESS Soft Hard 1 2 3 4 5					
PARTICLE FEEL Smooth Coarse 1 2 3 4 5					
FLAVOUR Bad Good 1 2 3 4 5					
SAMPLE BEST LIKED 1–10					
SAMPLE LEAST LIKED 1–10					

COMMENTS

Figure 1. Taste panel questionnaire.

which are based on meat or meat products, are themselves relatively darkly coloured.

When the texture of the samples was compared it was found that the methods of evisceration used to prepare fish did not affect the particle feel of pâtés. A similar result was obtained for the characteristic of hardness excepting pâtés prepared from manually eviscerated orangemouth corvina which were significantly softer than those prepared from acid eviscerated fish (Table 2). However, the method of evisceration had no significant effect on the flavour scores for those pâtés fabricated from this species or from Gulf croaker and an average score 3.17 was obtained. Significant differences in this

organoleptic character were, however, obtained for pâtés prepared from cabaicuchos and bronze striped grunt although no consistent trend was discernible.

Effects of formulation

It was considered that the inclusion of different fat types might particularly affect both the texture and flavour of the developed pâtés. From the results presented in Table 2, however, it is apparent that the particle feel of pâtés prepared from the different fish species was unaffected (average score 2.92). Furthermore, only pâtés formulated from manually eviscerated orangemouth corvina showed significant differences in their hardness; Formulation 1 being relatively softer than Formulation 2. Scores obtained for the flavour of bronze striped grunt and orangemouth corvina pâtés did not show any significant differences between formulations. Differences in this character were, however, found for Gulf croaker and cabaicuchos, although again no consistent trend was evident. Similarly, no influence of formulation type on the colour of pâtés was found.

In general, therefore, it would appear that the inclusion of a lower cost fat type, e.g. hydrogenated soya oil, into the pâté product may be feasible since, despite the few significant differences in organoleptic qualities that were identified, the overall acceptability scores were all high (Table 2).

Species effects

Panelists were able to detect differences between pâté samples prepared from the various species of shrimp by-catch fish although little variation in the chemical composition of the deboned minces from these same species was previously found (Poulter & Trevino, 1982). The major differences noted were in the colour and hardness of pâtés (Table 3). For example, pâté formulated from manually eviscerated lenguado (Formulation 2) gave a relatively high colour score. This could be explained by the oxidation of blood constituents which were found to contaminate the deboned material obtained from the fish frames. Conversely, pâtés prepared from acid eviscerated mojarras (Formulation 2) were significantly lighter in colour than those prepared from bronze striped grunt as well as those from the lighter fleshed fish species, e.g. orangemouth corvina. The characteristic of hardness also varied considerably between species, whereas that of particle feel was not significantly affected. The flavour of pâtés was also very similar though a notable exception was found for those based on deboned minces derived from acid eviscerated mojarras (Table 3). In this case very high mean flavour scores were obtained and this seemed to be reflected in the very high overall acceptability scores given to these samples by panelists.

Although the overall acceptability scores for the various pâtés prepared from the different fish species were all high (range 5.00 to 8.83), the significant differences that were found to occur in the organoleptic characters assessed

may present a problem if pâtés of a uniformly high quality are to be produced. There may, therefore, be a requirement to pre-mix the raw deboned minces from the various fish species in a certain set proportion prior to processing or, more appropriately, to apply a washing procedure to the minces in order to standardize their physical characteristics, particularly with respect to colour.

None the less, it is evident from this study that pâtés formulated with deboned mojarra minces have a very high acceptability. Furthermore, mojarras are to be found in the shrimp by-catch from the Gulf of California in high frequency, constituting over 26% by weight of the total (Young & Romero, 1979) and they alone may, therefore, be an appropriate source of raw material for the production of high quality pâtés.

Conclusions

Deboned minces derived from acetic acid eviscerated and cleaned by-catch fish served as a suitable raw material for the production of canned pâtés with high acceptability. No consistent differences in the organoleptic qualities of the pâtés prepared from acid or manually eviscerated fish were found.

The type of fat used in the formulations developed did not significantly affect the organoleptic qualities or the acceptability of the canned pâtés indicating the desirability of using lower cost fats.

Panelists were able to detect differences in the canned pâtés prepared from the various by-catch fish species studied, particularly with respect to their colour. This variation may present a problem if canned pâtés with uniformly high qualities are to be produced and it is suggested that a washing procedure to standardize the physical characteristics of the deboned minces may be a necessary requirement. This might then allow for an increased utilization of the various fish species to be found in the shrimp by-catch from the Gulf of California.

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Composition of bacterial flora in sliced vacuum packed Bologna-type sausage as influenced by nitrite

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Summary

The influence of the addition of 0, 100 and 200 p.p.m. nitrite to sliced vacuum packed Bologna-type sausage was studied at 2, 5, 10 and 20°C. An increasing inhibition of growth of *Brochothrix thermosphacta, Enterobacteriaceae* and *Moraxella/Moraxella*-like organisms was found with increasing nitrite concentration and/or decreasing temperature. The gram positive cocci, the yeasts and lactic acid bacteria were only inhibited to a limited extent. Consequently the lactic acid bacteria became more dominant in vacuum packed cooked meat product with added nitrite. The gram negative bacteria often constitute the major flora in packages produced without nitrite, and may proliferate at higher temperatures even in samples produced with nitrite.

Introduction

During the last 20 years many experiments have been made dealing with the influence of addition of nitrite to meat on pathogenic bacteria, especially on *Clostridium botulinum*, see Sofos, Busta & Allen (1979a), but relatively few relating to the normal spoilage flora of vacuum packed cured meat under commonly used storage conditions. The recent demand for a reduction in the nitrite added to meat products necessitates studies of the influence of nitrite to these products produced under commercial conditions.

Studies of the aerobic counts on the surface of vacuum packed frankfurters (Simon *et al.*, 1973) stored at 4.5°C showed no effect of addition of up to 156 p.p.m. nitrite. Bayne & Michener (1975) found only a slightly better keeping quality of vacuum packed frankfurters stored at 20°C made with the addition of nitrite, but Hallerbach & Potter (1981) observed an effect of 140 p.p.m. nitrite added to frankfurters stored under aerobic conditions at

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7–9°C. Increasing the storage temperature to 20°C made the effect of nitrite negligible.

The effect on the development of the total aerobic flora of 100–200 p.p.m. nitrite added to pasteurized minced meat in cans has been reported as inhibitory (Nilsson & Erlandsson, 1973) and insignificant (Sofos, Busta & Allen, 1979b). No effect of nitrite was seen in dry-cured sliced vacuum packed ham at 1 or 24°C (Kemp *et al.*, 1975), while there was an improved microbial quality of 'Braunschweiger' sausages if 156 p.p.m. nitrite were added (Chyr, Walker & Sebranek, 1980).

This study was undertaken to determine the effects of adding nitrite to vacuum packed cooked, cured and sliced Bologna-type sausage on the development of the microbial flora, both qualitatively and quantitatively, at normal storage temperatures and at temperature abuse.

Materials and methods

Sausage production

Three batches of Bologna-type sausage were produced using a standard recipe with the following composition: beef (15%), pork (15%), trimmings (25%), dried milk (3.9%), potato flour (3.9%), caseinate (1.9%), vegetable protein (1.9%), spices (0.3%), dried onion (0.1%), polyphosphates (0.25%), food colours (0.25%), and water (32.5%).

The beef and pork meat were pre-salted with sodium chloride the day before the production of the sausage. The remaining sodium chloride was added to the sausage emulsion either as pure salt or as a nitrite-salt mixture (sodium chloride containing 0.5% sodium nitrite) resulting in a total chloride concentration of *c.* 2.2% and a sodium nitrite concentration of 0, 100 or 200 p.p.m. in the finished sausage mix. After stuffing the emulsion in casings the sausages were cooked in a steam cabinet until a temperature of 75°C was reached in the centre. After cooling, the sausages were stored overnight at 2–4°C. After removing the casings the sausages were sliced and vacuum packed at a commercial manufacturer. The packaging film consisted of polyamide laminated to polyethylene (Rilotene, Denmark) which has an oxygen permeability of *c.* 52 ml/m^2/24 hr/1 atm. at 25° and 75% r.h.

Packs representing the three batches (with 0, 100 and 200 p.p.m. nitrite) were held at 2 ± 1°C, 5 ± 1°C, 10 ± 1°C and 20 ± 1°C respectively.

Sample preparation

A sample of *c.* 40 g was obtained by cutting aseptically through slices from three packs from the same batch. The total sample was homogenized with 150 ml sterile peptone water (0.1% peptone and 4% sodium chloride) for 1.5 min using a Stomacher.

Bacterial analyses and identification

Appropriate dilutions of the slurry were prepared in sterile peptone water with 4% salt and plated onto growth media to determine the following counts: total aerobic colony counts on Plate count agar (Difco) with 4% sodium chloride (PCA) and on all purpose medium with Tween (APT) (Difco); lactic acid bacteria on nitrite actidione polymyxin agar (NAP) (Davidson & Cronin, 1973); *Brochothrix thermosphacta* on streptomycinsulphate thallousacetate actidione agar (STAA) (Gardner, 1966); gram negative bacteria on desoxycholate hydrogensulphide lactose agar (DHL) (Eiken Chemical Co., Japan) and yeasts on APT medium with addition of 100 p.p.m. oxytetracycline. NAP was used as pour plates, the inoculated plates were overlaid with NAP. The drop and spread technique was used for all other media. All media were incubated at 20±1°C for 3–5 days.

Isolates from plates were identified using standard procedures (Cowen, 1974; Gibbs & Shapton, 1968). The gram positive flora was identified primarily by microscopy, catalase, motility and degradation of glucose and other carbohydrates in Hugh Leifson medium (Hugh & Leifson, 1953). The gram negative flora was identified by oxidase and catalase reaction, fermentation of carbohydrates, motility and sensitivity towards penicillin.

The qualitative as well as the quantitative microbial composition at the different sampling stages were obtained by pooling the colony counts from selective and non-selective plates.

Chemical analyses

At the beginning of the storage period the sausages were analysed for sodium chloride by the Volhard titration method (Anon, 1974), for water content by drying at 104°C to constant weight (Anon, 1955). The nitrite content was determined as follows: the samples were extracted with boiling water, the proteins precipitated and filtered and the nitrite content measured in the filtrate by means of sulphanilamide and *N*-(1-naphtyl)-ethylenediamine. The development of a red colour was measured in a Beckmann DB-G Spectrophotometer at 540 nm. A standard curve was made with known concentrations of sodium nitrite (Anon, 1963).

The pH was determined on the food homogenates using a combination electrode (Radiometer, Denmark).

Results

The results of the chemical analyses for the three batches are shown in Table 1, and the initial microbial levels in Table 2. Bacterial growth curves are only shown for *B. thermosphacta*, the lactic acid bacteria and the *Enterobacteriaceae* (on DHL) for the four temperatures (Figs 1–6).

Table 1. Composition of the Bologna-type sausages

Nitrite addition (p.p.m.)	Salt (%)	Water (%)	Salt:water (%)	Nitrite (p.p.m.)
0	2.2	59.8	3.7	0
100	2.2	57.6	3.9	66.8
200	2.3	59.8	3.8	133.5

Table 2. Initial numbers of micro-organisms in the sausages

Micro-organisms (No./g)	Nitrite added (p.p.m.)		
	0	100	200
Total APC	6	3	18
Lactic acid bacteria	3	3	5
B. thermosphacta	< 3	< 3	< 3
Gram negative bacteria	< 3	< 3	< 3
Yeast	< 3	< 3	< 3

Two media, PCA and APT were used to determine the total count. APT in contrast to PCA includes the lactic acid bacteria and was used in order to get an assessment of the total flora of lactic acid bacteria which not always appears on NAP or any other selective medium for these bacteria. The results stated below are for the total plate count on PCA. Results from APT plates were, however, used in making Table 3 showing the composition of the total flora during storage. This was because part of the flora of lactic acid bacteria was made up of *Streptococcaceae* (*L. mesenteroides*) and these bacteria only show moderate growth on NAP (Geraldini *et al.*, 1979; Nielsen, 1982).

The total aerobic plate count (APC) (from PCA) at 2°C showed a considerable dependence on whether nitrite was present or absent at the beginning of the experiment. While the APC in the series made without nitrite showed a steady increase with time, there was a lag period of about a week in the series where 100 or 200 p.p.m. nitrite were added to the sausages. After that period, however, the bacteria growth rate was very similar to the series produced without added nitrite, although with 200 p.p.m. nitrite added counts were *c.* 4×10^4/g compared with 10^7/g in the series without added nitrite during a 5 weeks' storage period. In the series without nitrite there was an increase in counts of *B. thermosphacta*, numbers exceeding 10^6/g within 3 weeks (Fig. 1). Addition of 100 p.p.m. nitrite considerably inhibited growth of *B. thermosphacta*, with a colony count of 10^5/g after 5 weeks' storage, and adding 200 p.p.m. completely inhibited this growth (Fig. 1).

Addition of 100 p.p.m. nitrite had little effect on the growth of the lactic acid bacteria (Fig. 1), and 200 p.p.m. nitrite showed slight inhibition.

Table 3. Composition of microbial flora on vacuum packed Bologna-type sausage stored at 2, 5, 10 and 20°C

		Nitrite added																						
		0							100							200								
Temp. (°C)	Days	A*	B	C	D	E	F	G	A	B	C	D	E	F	G	A	B	C	D	E	F	G		
2	0	100							50						50	64		18				9		
	6	46	9		15		30		33						66	100								
	13		43		56	1			72	8	10	10				59		35						
	20		20		79					12	83	5						100						
	27		26	66	9				2		60	34						100						
	34		43	55	13	2				8	87	5				7		92						
5	6	25	22		53									100		50						50		
	9		64		36					46	16	38				80						20		
	13		45	3	52				32		63							35		6	59			
	20	46	12	26	13	3			13	47	23	17						73		24	3			
	27		16	43	5	4			2	35	57	7						100						
	34		5	22	3	70			29	12	41	14				4	6	86		4				
10	2	44	25		31				100													100		
	6	6	28	4	62				15		41	36				2		95			2			
	9	12	45	20	13	10			8	3	29	16	1			6		86	5		2	1		
	13	3	9	33	7	49			45	2	38	2	13					100						
	20	4	3	90	2	1			63	9	23	5				32		38						
	27															1		97						
	34								6		82		3			48		39						
20	1	69							60						30	100								
	2	34	36	2	19		11	27			40				60			97				2		
	5		30	52		4		13	16	36	4		3		40	62		9				29		
	6	1	9	60				24	12	10	28		13		37	69	9		4		3	15		
	9		7	62				1	10	4	49		9		29	26		54				26		

* Percentages of A, *Micrococcaceae*; B, *B. thermosphacta*; C, lactic acid bacteria; D, Moraxella/Moraxella-like bacteria; E, *Enterobacteriaceae*; F, yeast, G, 'other bacteria'. The isolates A, D and G were obtained from PCA, B from STAA, C from NAP and/or APT, E from DHL and F from APT with oxytetracycline.

Irrespective of the nitrite level, there was a period of about 2 weeks with almost no increase in numbers of lactic acid bacteria. In spite of the negligible count of *Enterobacteriaceae* at the beginning of the storage period, these bacteria increased during storage in the series without added nitrite to 10^6/g within 5 weeks (Fig. 2). Addition of nitrite to the sausage completely inhibited the growth of these bacteria. Similarly the yeast counts increased in the series produced without nitrite (to *c.* 10^4/g after 4 weeks) but not in the series produced with nitrite.

The quantitative composition of the flora developing during storage at 2°C is presented in Table 3 which shows that while the *Micrococcaceae* constituted

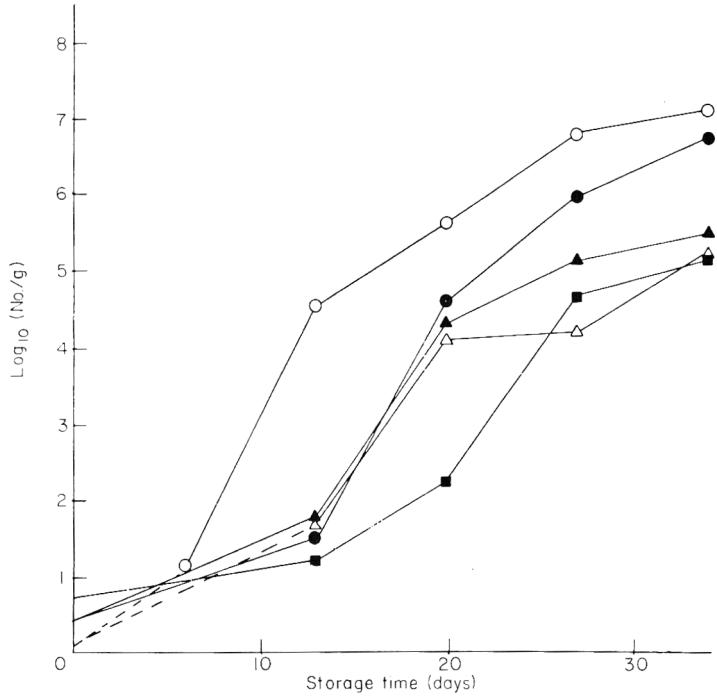

Figure 1. Growth of *B. thermosphacta* and lactic acid bacteria at 2°C. ○, 0 p.p.m. nitrite; △, 100 p.p.m.; □, 200 p.p.m. Open symbols: *B. thermosphacta*; closed symbols: lactic acid bacteria.

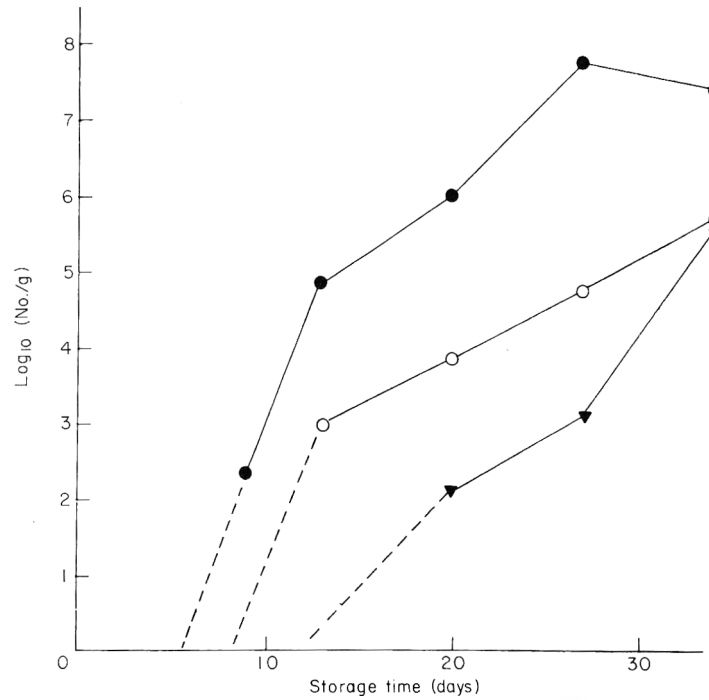

Figure 2. Growth of *Enterobacteriaceae* on DHL at 2 and 5°C. ○, 0 p.p.m. nitrite; ▽, 100 p.p.m. nitrite. Open symbols: 2°C; closed symbols: 5°C.

a large proportion of the flora at the beginning of the storage period, in fact they initially dominated the flora, they were soon overgrown by other bacteria. At any time during the experiment the ratio of lactic acid bacteria: *B. thermosphacta* increased with increasing nitrite concentration. The group of *Moraxella*/*Moraxella*-like organisms constituted a considerable proportion of the initial flora. Their growth was slightly inhibited by 100 p.p.m. nitrite and almost completely by 200 p.p.m. nitrate. The growth of these bacteria on DHL was very limited and they were therefore determined primarily on PCA.

Results at 5°C for the APC were similar to those at 2°C, but 100 p.p.m. nitrite inhibited less than 200 p.p.m. The maximum counts were therefore *c.* 9×10^7/g, 4×10^6/g and 10^5/g with 0, 100 and 200 p.p.m. nitrite respective. The counts of *B. thermosphacta* increased rapidly in the series without nitrite to more than 10^6/g after 2 weeks (Fig. 3). While the same numbers were reached after more than 4 weeks in the series with 100 p.p.m. nitrite, addition of 200 p.p.m. resulted in counts of not more than 10^4/g after *c.* 5 weeks. Counts of 10^7-10^8/g lactic acid bacteria were only observed in the absence of nitrite (after *c.* 3 weeks) while these bacteria did not exceed 10^6/g in series with nitrite (Fig. 3). In the product without nitrite, numbers of *Enterobacteriaceae* increased rapidly after a week, reaching 10^6/g after 3 weeks (Fig. 2). Adding 100 p.p.m. nitrite reduced their growth and 10^6g was not reached within the

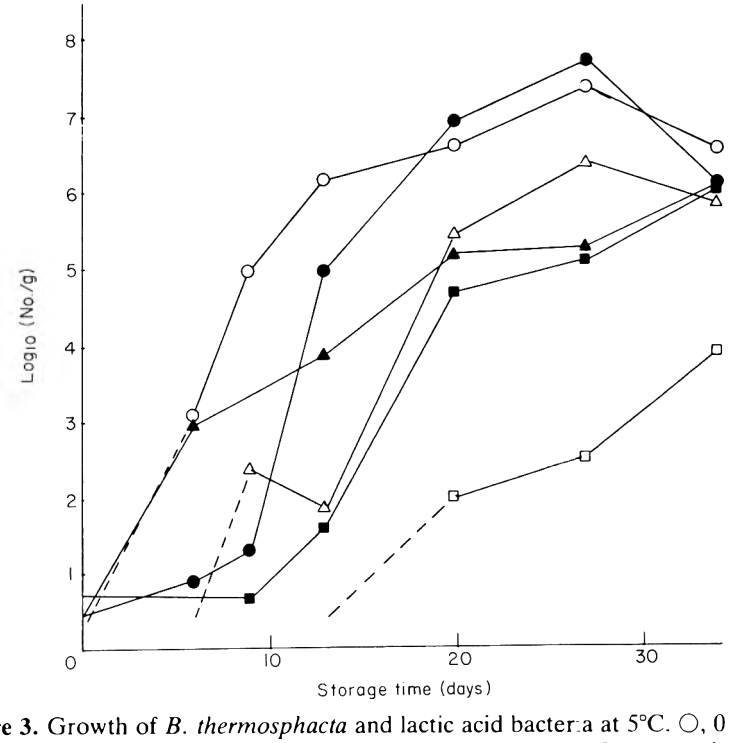

Figure 3. Growth of *B. thermosphacta* and lactic acid bacteria at 5°C. ○, 0 p.p.m. nitrite; △, 100 p.p.m. nitrite; □, 200 p.p.m. nitrite. Open symbols: *B. thermosphacta*, closed symbols:lactic acid bacteria.

storage period. Increasing the nitrite content to 200 p.p.m. completely inhibited growth of these bacteria. After 9 days the yeast counts increased both in the series without added nitrite and with 200 p.p.m., while for unknown reasons the counts for the series with 100 p.p.m. nitrite were less than 10^2/g until after 4 weeks. In series with added nitrite yeast counts were slightly lower than in the series without nitrite.

Table 3 shows that the proportion of *Micrococcaceae* in the total flora was only appreciable during the initial stage of storage, and that they were overgrown by other bacteria after 1–1½ weeks. At 5°C the proportion of *B. thermosphacta* was less and that of the lactic acid bacteria was greater with increasing nitrite concentration. At this temperature the proportion of *Moraxella*/*Moraxella*-like organisms did not seem to be influenced by 100 p.p.m. nitrite but inhibited by 200 p.p.m., and in all series these bacteria were overgrown by other bacteria during storage. Table 3 shows that *Enterobacteriaceae* constituted a larger part of the flora with time in the series without nitrite, making up the greater part after 5 weeks of storage.

The influence on APC of 100 p.p.m. nitrite to the Bologna-type sausage stored at 10°C was small, numbers being depressed *c.* ten-fold. Addition of 200 p.p.m. nitrite caused much greater inhibition, and counts of 10^6/g were reached 2 weeks later than in the other series. Addition of nitrite delayed

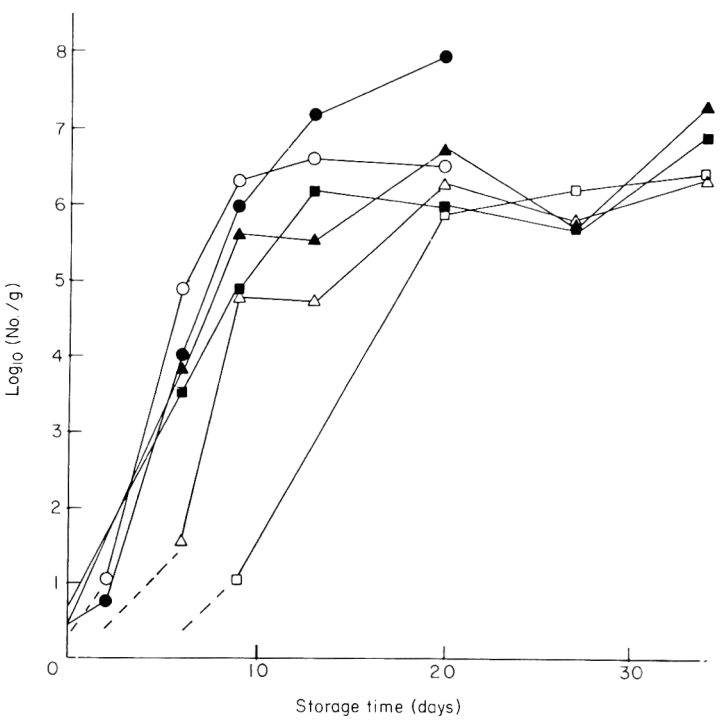

Figure 4. Growth of *B. thermosphacta* and lactic acid bacteria at 10°C. ○, 0 p.p.m. nitrite; △, 100 p.p.m. nitrite; □, 200 p.p.m. nitrite. Open symbols: *B. thermosphacta*; closed symbols: lactic acid bacteria.

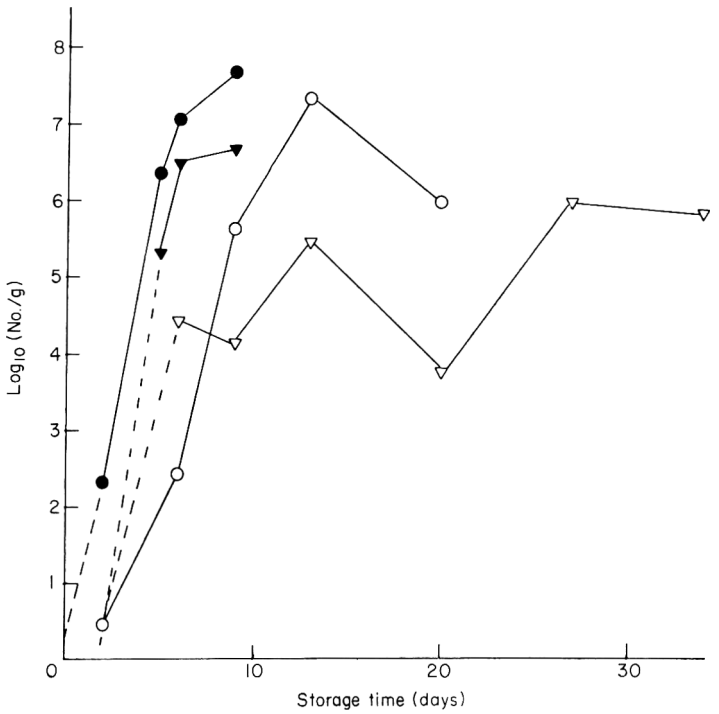

Figure 5. Growth of *Enterobacteriaceae* on DHL at 10 and 20°C. ○, 0 p.p.m. nitrite; ▽, 100 p.p.m. nitrite. Open symbols: 10°C; closed symbols: 20°C.

growth of *B. thermosphacta* (Fig. 4) numbers reaching 10^6/g in *c.* 1 week in its absence, and *c.* 3 weeks in its presence. Nitrite had little effect on the growth of lactic acid bacteria until numbers reached *c.* 10^6/g when counts increased in the series without nitrite, but nitrite restricted further growth. Counts of 10^7-10^8/g are therefore reached much later in the two series with added nitrite (Fig. 4). Adding 100 p.p.m. nitrite reduced the maximum counts of *Enterobacteriaceae*, while 200 p.p.m. completely inhibited their growth (Fig. 5). Yeasts were only inhibited to a limited extent, counts of 10^4-10^5/g were reached within 3 weeks.

At 10°C *Micrococcaceae* were a significant part of the bacterial flora in all series, but were always outgrown by lactic acid bacteria (Table 3). As at 2 and 5°C the proportion of lactic acid bacteria increased and that of *B. thermosphacta* decreased with time. At times the *Moraxella*/*Moraxella*-like bacteria and *Enterobacteriaceae* constituted a considerable proportion of the flora but in the end these bacteria were overgrown by the lactic acid bacteria.

Storing the vacuum packed sausage at temperature abuse (20°C) showed much the same picture for the APC as storage at 10°C, 100 p.p.m. nitrite added having a negligible influence on the growth while 200 p.p.m. nitrite reduced the growth rate and the maximum counts. Counts of *B. thermosphacta* were reduced with addition of 100 p.p.m. nitrite and the growth rate slower with 200 p.p.m. nitrite, resulting in lower counts in the series with

added nitrite. Counts of *c.* 10^6/g were reached in *c.* 4, 5 and 8 days in the three series respectively (Fig. 6). Growth rates of lactic acid bacteria were slightly reduced by the addition of nitrite resulting in lower counts in the series with added nitrite (Fig. 6). The *Enterobacteriaceae* were slightly inhibited by the addition of 100 p.p.m. nitrite, and counts of 10^6/g were reached in *c.* 1 week dependent on whether nitrite was present or absent (Fig. 5). There was no effect of nitrite addition on the yeasts at 20°C, in all series they grew to *c.* 10^4/g within a week.

Table 3 shows that the *Micrococcaceae* constituted a greater proportion of the total flora during storage at 20°C in nitrite containing series. The proportion of *B. thermosphacta* became less with time and with increasing nitrite concentration. The proportion of lactic acid bacteria again increased with time, but not with nitrite concentration, because growth of *Micrococcaceae* and/or 'other bacteria' accounted for a greater proportion in series with added nitrite. 'Other bacteria' were gram positive aerobic spore-forming bacteria (*Bacillus* spp.). The *Bacillus* spp. counts increased within 5 days to more than 10^5/g in all series. Maximum counts were 5×10^6/g, 10^7/g and 5×10^5/g with 0, 100 and 200 p.p.m. nitrite respective. The Bacilli comprised *B. cereus*, *B. subtilis* and *B. licheniformis*.

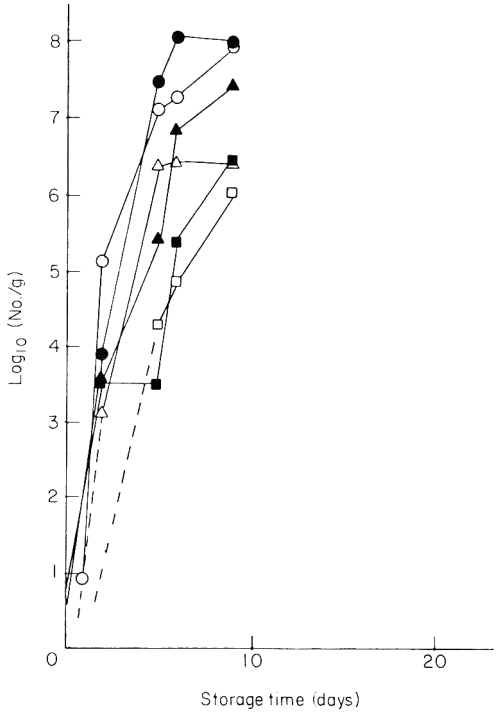

Figure 6. Growth of *B. thermosphacta* and lactic acid bacteria at 20°C. ○, 0 p.p.m. nitrite; △, 100 p.p.m. nitrite; □, 200 p.p.m. nitrite. Open symbols: *B. thermosphacta*; closed symbols: lactic acid bacteria.

Discussion

There was a considerable effect of nitrite in the Bologna-type sausage on the development of the total APC at all temperatures. Increasing the storage temperature decreased inhibition at all temperatures. Thus, at 2°C, while 100 p.p.m. added nitrite had nearly as great an effect as 200 p.p.m., the same concentration of nitrite had negligible effects at 10 and 20°C. The inhibition caused by 100 p.p.m. added nitrite was almost entirely due to an increase of the lag period, while once the growth had started the flora developed more or less as in series produced without nitrite. Adding 200 p.p.m. nitrite showed one additional effect, in these series the maximum counts reached were less than in other series. Again this effect was less with increasing temperature and may have been caused by the faster depletion of nitrite at higher temperatures (Nordin, 1969). These effects are in agreement with results of Hallerbach & Potter (1981) on frankfurters with or without 140 p.p.m. added nitrite and stored under aerobic conditions.

The gram positive cocci could only be followed during part of the storage period because they were overgrown by other bacteria. The study showed, however, that the cocci could compete with other bacteria in a meat product with a relatively low salt:water ratio and with added nitrite. These bacteria, constituting a greater proportion of the total flora in series with added nitrite, are analogous to results with vacuum packed sliced bacon (Wood & Evans, 1973) with a much higher salt:water ratio where the significance of the cocci were at least the same (Shaw & Harding, 1978) or greater (Hansen & Riemann, 1961).

The effect on *B. thermosphacta* of adding nitrite to the product resembled the total aerobic plate count. There was an increased lag period and lower maximum counts in series with added nitrite, and these effects were greater at lower temperatures. Thus, at 2°C and 200 p.p.m. nitrite there was no development at all of *B. thermosphacta* during 5 weeks. Comparing series with 100 p.p.m. added nitrite and series without nitrite stored at 5 and 2°C shows that counts of 10^6/g were reached 10 and 16 days later in the nitrite containing series. Counts of 10^6/g of *B. thermosphacta* seem to have an effect on the organoleptic quality of vacuum packed cured meat (Qvist & Fallesen, 1978; Qvist & Mukherji, 1979). The significance of the inhibition of *B. thermosphacta* caused by the nitrite addition is therefore obvious. Studies with *B. thermosphacta* in broth cultures showed similar results, i.e. decreasing inhibition with increasing temperature (Brownlie, 1966; Roberts, Britton & Shroff, 1979). Collins-Thompson & Lopez (1980) however, did not find a temperature effect. The greater Q_{10} values for bacterial growth than for gas permeability of the packaging films means that the accessibility of oxygen possibly is less at higher temperatures. The more anaerobic conditions which may arise makes the inhibition caused by nitrite greater as shown with broth cultures (Collins-Thompson, 1980).

There was no effect of nitrite on the lag period of the lactic acid bacteria,

but there was a more rapid change to the stationary phase, or a period with a very slow increase in bacterial counts in the series with added nitrite. This means that in series produced without nitrite high counts of lactic acid bacteria (10^7-10^8/g) are present for longer periods, resulting in a greater microbial activity. There was a trend for a flora dominated by lactic acid bacteria in series with added nitrite. The effect of nitrite addition on the lactic bacteria is inconsistent. Some are inhibited (Shaw & Harding, 1978; Taylor & Shaw, 1975), others only partly inhibited (Wood & Evans, 1973), while the atypical streptobacteria, a very common group of bacteria in vacuum packed sliced cured meat (Nielsen, 1982), are not affected by addition of 200 p.p.m. nitrite to vacuum packed Bologna (Mol *et al.*, 1971). These different effects of nitrite within the lactic acid bacteria may be due to differences in their abilities to reduce the added nitrite (Collins-Thompson & Lopez, 1981). The selective medium used for the lactic acid bacteria, NAP, was satisfactory for one *Lactobacillaceae* but not for *Streptococcaceae*. These latter bacteria are therefore only partly contained in the growth curves for NAP but were counted (when possible) on APT for use in Table 3. The influence of nitrite addition on the lactic acid bacteria were, however, the same irrespective of the media. Other selective media for instance MRS have been tried in the laboratory but they have not proved to be better. This is analogous to results by Geraldini *et al.* (1979).

The influence of nitrite addition on the *Enterobacteriaceae* was clearly a combination of nitrite concentration and storage temperature. Adding 200 p.p.m. nitrite caused complete inhibition at all temperatures, while 100 p.p.m. nitrite only showed total inhibition at 2°C. At other temperatures this addition caused prolongation of the lag period and lower maximum counts. In studies with vacuum packed 'Brühwurst', Leistner, Hechelmann & Uchida (1973a, b) inoculated *Klebsiella* spp., *E. aerogenes*, *E. cloaceae*, *E. hafniae* and *S. liquefaciens* were not inhibited by 100 p.p.m. nitrite. In studies with broth cultures, Collins-Thompson, Wood & Jones (1982) observed that 50 p.p.m. nitrite added permitted growth of *S. liquefaciens*, *E. aerogenes* and *E. hafniae* under anaerobic conditions with a concomitant depletion of added nitrite. Even 400 p.p.m. nitrite and 1% sodium chloride permitted growth of *S. liquefaciens* in studies by Roberts *et al.* (1979). In vacuum packed Bologna no growth was observed, however, before 6–7 weeks storage at 5°C at a time where the nitrite concentration was only *c.* 5 p.p.m. (Collins-Thompson *et al.*, 1982). Factors other than nitrite concentration may be important for the development of these bacteria in vacuum packed cured meat. Collins-Thompson *et al.* (1982) showed that the onset of *Enterobacteriaceae* growth coincided with the depletion of glucose in vacuum packed Bologna. The authors suggested that slower growth rates of the lactic acid bacteria when the glucose concentration decreases permit the growth of gram negative bacteria because of the wider range of nutrients available for these bacteria. The present study, however, shows that the growth of *Enterobacteriaceae* starts at a time where the lactic acid bacteria are still in the logarithmic phase, and

seem more correlated with the nitrite addition. Present studies in the laboratory support the assumption that the glucose content of the cured minced meat products, within the concentrations used in commercial products, have only a minor influence on the growth of these bacteria.

The study shows that the *Enterobacteriaceae* are not the only gram negative bacteria which may proliferate in the vacuum packed cured meat. The *Moraxella*/*Moraxella*-like bacteria constituted a considerable proportion of the total flora at 2, 5 and 10°C. These bacteria were also inhibited by a combination of low temperature and high nitrite concentration. Thus, while the addition of nitrite only had negligible influence at 10°C, the addition of 200 p.p.m. nitrite resulted in their complete inhibition at 2°C. The greater importance of the *Moraxella*/*Moraxella*-like bacteria in series produced without added nitrite and at earlier times at higher temperatures agrees with studies of the gas composition in packs with different nitrite levels where the percentage of oxygen was higher in series produced without nitrite (Nielsen, 1982). Reports on findings of these bacteria in vacuum packed hams are few (Shaw, Harding & Taylor, 1980; Patterson & Gibbs, 1978). In spite of their strict aerobic character these bacteria can as it is seen grow in vacuum packed meat products. These bacteria probably have only limited influence on the organoleptic quality of the product (Freeman *et al.* 1976; Patterson & Gibbs, 1978).

There are some development of yeasts at all temperatures, they were inhibited by nitrite addition only at 2°C; at all other temperatures the influence of nitrite addition was negligible. Counts of about 10^4-10^5/g were reached at all temperatures. Development of yeasts is not uncommon in vacuum packed cured meat under refrigerated storage (Reuter, 1970; Shay *et al.*, 1978; Daelman & van Hoof, 1975).

Due to the addition of dyes to the sausage only very small differences in colour between series with and without nitrite were observed. No discolourations of the sausages at 2, 5 and 10°C were seen but the series stored at 20°C and produced without nitrite had an objectionable colour at an earlier time than the series with added nitrite.

In summary the study has shown that considerable problems may arise if one completely omits nitrite in vacuum packed cured meat, while keeping other factors constant. The normal spoilage flora was affected to such a degree that spoilage might proceed at a faster rate. Decreasing the storage temperature and/or adding nitrite increased the lag phase or completely inhibited the growth of some important spoilage bacteria.

Acknowledgment

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Muscle colour deterioration in iced and frozen stored bonito, yellowfin and skipjack tuna caught in Seychelles waters

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Summary

The colour deterioration of muscle in iced and frozen stored bonito, yellowfin and skipjack tuna caught in Seychelles waters has been determined. Storage life on ice before noticeable browning occurred was 12–14 days for yellowfin tuna and 7 days for bonito and skipjack tuna. At cold storage temperatures above −30°C colour deterioration was very rapid. A new procedure has been devised for preparing the water extract necessary for the analysis of the ratio of met- to oxy- and deoxyhaemoglobin.

Introduction

The Republic of Seychelles consists of 96 islands spread over a wide area of the western Indian Ocean. The land mass occupies an area of 278 km^2 but the Exclusive Economic Zone declared in January 1978 covers an area of some 450 000 nautical $miles^2$. Expansion of the tuna fishing effort in this vast area of ocean is proceeding rapidly. Numerous problems have occurred during this development and poor handling of the catches with the resultant deterioration in muscle colour has been of particular concern.

When tuna and bonito are first caught the muscle is a pink (yellowfin, *Thunnus albacares*) to deep red colour (skipjack, *Katsuwonus pelamis*; bonito, *Euthynnus affinis*). During handling on deck, iced storage and frozen storage and intermediate handling stages this reddish colouration becomes progressively browner. Canneries and other processors of tuna and bonito desire that the muscle should be pink or red with as little brown discolouration as possible. For the Japanese product, Katsuobushi (cooked, smoked and dried skipjack and bonito) for instance, it is imperative that the fish are red muscled when entering production.

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The colour of tuna muscle is due to the presence of pigments and more specifically of myoglobin, the predominant pigment. Studies on yellowfin tuna have indicated that myoglobin constitutes 69–85% of the total haem pigment in light muscle and 81–95% in dark muscle (Brown, 1961). A water extract from raw fresh tuna muscle exhibits a spectrum characteristic of oxymyoglobin with two maxima, at 540 and 576 nm, while that from muscle kept iced or frozen stored for a period exhibits a mixed spectrum of metmyoglobin with maxima at 503 and 633 nm and that of oxymyoglobin. The discolouration of tuna meat during iced and frozen storage is for the most part due to autoxidation of oxy- and deoxyhaemoglobin to metmyoblobin. Bito (1976) has prepared a calibration curve of $E_{540\ nm}$ (β_{max} of oxymyoglobin)/$E_{503\ nm}$ (β_{max} of metmyoglobin) against percentage metmyoglobin for accurately measuring the proportion of metmyoglobin to the total myoglobin in a water extract (Fig. 1). To sensory observation the tuna meat appears brownish when

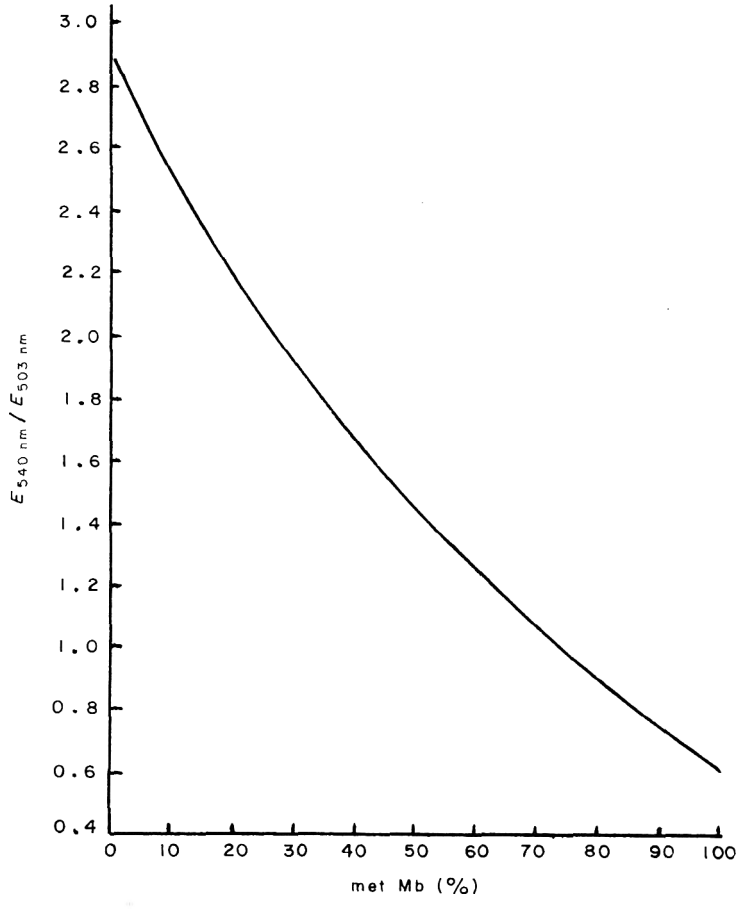

Figure 1. *Relation between the relative proportion of metmyoglobin to total myoglobin (metmyoglobin plus oxymyoglobin) and the ratio of the optical densities at 540 and 503 nm.

*Taken from Bito (1976).

the proportion of metmyoglobin to the total exceeds 50% (i.e. when the ratio E_{540}:E_{523} becomes less than 1.45).

The following work was carried out so that guidelines for the iced and frozen storage of tuna and bonito could be established for the local catching fleet and processors and a clear indication given of the effect on muscle colour of various types of storage.

Materials and methods

Origin and handling of fish

All fish were caught by the boats of the German Marine Research Institute, Bundesforschungsanstalt für Fisherei, trawling in the Seychelles EEZ during 1981.

Iced and frozen storage trials were carried out on yellowfin, bonito and skipjack tuna. For the iced storage trials fish were killed by a blow to the head and stored in boxes with a large excess of freshwater flake ice. The fish were re-iced as necessary during the period of the trial. For the frozen fish storage trials, fish which had been on ice for 12–16 hr (i.e. caught on the last day of fishing close to the home port) were blast frozen and stored in cabinets at −15, −20 and −30°C.

Sampling

Since Bito (1976) has pointed out that colour deterioration of fish muscle proceeds most rapidly near the skin of the fish and becomes progressively slower towards the centre, it was expected that along the length of the fish, colour deterioration would vary (i.e. near the tail, most rapid deterioration would occur since the muscle thickness is less). For a high proportion of the analyses, therefore, fish were sampled near the head, in the middle and at the tail. In order to average the colour, a quarter section (without dark muscle) from above the lateral line was taken and a fairly substantial weight of sample used (approximately 45 g). Frozen fish were not thawed before sampling as thawing can cause further colour deterioration. A sample was sawn out of the frozen fish, chopped finely, and the colour analysis started immediately.

Colour analyses

The methodology of Bito (1976) was not available and so a new rapid procedure was devised.

A quarter section of muscle was cut out from the fish, the skin and dark muscle removed and the section pared down until it weighed approximately 45 g (since the measurement taken is a proportional one and not an absolute value, accurate weighing is not essential). The sample was put into an

18 × 30 cm polythene bag and 150 ml of *chilled* distilled water added. The mixture was blended for 20 sec in a Colworth Stomacher lab blender Model 400. This machine is normally used for microbiology but its sponging action was very suitable for this particular purpose and extraction efficiency was very high—colourless fibres or bundles of fibres remaining. The resultant mixture was filtered through a 9 cm Whatman 42 filter paper into a clean *dry* test tube, placed in ice (it is imperative that the extract is kept cool as it becomes opaque if allowed to remain at room temperature for any length of time. Optical densities could not then be measured). After approximately 8 ml had filtered through, the test tube was placed in water at ambient temperature for 2 min and then the optical density of the extract was measured in a 1 cm cell at 540 and 503 nm against a water blank.

Results and discussion

Iced fish storage trials

Each result given in the tables is an average from five fish. In the case of yellowfin (Table 1) and bonito (Table 3), two separate trials are recorded and for skipjack (Table 2), three trials.

The results show that yellowfin can be stored for up to 12 days on ice before even the tail muscle ratio 540:503 nm falls below 1.45 and it is not until 14 days storage that serious colour deterioration had occurred. The colour storage life of skipjack and bonito is much shorter than that of yellowfin, of the order of 1 week on ice. This is of course the absolute maximum since by this time the muscle appears brown. Any further processing will give further deleterious colour deterioration making the fish completely unacceptable with respect to muscle colour. It is clear, therefore, that both skipjack and bonito should be landed if iced as soon as possible and certainly within 3 or 4 days of capture if

Table 1. Yellowfin tuna iced storage colour trials

		Ratio 540:503 nm		
Trial no.	Days in ice	Head	Middle	Tail
1	1		2.03	
2	2	2.31	2.18	2.27
1	4		1.90	
1	7	2.07	1.80	1.74
2	7	1.94	1.92	1.80
2	9	1.84	1.77	1.72
2	12	1.95	1.68	1.40
1	13	1.84	1.58	1.38
2	14	1.63	1.44	1.28
1	15	1.59		

Table 2. Skipjack tuna iced storage colour trials

		Ratio 540:503 nm		
Trial no.	Days on ice	Head	Middle	Tail
1	1		2.20	
2	2	2.11	2.07	2.16
1	4		1.84	
2	4	1.88	1.82	1.72
3	5	1.92	1.75	1.78
1	6	1.99	1.87	1.72
2	7		1.32	
3	8	1.61	1.18	1.24
1	9	1.55	1.50	1.52
2	11	1.29	1.09	0.92

Table 3. Bonito iced storage colour trial

		Ratio 540:503 nm		
Trial no.	Days in ice	Head	Middle	Tail
1	1		2.00	
2	2	2.08	2.11	2.26
2	4	2.23	2.14	2.01
1	6		1.66	
2	6	1.77	1.61	1.49
1	8		1.45	
2	8	1.55	1.40	1.31
2	9		1.38	

further processing is to be carried out or if the fish are to be frozen (where further deterioration will occur). Similarly, yellowfin tuna should be landed within 8 or 9 days if ice stored. In many instances in the Seychelles these times were being exceeded by a wide margin resulting in inferior quality tuna being landed.

Frozen fish storage trials

Each result (Table 4) is an average of five fish. Colour deterioration at temperatures above −30°C is obviously very rapid. Brine freezer boats operating their refrigeration at −15°C can expect therefore, a rapid drop in the colour quality of their catch. Transfer of the catch to a cold store operating at −30°C or lower should be carried out as soon as possible. These results indicate what can happen if careful, cold storage of the fish is not carried out and has serious implications for cold store operators. Fish exposed to cold storage temperatures above −30°C for even a short period are unlikely to be

Table 4. Frozen storage colour trial

Species	Months frozen	Temperature (°C)	Middle section ratio 540:503 nm
Yellowfin*	1	−15	1.13
Yellowfin*	1	−15	1.26
Yellowfin*	1	−15	1.29
Bonito	1	−15	1.01
Skipjack	1	−15	1.11
Yellowfin	1	−20	1.39
Bonito	1	−20	1.14
Skipjack	1	−20	1.47
Skipjack	2	−20	1.17
Yellowfin	1	−30	1.95
Bonito	1	−30	1.89
Skipjack	1	−30	1.92

* Separate trials.

suitable for Katsuobushi production and would expect to command a lower price on the world market.

The procedure developed for colour analysis of tuna muscle is both simple and rapid and was expected to become part of the Seychelles Fishery Department's routine tests on export fish. Since the results obtained indicate the differences to be expected between the head, middle and tail regions, it is considered that for routine testing it is only necessary to analyse the middle section.

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Book reviews

Nutritive Sweeteners. Ed. by G. G. Birch and K. J. Parker. London: Applied Science Publishers Ltd, 1982. Pp ix+316. ISBN 0 85334 997 5. £29.00.

This book contains the sixteen papers presented in March 1981 at the Symposium organized by the National College of Food Technology. The topics include reviews on the production, properties and various food uses of the range of carbohydrate sweeteners available today. The effect of such nutritive sweeteners on body weight response, obesity, blood glucose elevation and dental caries is covered. There are also papers on the physiological and psychological processes involved in the perception of sweetness.

In a collection of papers such as these there is bound to be a variation in the standard of individual contribution. On the whole, the quality is high with the reviews on malt and maltose syrups (P. D. Fullbrook), lactose and lactitol (P. Linko) and hydrogenated glucose syrups, sorbitol, mannitol and xylitol (P. J. Sicard) particularly noteworthy. The paper on sweet taste receptor mechanisms (A. Faurion and P. MacLeod) summarizes well the historical development of knowledge in this area, but rather surprisingly fails to include any specific reference to the role of cell membranes. In the reviewer's opinion the paper by R. S. Shallenberger postulating a relationship between chiroptical properties and sweetness is out of place here and would be better suited to a volume with a theoretical rather than applied bias.

Some overlap is to be expected when different authors deal with closely related topics. However, the editors have kept this to an acceptable level. Each paper provides a wealth of references and all authors would appear to have ensured that their coverage included recent literature. Overall, a fairly comprehensive and up-to-date account of nutritive sweeteners is provided which will be of interest and use to food scientists and technologists and a source of further reading. The book is clearly printed and illustrated and remarkably free from even minor errors. An adequate index is included but more useful are the abstracts which precede each paper. The National College of Food Technology organizes a Symposium on this scale annually and it is all credit to the editors that the proceedings are published to this high standard within the year. Prior to 1981 the proceedings included an account of the discussion which followed each paper. Since interesting and frequently

valuable points were raised in such discussions, it is regrettable that the editors or publishers have chosen to discontinue this practice.

D. McHale

Dairy Facts and Figures 1981.
Thames Ditton: Federation of United Kingdom Milk Marketing Boards, 1982. Pp. 217. £4.00.

This publication contains much information of interest not only to those working in the dairy industry but also to the food manufacturer and the consumer. Of 15 214 million litres of milk sold off farms 46.9% was used as liquid and 53.1% manufactured. Of heat treated milk 86.2% was pasteurized, 6.6% sterilized, 6.5% homogenized and 0.7% 'ultra heat treated', a strangely ungrammatical term.

The types of manufacture over 1959—81 make interesting reading. Butter production has increased enormously, cheese substantially, condensed milk, whole milk powder and sterilized cream have altered little, but fresh (which means pasteurized) cream increased steadily up to 1974. Other products increased up to 1970, but have remained stationary since then. The quantities of cheese by variety have altered little, Cheddar being by far the most important, followed by Cheshire, Leicester, Double Gloucester, Stilton, Lancashire and Wensleydale. The neglect of other indigenous varieties, particularly the semi-hard and soft, continues to be a blot on the record of an otherwise progressive industry. Production of farmhouse cheese is steadily increasing and perhaps enlightened farmhouse makers will fill the gap. There should be a market for low fat cheese in view of all the fuss about the danger of milk fat, nonsensical as it is.

The increased butter production has inevitably meant the release of more skimmed milk, 4616 million litres being utilized, most being spray dried. Of 1907 million litres of whey 654 million were used for stock feeding which is most appropriate, but it seems strange that we in Britain have no interest in using a highly nutritious liquid, containing about half the solids in milk in 93% water, as the basis for a beverage. Children are the chief consumers of soft drinks which are often no more than sugar, water, flavouring and colour, and not only have little nutritional value but are hardly good for the teeth.

The specialized nutritional value of dairy products is well established. They contribute 19.7% of our energy, 22.6% of protein, 60.1% of calcium and 23.8% of vitamin A. Most of these come from liquid milk, except vitamin A of which about half comes from butter. We spend £1.21 per head per week on dairy products, representing 16.78% of total expenditure on food. Annual consumption per head was 132.9 kg liquid milk, 6.1 kg butter, 5.6 kg cheese, 2.8 kg yogurt, 1.8 kg evaporated and condensed milk, 1.6 kg cream (40% fat) and 1.2 kg milk powder. As a nation we are top of the league for liquid milk for the countries listed, about average for butter, but low in our consumption

of cheese, being markedly inferior to France, Italy, Western Germany, Sweden, Belgium and the Netherlands, and below the U.S.A., Denmark, New Zealand, Canada and Australia. Bearing in mind our considerable milk production, the outstanding nutritional value of cheese and its cheapness as a source of protein, calcium and energy, there is obviously considerable scope for improved marketing of a first-class home produced food. We import 166 271 tonnes of cheese annually, most of it Cheddar, although this has been steadily falling in recent years. Imports of Blue veined and other types seem to remain stationary, but imports of processed cheese are steadily increasing.

Although the importation of butter has been more than halved in the last 5 years, we still import 203 533 tonnes, most of it coming from New Zealand. Strange items are the 9 427 724 litres of liquid milk imported, and the 2080 tonnes of yogurt, nearly all coming from Germany. Figures for the home production of yogurt are not given; perhaps they will be in the next issue.

The U.K. self sufficiency ratios are of great economic and nutritional interest. We are 82% self sufficient in milk fat (why does the dairy industry still refer to it as butter fat?) and 113% self sufficient in milk solids-not-fat. The figures are 52.2% for butter and 70.4% for cheese.

The efficiency of our farmers is well illustrated by the average milk yield per cow; at 4715 litres per cow per year we are excelled only slightly by the Netherlands and are superior to all other EEC countries. There is a considerable amount of information specifically of interest to milk producers.

This most useful and compact manual should be in the possession of all who have a serious interest in the dairy industry and in the use of dairy products for food manufacture.

J. G. Davis

The Problem of Dark-cutting in Beef. Ed. by D. E. Hood and P. V. Tarrant. (Current Topics of Veterinary Medicine and Animal Science, Vol. 10). The Hague: Martinus Nijhoff, 1981. Pp. xi+504. ISBN 90 247 249 5. US$43.00.

This publication contains the proceedings of a seminar held by the Commission of European Communities on 7 and 8 October 1980 in Brussels on the topic of dark cutting beef. In response to the increasing consumer concern for animal welfare and the marketing requirement for improved meat and carcass quality the seminar was planned by the Carcass and Meat Quality Expert Group for the CEC Beef Research Programme to bring together specialist opinion in the areas of aetiology of the condition, heritability characteristics, identification and prevention, microbiology, eating quality and technological potential. Dark cutting beef occurs as a result of low muscle store leading to a high ultimate pH in meat. This is a persistent condition in all cattle with the highest incidence in young bulls which may be because of their excitable temperament. It may reach a level of greater than 25%.

Fundamental information is presented of the underlying biochemical processes in the areas of carbohydrate metabolism, energy supply, enzyme control mechanisms and muscle fibre differentiation in metabolic activity. The dark cutting condition may be avoided by carefully minimizing pre-slaughter stress particularly in young bulls. Exact recommendations are not formulated but areas for attention are time in transport, mixing with strange animals and the period spent in the lairage. Animal behaviour studies indicate precisely the areas for improved techniques. In the examination of organoleptic and processing properties the increased water holding capacity of high pH meat was noted and the prevention of early spoilage was discussed. The DFD (dark firm and dry) condition requires early diagnosis preferably by non-destructive tests and pH indicator and glucose strips were displayed. Prediction in pre-rigor muscle is done by freeze thaw pH determination and the fibre optic probe for light scatter measurement is recommended. The financial losses of dark cutting beef are high in all countries and double muscle cattle breeds are susceptible. As yet a marker gene such as the 'Halothane gene' in pigs has not been found in cattle. The dark cutting defect in beef occurs progressively as the ultimate pH exceeds 5.8. Up to 6.3 the extent of the effect depends on processing and marketing factors and above 6.3 an unacceptable degree of DFD is present.

As with the proceedings of most symposia the book is an information reference source. This book will be of great value to food technologists/scientists, veterinarians and students of meat and animal products. The volume will rarely be purchased except by the specialist, but it must be a compulsory resident in all libraries of meat research establishments. The contributors to the Symposium and the editors have done a professional job in presenting (in a digestible form) this mass of information from several disciplines. In particular the discussion sections are pertinent and incisive. There is a nice balance between the types of specialists giving the papers, and the material is organized to enable a clear picture to emerge during reading or alternatively the information is easily available for selection. Each contribution includes a comprehensive list of references.

the ultimate pH exceeds 5.8. Up to 6.3 the extent of the effect depends on processing and marketing factors and above 6.3 an unacceptable degree of DFD is present.

A. Dilworth

Effects of Low Temperatures on Biological Membranes. Ed. by G. J. Morris and A. Clarke.
London: Academic Press, 1981. Pp. xxii + 432. ISBN 0 12 507650 9. £22.00.

This book covers many aspects of the effects of temperature on the membranes in a wide range of organisms including bacteria, plants, fish and mammals. Much evidence is presented to support the hypothesis that both

thermal adaptation and the deleterious effects of low temperatures manifest themselves as changes in cellular membranes. In general, the temperature range that is dealt with is that found naturally in the environment, and topics such as cryopreservation are not dealt with directly.

The material is organized in four main sections: basic principles, adaptation to low temperature, chilling injury and freezing injury. The section on basic principles contains two chapters, one on the biophysics of low temperatures that deals largely with solvent effects, and a second that covers membrane structure and the effects of temperature on lipid fluidity and lipid protein interactions. The second section on adaptation to low temperature contains chapters on adaptation in bacteria, *Tetrahymena*, fish, several animal tissues and hibernating mammals; the emphasis is on temperature-induced changes in lipid composition and membrane fluidity, although some authors discuss direct effects of temperature on the Na^+, K^+-ATPase. The third section on injury at low temperatures contains chapters on chilling injury in plants, spermatozoa and heart cells. The last section on freezing injury begins with a consideration of the use of liposomes as a model system, followed by chapters on freezing injury in thylakoid membranes, *Chlamydomonas*, plant protoplasts and red cells. The final chapter deals with the thermodynamics of membrane structure and freezing damage, and, appropriately perhaps, concludes with some computer simulations of membrane damage.

In addition there is a prefatory section dealing with nomenclature. It was particularly helpful to have a standard definition of the terms 'acclimation', 'acclimatization' and 'adaptation'. The remainder of this section on the structure and terminology of lipids is marred by a lack of consistency in the presentation of lipid formulae, particularly the glycerol-oxygens of phosphoglycerides. Nevertheless, I thought this a useful addition to the book.

A number of the topics were presented originally as papers at a symposium held by the Society for Low Temperature Biology in September 1980. Despite the diversity of authors and its origins, the book is definitely not a collection of conference papers. There is a large degree of consistency in the style and presentation; this unity is helped by abundant cross-referencing between chapters. Inevitably there are discrepancies; for example, some authors interpret effects in terms of boundary lipids (lipid annuli) whose existence is disputed in the earlier chapter on basic principles of membrane structure.

Although there is a large amount of data, for example, on temperature dependent changes in lipid composition, authors have generally adopted a critical approach. In particular, several criticize, on fundamental and practical grounds, the use of Arrhenius plots to monitor lipid phase transitions and point out that simplistic interpretations of thermal injury based upon bulk phase transitions are invalidated generally by the complex lipid composition of cellular membranes. In this respect the book is particularly timely. Just as recent advances in our knowledge of membrane lipids emphasize their heterogeneity and the presence of micro-environments and specific lipid protein interactions, so must theories of thermal injury acquire more

sophistication. One casualty of this advance is the phase change hypothesis of chilling injury in plants, and a chapter is devoted to this topic.

The other major contribution the book makes is in relation to freezing injury, where an attempt is made to provide a unitary hypothesis. However, despite the undoubted changes in membranes that occur in this and other low temperature phenomena, one is left with the final impression that an explanation of the *mechanisms* underlying these changes is still some way off despite the rapid expansion in knowledge in this area.

The editors' stated intent is to present an integrated approach to low temperature effects on membranes, and they have achieved that aim in good measure. A wide spectrum of examples is used, generally in a well balanced and critical manner. The extensive information is well documented and has been up-dated since the original presentation of some in 1980. Despite the camera-ready format, there are few inconsistencies or typographical and referencing errors, although I found the frequent hyphenation in places rather annoying. The style of the figures is not uniform, but they are clear, as are the tables and electron micrographs; the few light micrographs are more difficult to interpret, particularly that on p. 294. Any such criticisms must be tempered by the fact that the use of this production style means that the price (£22.00) is not unreasonable for a book of this nature, and may even be within the range of purchasers other than libraries.

I enjoyed reading this book and, as a 'membrane biochemist', found much to interest me. The book should appeal to workers in a wide range of disciplines, both basic and applied, including ecologists, biochemists and those in the food industry. Food technologists will find information pertinent to the low temperature storage of foodstuffs and their spoilage by bacteria and fungi; the survival and recovery of frozen foodstuffs; and the problems of germination and frost-hardiness in plants. All in all, a book to be recommended.

N. J. Russell

Developments in Food Proteins. 1. Ed. by B. J. F. Hudson.
London: Applied Science Publishers Ltd, 1982. Pp. x + 335. ISBN 0 85334 987 8. £35.00.

This is one of a series covering developments in food science and technology intended to deal speedily with the latest trends and published within 6 months of being written—this last, if it is true, is a rare feat in the field of publishing.

The term 'food proteins' covers a vast diversity of topics as, indeed, is illustrated by the nine chapters. They range from basic scientific aspects through the uses of milk and novel proteins to consumer acceptability of novel protein foods. The authors range from the Department of the Government Chemist and a County Analyst's Laboratory to university departments in Ireland, Britain and South Africa.

An article by Professor Fox and his colleagues from University College, Cork, discusses the problems of understanding, and thus being able to modify, the physico-chemical properties so as to be able to make use of, for example, milk, meat and wheat proteins, and isolated proteins. The 440 papers referred to in this chapter indicate the work in progress in attempting to understand what has been achieved accidentally in the past and what is being attempted now.

The other chapters are not so extensively documented, but the first one is supplemented by chapters on techniques such as the use of differential scanning calorimetry to improve our understanding of protein behaviours, and another on the concentration of proteins by ultrafiltration.

These discussions lead naturally to the uses of milk proteins in foods during the last decade—which require the basic information lacking in many areas of food technology. This article appropriately comes from Dr Evans of the National Institute for Research in Dairying who touches upon an omission when he states that industry has only gradually realized that customers' choice is governed by price and expectation of flavour 'invariably at the expense of nutritional quality'. Whether or not this is true it points the omission of a section on nutritional value in the book, especially notable since there is so much misconception about protein quality, its meaning and its relative importance.

There are two sections which touch upon the fringe of this area—one by Dr Richardson of Cadbury-Schweppes who presents a must unusual and in-depth discussion of consumer acceptability, some of which apparently derives from practical experience of his own company. He points out that there is no nutritional evidence to justify an increased protein consumption in western countries and that even worldwide 'protein problems' have been overstated. Novel proteins intended to replace meat become expensive rather than cheap substitutes when they are made acceptable.

A logical follow-up is the case history of the use of vegetable protein in a school meal programme, with its technical, administrative and economic considerations.

Finally, there is a chapter on leaf protein and another on algae as future sources of protein foods.

Overall this is a thoughtful and thought-provoking book living up to the expectations of the reader and the intentions of the publishers in attempting to produce a book on the latest trends in the field. The choice of topics might not suit everyone but a second volume is intended to cover some of the gaps including the fields of raw materials, analytical and processing techniques and product attributes or disciplines. Perhaps nutrition will be reserved for a later book all to itself, when such esoteric terms as BV, GPV, NPR and so forth might be explained to the food scientist, technologist and even to the potential consumer of the protein foods.

A. E. Bender

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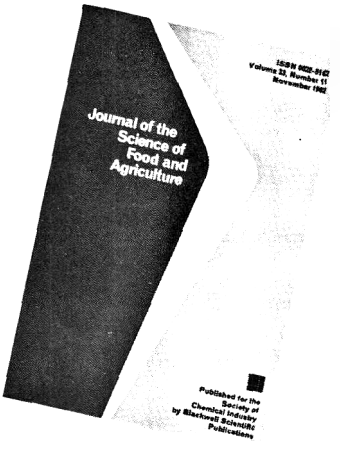

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Standard usage. The *Concise Oxford English Dictionary* is used as a reference for all spelling and hyphenation. Statistics and measurements should always be given in figures, i.e. 10 min, 20 hr, 5 ml, except where the number begins the sentence. When 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 is 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		

NON SI UNITS

inch	in	= 25·4 mm
foot	ft	= 0·3048 m
square inch	in^2	= 645·16 mm^2
square foot	ft^2	= 0·092903 m^2
cubic inch	in^3	= 1·63871 × 10^4 mm^2
cubic foot	ft^3	= 0·028317 m^3
gallon	gal	= 4·54611
pound	lb	= 0·453592 kg
pound/cubic inch	lb in^{-3}	= 2·76799 × 10^4 kg m^{-3}
dyne		= 10^{-5} N
calorie (15°C)	cal	= 4·1855 J
British Thermal Unit	BTU	= 1055·06 J
Horsepower	HP	= 745·700 W
Fahrenheit	°F	= 9/5 T°C + 32

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 bear a 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'.

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Contents

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