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Composition and properties of shark liver oil and liver residue

A. O. BANJO

Summary

The livers of *Carcharias taurus* and *Sphyrna diplana* were studied in detail, particular attention being paid to the potential uses of the lipid. The food value of shark, technological characteristics and the uses to which shark resources could be put are presented.

Seasonal variation in the composition of the shark liver oil was also observed. The detailed chemical composition of the extracted shark liver oil was compared with that of the commercial cod liver oil. The currently discarded liver residue left after oil extraction was found to be rich in protein and ash.

Introduction

A great potential for the shark fishery exists in Nigeria, for shark and rays have been found to constitute 16% by weight of the inshore trawl landings in Nigeria with an annual capacity of 7215 metric tons in 1975. Yet, little or no information is available on the chemical properties and the economic potentialities of this shark resource.

Shark is consumed in Nigeria either fresh or smoked, its flesh being processed to rid it of urea and ammonia. The liver which is not eaten contains extractable oil that can be used in the preparation of pharmaceutical food, paint, soap, cosmetics, leather tanning, printing ink, oilcloth, linoleum and in tempering steel parts such as steel springs.

This paper gives a brief account of the seasonal variations in the weight, lipid and water contents of shark livers and provides the basic information on the proximate analysis of the liver residue left after oil extraction.

Materials and methods

The livers of *Carcharias taurus* and *Sphyrna diplana* trawled off the Lagos coast by the research vessel, 'R. V. Kiara' or obtained from gillnet catches set off the Lagos coast were used as the sources of the liver oil.

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Sharks weighing from 1.2 to 98.5 kg provided the sources of liver. The livers were removed immediately after landing the fish, each was weighed, and then minced and well mixed after noting its colour. Samples of the minced livers were then used for the different analyses.

The seasonal variation in the weight percentage of the livers in proportion to the total body weight of shark was then determined.

Extraction of liver oil and studies on its chemical and Physical properties

Oil was extracted from the minced liver either by the cold solvent extraction method (Bligh & Dyer, 1959) or by steaming.

The oil and water contents of the liver were determined throughout the year for possible seasonal fluctuations. The water content was determined by drying to constant weight 5 g of liver tissue for 20 h at 100°C in an oven.

Iodine value of the extracted oil was determined throughout the year, by Wij's method while the free fatty acid content was found by titrating the oil absorbed in 25 ml of neutral ethyl alcohol against N/10 potassium hydroxide using phenolphthalein as indicator.

Saponification value was assayed by boiling for one hour under reflux 2 g of the oil with 25 ml of alcoholic potassium hydroxide solution and then titrating hot the excess alkali with 0.5N hydrochloric acid using 1 ml of 1% phenolphthalein as indicator.

Unsaponifiable matter was also determined from the mixture left after saponification according to AOAC method (1975).

Fatty acid composition

Gas–liquid chromatographic analysis was carried out on the lipid in order to get a full picture of the distribution of its fatty acids.

The fatty acids present in the oil were separated as their methyl esters. 0.1 ml of the ester mixture was then resolved at 190°C using acetone as the solvent, on a Beckman GC 72–5 instrument with a column of 20% diethylene glycol succinate on Chromosorb WHMDS (mesh 80–100) and a highly sensitive flame ionisation detector. Helium was the carrier gas at a flow rate of 40 ml/min (Fig. 1).

Calculation was based on peak height times width at half height. Peak areas were determined from three runs of each sample and averaged. The percentage composition of the fatty acid component of the lipid was calculated on the basis of the peaks on the chromatogram, in proportion to the total area.

Comparison of extracted shark liver oil with commercial cod liver oil

Some comparative studies were carried out on the shark liver oil and commercial cod liver oil. These included the determination of iodine value, free

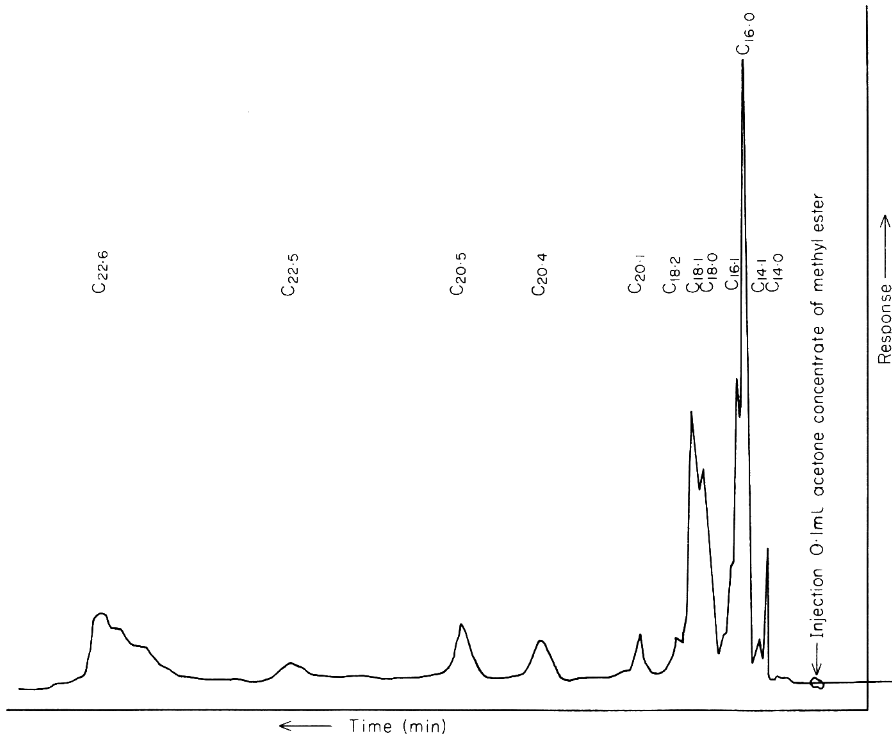

Figure 1. GLC-chart of fatty acid methylesters of shark liver oil.

fatty acid (FFA), moisture content (i.e. moisture entrained in the oil), saponification value, fatty acid composition, unsaponifiable matter and melting point.

Liver residue

The liver residues left after oil extraction were analysed for moisture, ash and protein contents and for the percentage of residual oil.

The protein content was determined as Nx6.25 using the Kjel–Foss automatic protein nitrogen analyser.

Results and discussion

The shark livers used in this study were pale cream coloured or dark brown with mottled patches. The mottled livers were found to contain less oil than the cream coloured ones. Bailey (1952) discovered that mottled livers had lower oil contents than putty grey ones. Livers procured from sharks throughout the year were found to constitute 3.1 to 6.5% of the shark (see Table 1).

The oil content of the shark livers ranged from 25 to 44% using the method of Bligh & Dyer (1959) (see also Table 1). The rendering of the oil by steaming was found to be incomplete.

Table 1. Seasonal variation in weight percentage, oil content (Bligh & Dyer, 1959) water content and iodine value of shark liver

Month	Weight (%)	Oil content (%)	Water content (%)	Iodine value
January	4.8	35	40	146
February	5.8	38	39	172
March	4.3	25	43	120
April	6.5	32	40	141
May	3.6	26	51	171
June	3.1	25	62	124
July	3.9	29	52	161
August	5.6	44	40	166
September	4.9	40	43	171
October	4.7	35	45	157
November	3.4	29	57	131
December	4.4	31	49	132

Table 1 also illustrates the seasonal variation in the moisture content of the livers and iodine value of the extracted oil.

It can be seen from Table 1 that oil and moisture contents were inversely proportional in shark liver as generally established in fish (Burgess, 1965). The combined moisture and oil content was found to be between 68 and 87%.

The sharp decline in oil content observed between August and November (Table 1) might be due to spawning during this period when there was a depletion in the oil reserve of the liver. Many gravid sharks were actually caught during this period. Correspondingly, there was a sharp rise in moisture content as expected (Table 1). The iodine value was between 120 and 172 throughout the year (Table 1). The high values indicated that the shark liver oil was highly unsaturated.

The drop in iodine value towards the end of the year could be due to maximum depletion. Lovern (1938) noted that some fish preferentially utilize the unsaturated fatty acids from their lipid stores during starvation and presumably leading to less unsaturation in their lipids. Lovern also, went further to demonstrate that lipid unsaturation rises with increasing and falls with decreasing lipid content. This trend is well illustrated by Table 1. For example, in February when the oil content was very high at 38%, the iodine value was at a maximum at 172. In March and June when the oil content was at a minimum level of 25%, the iodine values were also low at 120 and 124 respectively. Conversely in August and September when the oil contents were high at 44 and 40% respectively, the iodine value was again high at 166 and 171 respectively for the two months. Also, between November and December when the oil content increased from 29 to 30%, there was a corresponding small rise in lipid unsaturation (Table 1).

The free fatty acid (FFA) values of the oil were very low at 1.84. This low initial level of free fatty acid indicated a good quality oil.

The saponification value was between 144 and 172.90, whilst the unsaponifiable matter was between 8 and 15.31%. The high level of unsaponifiable matter indicated probably high levels of hydrocarbons (e.g. squalene) in the oil.

Fatty acid composition

The dominating fatty acid as shown in Table 2 is palmitic acid $C_{16:0}$ (29.0%) followed by nisinic acid $C_{22:6}$ (17.20%). The high values of $C_{16:1}$, $C_{18:1}$, $C_{20:4}$, $C_{20:5}$, and $C_{22:6}$, account for the high unsaturation in the liver oil. The low level of linoleic acid $C_{18:2}$ (0.28) is due to the conversion of this acid to arachidonic acid $C_{20:4}$ (the most essential fatty acid in the liver).

Table 2. Fatty acid composition of shark liver oil

Fatty acid	Composition* (%)
$C_{14:0}$	6.44
$C_{14:1}$	0.46
$C_{16:0}$	29.07
$C_{16:1}$	6.16
$C_{18:0}$	4.42
$C_{18:1}$	17.02
$C_{18:2}$	0.28
$C_{20:1}$	3.04
$C_{20:4}$	5.34
$C_{20:5}$	6.90
$C_{22:5}$	3.68
$C_{22:6}$	17.20

* = average

Table 3. A comparison of chemical and physical properties of shark liver oil and commercial cod liver oil

Biochemical factors	Cod liver oil	Shark liver oil
Iodine value	146.6–152.0	120.0–172.0
Free fatty acid	1.20	1.84
Peroxide value (m.eg/kg)	1.01	3.20
Moisture content entrained in oil (%)	0.91	1.47
Melting point 0°C	17	4
Saponification value	192.3–193.8	144.0–172.90
Unsaponifiable matter (%)	1.8–2.0	8.0–15.30

Table 4. Mean values for the main fatty acids in cod liver oil and shark liver oil based on gas–chromatographic studies

	Composition* (%)	
Fatty acid	Cod liver oil	Shark liver oil
$C_{14:0}$	3.24	6.44
$C_{14:1}$	–	0.44
$C_{16:0}$	10.78	29.07
$C_{16:1}$	8.92	6.16
$C_{18:0}$	2.49	4.42
$C_{18:1}$	23.90	17.02
$C_{18:2}$	1.97	0.28
$C_{18:4}$	2.29	–
$C_{20:1}$	11.34	3.04
$C_{20:4}$	0.7	5.34
$C_{20:5}$	9.01	6.90
$C_{22:5}$	0.2	3.68
$C_{22:6}$	10.43	17.20

* = average.

The comparison of shark liver oil with commercial cod liver oil showed that striking similarities exist in their chemical and physical properties as shown in Table 3. However shark liver oil could be said to be more unsaturated than cod liver oil as depicted by its higher iodine value. Moisture entrained in shark liver oil is higher, thus this oil will probably need more purification to prevent it from going rancid rapidly. This higher moisture value could also account for the slightly turbid colour observed in the shark liver oil. Unsaponifiable matter is also higher in shark liver oil indicating a higher level of hydrocarbons.

As shown in Table 4, shark liver oil contains higher levels of $C_{14:0}$, $C_{16:0}$, $C_{18:0}$, $C_{20:4}$, $C_{22:5}$, and particularly $C_{22:6}$ than cod liver oil. $C_{16:1}$, $C_{18:1}$, and $C_{18:2}$ are however lower in shark liver oil. The higher unsaturation found in shark liver oil (Table 3) could be attributed to the occurrence of more polyunsaturated fatty acids as seen in Table 4.

Liver residue

Liver residue analysis gave the following results:

Moisture content: 2–7%
Ash content: 2–4%
Protein content: 54.2–74.3%
Residual oil: 0.1–0.3%

The above results demonstrate the potentiality of the liver residue as a foodstuff for animal consumption because it is rich in protein.

Acknowledgments

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Decrease in the cold store flavour developed by frozen fillets of starved cod (*Gadus morhua* L.)

DEREK A. ROSS AND R. MALCOLM LOVE

Summary

Frozen fillets from cod which had been starved in an aquarium developed less cold store flavour or odour at −10°C than was developed by fed controls. Cis-4-heptenal, the major compound responsible for the off-flavour, was found to be present at a much lower level in the starved fish. At the same time the relative proportions of phospholipids and of the most important polyunsaturated fatty acids were found to be reduced by starvation. It was concluded that selective mobilization of precursors reduced the formation of the flavour compound during subsequent frozen storage. A seasonal variation in cold store flavour (and odour) therefore seems almost inevitable.

Introduction

A characteristic off-flavour develops in cod muscle during frozen storage, on occasion being so strong as to make the product unacceptable (Love, 1975). A similar odour develops at the same time, and whenever 'flavour' is mentioned in this account, it is implied that off-odours are present as well.

Lipid oxidation is the source of the undesirable characteristics (McGill, Hardy & Gunstone, 1977), even though lipids account for less than 1% of the white muscle of this species. The major substance responsible for the flavour (and odour) has been identified by McGill (1974) and McGill *et al.*, (1974) as cis-4-heptenal, which arises from the oxidation of unsaturated fatty acids located primarily in the phospholipids (A. S. McGill, Torry Research Station, personal communication).

The purpose of the present work was to study the effect of starvation on the quantity of cod muscle lipids and the relationship between any change in their composition and the flavour of the cooked product after cold storage.

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Material and methods

For the study of lipids and lipid fractions, trawled cod (*Gadus morhua* L.) caught off Aberdeen, were placed in a sea-water aquarium maintained at 9° and starved for various periods.

For the frozen storage and taste panel experiment, five cod which had been maintained for about a month on a diet of squid muscle in the stock tank were transferred to a separate but similar tank and kept without food for 2 months at 9°. At the end of this time, they, together with five fish from the stock tank which had continued to feed on squid muscle, were killed by a blow on the head, gutted and filleted. The fillets were then wrapped in aluminium foil and placed in a room at −10° where they remained for 5 or 10 weeks (one fillet from each fish for each period).

Muscle lipid was extracted by the method of Bligh & Dyer (1959) as modified by Hanson & Olley (1963) in the presence of anti-oxidant BHT.

The concentration of lipid in the muscle was determined gravimetrically and the water content, used as a measure of depletion, was calculated from the weight lost by about 10 g of chopped muscle in 7 days at 100°C in an open basin (Love, 1960). The pH was measured with a pH meter on a homogenate of 10 g of muscle in 20 ml distilled water.

The phospholipids were determined (as % of total lipid) by thin-layer chromatography, quantified by densitometry using a natural phospholipid mixture as a standard. The phospholipids themselves were separated into subfractions by the thin-layer system of Rouser, Fleischer & Yamamoto (1970).

The total lipid was transesterified for 1 hr at 80°C by methanol containing 3% H_2SO_4 and 0.5% benzene, under nitrogen, and the methyl esters were extracted into redistilled pentane.

Gas lipid chromatography of the methyl esters was performed with a Perkin Elmer F17 instrument fitted with a 2 m, 3.75 mm ID, 6.4 mm OD glass column coated wth SP 222 PS (Supelco Inc). Helium (99.99% pure) was used as carrier gas at a flow rate of 25 ml/min. All runs were isothermal. Components were identified by measuring the retention times relative to standard mixtures on columns of differing polarities. Results were quantified from the peak height × width at half peak height. Reproducibility of the method for the major components (those which formed more than 10% of the total acids) was ± 5% and ± 20% for acids that were less than 10% of the total.

Isolation and quantification of volatile flavour components, notably cis-4-heptenal, was performed according to the procedure of McGill *et al.* (1974) and McGill & Hardy (1977) except that the components were obtained from the extracted lipid and not directly from the muscle.

Four taste panel members trained in the detection and quantification of cold-store flavour and odour examined the steam-cooked muscle under code and submitted values for the flavour and odour within a range of 0 to 6 in order of increasing strength. The results obtained were submitted to analysis of variance.

Results

Changes in lipid composition during starvation

Figure 1 shows that starvation reduces the lipid content of cod white muscle, but that there is considerable scatter among individuals. Minimal values of 0.43% are reached, initial values ranging from 0.6 to 0.92. The water content of the white muscle is used in this work as a measure of starvation, rather than 'time without food'. This is because the composition of the muscle is affected by starvation only after more readily mobilized materials, such as liver lipid, have been used up, and the time-lag before the composition of the muscle changes will depend on the initial reserves in the liver (Love, 1970).

The relative proportions of total phospholipid in the muscle lipids of cod are also shown (Fig. 2) to fall during the course of starvation, confirming the unpublished observation of Olley (quoted by Wilkins, 1967) on the same species. Such a result is surprising, since the lipid fraction usually mobilized by starving animals is triglyceride, the 'storage' or 'depot' lipid, in contrast to phospholipid which is considered to form part of the structure of the cell. However, analysis of the muscle of replete cod (water content 81.0%) showed that only 1.3% of the lipid was triglyceride while 87.7% was phospholipid, so the latter is by far the most important lipid fraction in the flesh of this species. The main relative increase occurring during starvation is in the cholesterol fraction. The lipids of cod liver are more like the storage lipids of other animals, consisting mainly of triglycerides, and it is mainly triglycerides which are taken from the liver during starvation.

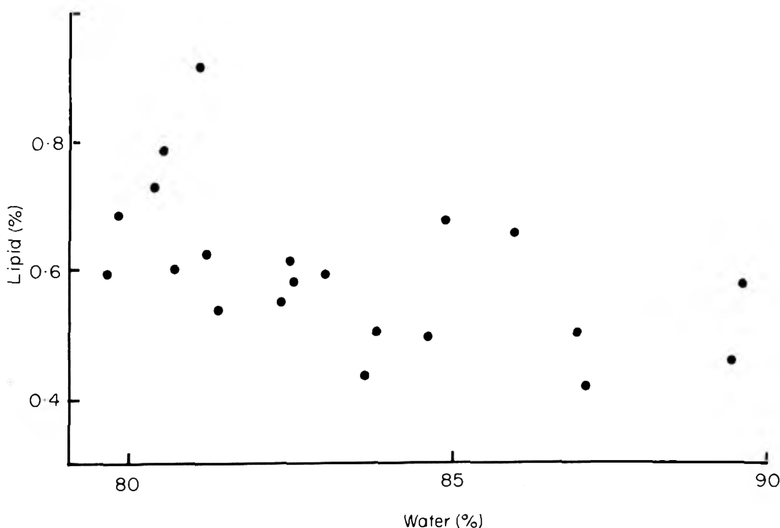

Figure 1. The decrease in the lipid content of the white muscle of cod during the course of starvation, which is shown by the increase in the water content.

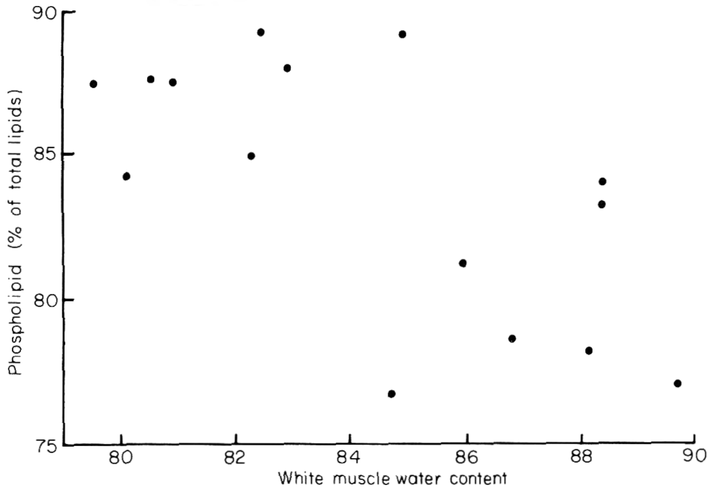

Figure 2. The decrease in the proportion of phospholipids in the lipids of cod muscle during starvation, which is shown by the increase in the water content. The phospholipid values were obtained by densitometry of TLC plates.

As phospholipids are mobilized, the actual composition of those still remaining in the muscle does not change greatly, apart from a relative increase in sphingomyelin (Table 1).

However, starvation does cause a steady decrease in the unsaturation of the remaining total muscle lipids. This is shown in Fig. 3, expressed as the sum of 22 : 6, 22 : 5, 20 : 5 and 20 : 4 acids. As well as being the major polyunsaturated acid, 22 : 6 is also the most unsaturated, and in these experiments it was found to decrease the most among the fatty acids. There was therefore a slight relative increase in 20 : 5 and 20 : 4 acids during starvation, though all decreased in absolute terms.

Table 1. Effect of starvation on the white muscle phospholipid composition

Phospholipids	Water content								
	80.59	80.67	81.09	81.31	82.4	83.02	85.61	88.98	89.67
LPC	0.29	0.24	0.3	0.65	0.49	0.57	0.46	0.54	0.32
S	1.64	2.28	1.92	1.87	2.36	4.03	3.96	5.73	4.31
PC	75.2	74.96	81.62	72.69	67.02	65.72	76.04	73.91	67.71
PI + PS	5.65	4.58	2.62	4.27	3.32	7.45	5.35	6.07	7.6
PA	0.68	0.28	1.11	1.19	0.55	0.53	0.55	0.57	0.72
C	0.46	0.85	0.89	0.74	0.47	1.01	0.77	0.72	0.95
PE	15.99	16.63	11.32	18.59	25.77	20.7	12.86	12.47	18.33

LPC = Lysophosphatidyl choline; S = Sphingomyelin; PC = Phosphatidyl choline; PI = Phosphatidyl inositol; PS = Phosphatidyl serine; PA = Phosphatidic acid; C = Cardiolipid; PE = Phosphatidyl ethanolamine.

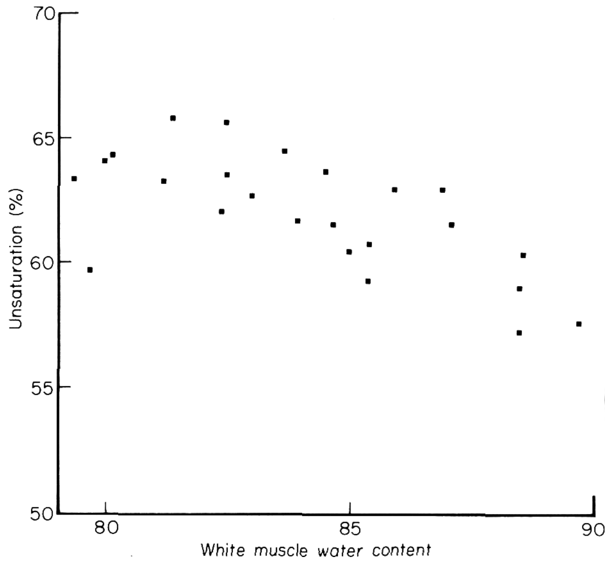

Figure 3. The decrease in the polyunsaturation of cod white muscle lipids during starvation, which is shown by the increase in the water content. The unsaturation is measured as the summed fatty acids 22 : 6 + 22 : 5 + 20 : 5 + 20 : 4 expressed as a percentage of the total fatty acids.

Cis-4-heptenal is derived from the oxidation of ω3 acids (A. S. McGill, personal communication), and 22 : 6 is the dominant, though not exclusive, ω3 acid in cod muscle. Its depletion might therefore be expected to reduce the quantity of cis-4-heptenal formed during cold storage, as the next experiment demonstrated.

Formation of off-flavours (and odours) during cold storage

The characteristics of the five cod fed continuously and four cod starved for 2 months are shown in Table 2. In addition, a single much larger cod was

Table 2. Characteristics of the fish used for cold storage at −10°

	Starved group*		Fed group†	
	Mean	Standard deviation	Mean	Standard deviation
Length (cm)	52.0	8.1	44.2	7.7
Ungutted weight (g)	935	468	816	415
Muscle water content	84.3	1.2	79.9	0.4
Muscle pH (post-mortem)	7.2	0.1	6.9	0.2

* 2 months at 9°C without food.
† Fed on squid muscle on alternate days.

Table 3. Taste panel values for off-odour and flavour in muscle from fed and starved cod after cold storage at −10°C

	Odour		Flavour	
	5 weeks	10 weeks	5 weeks	10 weeks
Fed controls (5 fish)	1.5	1.55	3.43	3.55
Starved fish (5 fish)	0.55	0.4	1.28	1.8
Difference	0.95	1.15	2.15	1.75
Significance level	1%	5%	0.1%	5%

Cis-4-heptenal values were as follows: starved cod muscle, 3.5 nmol/1000 g wet muscle; fed cod muscle, 23.0 nmol/1000 g wet muscle.

starved along with the others. When killed, its length was 97 cm, ungutted weight 7760 g, muscle water content 84.4%, pH 6.93. Since the water content was very close to that of the other four fish, it was taken as a fifth member of the starved group in spite of the disparity in sizes. Equal weights of muscle were taken from each fish for the pooled samples used for cis-4-heptenal determinations. The water contents show that the starvation had been quite severe but was well within the limits found in wild fish; they also show that the feeding of the 'fed' group was adequate.

The results of tasting are summarized in Table 3, from which it is clear that the fed fish give rise to more intense odour and flavour than the starved.

The cis-4-heptenal results are from single determinations carried out on pooled samples each representing the five fish after storage for 10 weeks. The striking difference between the values clearly accounts for at least part of the differences observed by the taste panel. The precision of the method used for trapping and flushing the cis-4-heptenal is better than 2% (McGill *et al.*, 1974), and the flame ionization detector on the gas chromatograph is sensitive to 10^{-10} g of this compound.

Discussion

The characteristics which are important in considering fish muscle as a foodstuff are colour, taste, odour, surface appearance (gaping or its absence) and texture in the mouth after cooking, and all of these have now been shown to vary seasonally and from ground to ground (Love, 1975). Genetic factors between races or stocks of fish seem to be relatively unimportant in this context, but physical activity influences the colour of the dark muscle which lies immediately under the skin (Love, Munro & Robertson, 1977) and the post-mortem pH of the muscle, itself governed by the feeding level in relation to previous starvation (Love, unpublished), influences gaping and texture (Love, 1975).

It is now clear that small changes in the muscle lipid content profoundly in-

fluence the tendency to form cold-store flavours during storage. A hint of this was suggested by our observation that cod caught on the Faroe Bank developed a strong off-flavour even when stored for only 3 months at −30°C (Love, 1975), and at the same time contained about 0.1% more lipid in their flesh than those from Aberdeen Bank, which maintained a better taste and smell under the same conditions (Love, Hardy & Nishimoto, 1975).

If the muscle lipid in this species had been like that of fatty species such as herring and mackerel, consisting largely of triglycerides, starvation would probably have had a smaller effect on the formation of cold-store flavour. Production of cis-4-heptenal depends on the presence of unsaturated fatty acids, and in cod the most unsaturated acid (22 : 6) forms only 6–7% of triglycerides in contrast to about 40% of, for example, phosphatidyl ethanolamine (Addison, Ackman & Hingley, 1968).

The fact that phospholipid is an essential component of cellular structures may seem hard to reconcile with its mobilization during starvation (Fig. 2). However, there is no doubt that it is mobilized: Dambergs (1964) has demonstrated a clear fall in cod muscle lipid, which as we have seen is nearly all phospholipid, in April, around the spawning time. This is consistent with the actual destruction of cellular material which occurs during starvation, described by Love, Robertson & Strachan (1968) and in a more extreme form by Love & Lavéty (1977).

The oxidation of lipids in biological materials is a complex process, since the rate is influenced not only by their composition but also by the incidental presence of amino acids, proteins, metal ions and antioxidants (reviewed by Labuza, 1971). While starvation preferentially removes unsaturated fatty acids, its effect on the proportions of other constituents could also be important in influencing indirectly the stability of the lipids to oxidation.

A seasonal variation in the unsaturation of cod liver lipids has already been observed by DeWitt (1963), who found that an abrupt decrease occurred at the spawning season, after which the unsaturation was gradually restored during the summer period of intensive feeding. In the case of muscle the annual increase in water content that occurs naturally at this time (Love, 1960) shows that depletion occurs, so that the unsaturation of muscle lipids, also, would almost certainly change seasonally. This means both that North Sea cod caught from March to May, frozen and cold stored, will probably develop less off-flavour than those caught at other times, and also that there would be times of the year when even the cod from Faroe Bank would develop less cold-store flavour and retain a quality during storage more like that of those from other grounds.

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The control of the botulism hazard in hot-smoked trout and mackerel

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Summary

The growth and toxin production of *Clostridium botulinum* types B, C, E and F in hot-smoked trout and mackerel has been studied. Using whole trout which were naturally contaminated with *Cl. botulinum* type E it was established that salt was the major inhibiting factor; a minimum concentration of 2.5% salt-on-water phase prevented the production of toxin for 30 days when fish were stored at 10°C. When whole and minced fish were inoculated with spores of *Cl. botulinum* types, B, E and F at a concentration considerably higher than that found in nature ($10^2 g^{-1}$) a minimum salt concentration of 3% was required to achieve a similar effect. Further studies using trout which were inoculated with suspensions of a number of strains of *Cl. botulinum* containing both spores and vegetative cells ($10^2 g^{-1}$) showed that fish smoked to produce a minimal salt concentration of 3% had a safe shelf life of 30 days at 10°C and 1 day at 20°C.

Introduction

Although the often fatal food poisoning disease, botulism, rarely occurs in Britain, over 20 years elapsing between the recent outbreak (Anon, 1978) and the last authenticated case in 1955 (McKay-Scollay, 1958) recent studies have shown a high incidence of the causative organism, *Clostridium botulinum*, in both home-grown and imported farmed trout (Huss, Pedersen & Cann, 1974, Cann, Taylor & Hobbs, 1975). The organism is also present to a lesser extent in mackerel (TRS unpublished results). Despite conservative measures which may be made for the control of *Cl. botulinum* in trout complete elimination of this organism from fish is not a practical proposition (Huss *et al.*, 1974). It must, therefore, be accepted that even under the most hygienic conditions of handling and processing some fish will remain contaminated.

In the case of wet fish which is distributed through the cold-chain there are, in practice, two safeguards. The rate of growth and toxin production by *Cl.*

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botulinum at temperatures below 10°C is such that putrefaction is usually evident before toxin is produced. Furthermore any preformed toxin is destroyed during cooking thus ensuring adequate safety to the consumer (Cann, 1977). Unfortunately, this is not necessarily so in the case of hot-smoked fish products.

Traditionally, smoked fish were heavily cured to the extent that *Cl. botulinum,* where present, could not grow and produce toxin in such a heavily salted, smoked and dried product. However, public taste has changed over the years with the introduction of lighter curing methods. Nowadays, smoked fish are distributed with much less smoke and salt and more moisture thus providing a suitable substrate for the growth of *Cl. botulinum*. Furthermore, hot-smoked fish are produced for the table to be eaten without further cooking and are, therefore, potentially dangerous if any errors should arise in temperature control during storage and distribution.

The danger of changes in traditional practices without adequate quality control has been reflected in outbreaks of botulism from hot-smoked fish in North America in the 1960s (Anon., 1963, 1964) and more recently in Germany in 1970 (Baumgart, 1970). Consequently, using both naturally and artificially contaminated fish the present work was undertaken to establish safe, commercially practicable, processing and storage procedures compliant with present day public taste for smoked fish.

Materials and methods

Of the factors affecting the ability of *Cl. botulinum* to grow and produce toxin in smoked fish products, those of major importance are the degree of contamination, the concentration of salt in the product, the temperature of storage and the salt resistance of the causative organism. To investigate their interaction this work was carried out in a series of storage studies using firstly, trout naturally contaminated with *Cl. botulinum* type E; secondly, trout and mackerel inoculated with either spores or mixtures of spores and vegetative cells of *Cl. botulinum* and thirdly, minced trout flesh inoculated with spore suspensions only.

Naturally contaminated trout

Fish were collected from a farm wherein the incidence of *Cl. botulinum* type E is known to be consistently higher than 80% (Cann *et al.*, 1975). They were prepared in the following ways: (a) ungutted unsmoked (b) gutted unsmoked (c) ungutted smoked and (d) gutted smoked. Fish were brined during the curing process to give individual batches ranging from 1.5 to 4% salt concentration expressed as salt-on-water phase.

Inoculated fish

Whole fish (*trout and mackerel*). Fish were eviscerated and washed with tap water prior to brining, smoking and vacuum packaging.

Minced trout. The flesh of smoked trout was stripped from the bone, minced in bulk, the salt concentration checked and adjusted to the required concentration where necessary, prior to vacuum packaging in 100 g amounts.

Smoke curing of fish

The control of salt levels in smoked fish is difficult but was effected by the development of a standardized curing procedure (Mills, 1978).

Strains of Cl. botulinum

Three types of *Cl. botulinum* were used in the major inoculation studies. They were National Collection of Industrial Bacteria (NCIB) 4207, a strain of type E isolated from canned sprats by Hazen; Torry Research Station (TRS) FT11, a strain of type B isolated from Irish Sea herring and NCIB 10641, a strain of type F isolated from herring caught in the Moray Firth of Scotland. Further strains used in additional studies were *Cl. botulinum* type B, NCIB 4301, TRS-FT 50 and TRS-FT 236; type C, NCIB 4218 and 4225; type E, NCIB 4206, 4214, 4248 and 10660 and type F, NCIB 10658, TRS-FT 32, 237 and 238.

Vacuum packaging and inoculation of fish

The preparation of spore suspensions, the vacuum packaging and inoculation of the various fish products were carried out using the methods described by Cann *et al.* (1966). All packages were inoculated with $10^2 g^{-1}$ spores of *Cl. botulinum*.

Incubation

The temperature at which smoked fish are held during production and distribution throughout the commercial cold-chain is known to vary considerably. An incubation temperature of 10°C was chosen based on the British Standard specification (BSI 3918:1965) requiring domestic refrigerators to function at or below this temperature.

Assay of toxin

For statistical purposes whole fish were sampled in batches of $\nless 35$ (Cann *et al.*, 1975). Separate batches from each treatment were examined after 10, 20 and 30 days. Minced trout were examined daily in duplicate over a 30 day period. Toxin was assayed using the method of Cann *et al.* (1966).

Results

Naturally contaminated trout

The production of toxin by *Cl. botulinum* type E was inhibited at a salt concentration of 2.5% over a period of 10, 20 and 30 days for all treatments of fish. At a lower concentration of 2.0% salt only the ungutted fish became toxic, the added effect of smoking reducing the incidence from 19% (s.e. 6.1) in brined fish to 5.6% (s.e. 3.8) in the fully cured product.

Inoculated whole fish

Toxin was produced in trout at a salt concentration of 2.5%; the degree of toxicity varying with the type of *Cl. botulinum* that was used. After a storage period of 30 days at 10°C, type B (FT 11) produced toxin in 90% of fish, type E (NCIB 4207) in 20% and type F (FT 10) in none of the fish. No toxin was produced by any strain when the salt concentration in the fish was raised to 3.0%. Additional studies using inocula of mixed vegetative cells and spores of *Cl. botulinum* types B, C, E and F showed that a salt concentration of 3.0% was inhibitory to the production of toxin in both trout and mackerel over storage periods of 1 day at 20°C and 10, 20 and 30 days at 10°C.

Minced trout

The production of toxin in minced trout stored at 10°C is shown in the figure. At a salt concentration of 2.0% in fish inoculated with types E and F, toxin was detectable after 10 and 21 days of storage respectively. Toxin was not found at 2.5% salt concentration. *Cl. botulinum* type B produced detectable toxin in fish after 10 days at a salt concentration of 3.0%. Toxin production was, however, erratic and of low titre during subsequent storage and

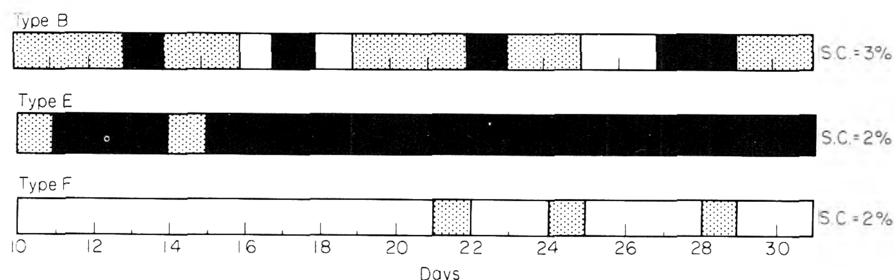

Figure 1. Comparison of toxin production in minced smoked trout during storage at 10°C after inoculation with $10^2 g^{-1}$ of viable spores of *Cl. botulinum*. S.C. = Salt Concentration; □, toxin not detected; ▨, some toxin present (insufficient to kill all inoculated mice); ■, duplicate samples toxic.

never attained levels higher than 100 Mouse Lethal Doses (MLDs) per gram of mince compared with the 4000 MLDs per ml of a control Robertsons meat broth culture.

Discussion

The results of storage studies of naturally contaminated trout clearly show the value of conservative measures in the control of toxin formation; thorough evisceration and washing of fish reduces the number of contaminating organisms. However, even in ungutted fish, brining to a level of 2.5% salt was sufficient to inhibit toxic outgrowth of the low numbers of *Cl. botulinum* type E present. This is in accordance with previous findings (Cann *et al.*, 1965) which showed that little or no toxin was produced in marine fish that were inoculated with spores of the order shown to be present naturally in Danish trout (0.34–5.3 g^{-1}) by Huss, Pedersen & Cann (1974) and in British trout (0.7 g^{-1}) and North Sea herring (0.66 g^{-1}) by Cann & Taylor (unpublished results) and Cann *et al.* (1966) respectively.

Comparison of the results between the naturally contaminated whole trout and those inoculated with $10^2 g^{-1}$ viable spores of *Cl. botulinum* types B, E and F clearly demonstrates that even with an increase of contamination of more than two orders of magnitude toxin development was inhibited for 30 days in smoked fish of 3.0% salt concentration.

The picture of toxin production was less clear cut in the inoculated minced trout. Here differences in salt tolerance between type B and types E and F were reflected by toxin production in 10 days by type B in fish with a 3% salt concentration. The disparity between the results with whole and minced fish can be explained by the method of production. On the one hand the salt concentration within the minced fish was uniform and stable. On the other hand during the smoking of whole fish a salt concentration gradient is created between the skin and the flesh with subsequent diffusion of salt into the flesh until equilibrium is reached. Thus the flesh of whole fish with an initial minimal salt level of 3% will later have an increased salt concentration depending on the length of storage. In addition, the fine particulate nature of mince in affording intimate contact between the bacteria and available nutrients may afford more favourable conditions for its growth.

The sensitivity of *Cl. botulinum* to salt varies with the type and strain under consideration. Proteolytic strains of types A and B are resistant to about 10% whereas non-proteolytic types B, E and F are susceptible to 5% salt concentration (Hobbs, 1976). These figures, however, only apply to studies of the organism at optimum temperatures for growth and toxin production; at lower temperatures the sensitivity to salt increases (Ohye & Christian, 1967; Baird-Parker & Freame, 1967). Data available for type C show a limiting salt concentration of 3% for growth at 30°C and for four strains of type E of 4.5% at 10°C. The latter figure was however based on outgrowth of spores at an inoculation

level of 10^6 ml^{-1} in culture medium over a period of 365 days. A study of one strain of type E showed that 11 days were required for outgrowth of spores in 3% salt medium at 10°C; the presence of toxin was not tested for (Segner, Schmidt & Boltz, 1966, 1971).

The variation of salt resistance and toxigenicity between strains of *Cl. botulinum* of different geographical origin is significant when it is considered that quantities of contaminated Danish and Japanese trout are imported into Britain (Huss *et al.*, 1974). In this context the results of the additional inoculation studies clearly show that a 3% salt concentration in smoked fish is effective against a variety of such strains of types B, C, E and F.

In practice, therefore, the control of the botulism hazard in hot-smoked fishery products that are marketed in the United Kingdom can be effected by the combined use of a light smoke cure and some degree of refrigeration; legislative and consumer requirements of today excluding the use of other preservatives, irradiation and the traditional heavy cures with their high salt and smoke content. Similarly, modification of the smoking process to bring about a pasteurization effect, as introduced into the regulations of certain American States requires such a level of heating that the finished product is no longer of the texture and appearance demanded by the consumer and even then is not always sufficient to kill all residual spores of *Cl. botulinum* (Hobbs, 1976).

When considering the storage and transport of lightly cured, hot-smoked fishery products it must be remembered that they should be regarded as very short life foods as defined by the Foods Standard Committee (1972). Such foods require chill conditions at all stages from production to consumption. Regrettably this is often not the case either in industry or the home. Concern about the possible mishandling of hot-smoked fishery products has led the Department of Health and Social Security to publish Recommended Practices for the Processing, Handling and Cooking of Fresh Hot-Smoked and Frozen Trout.

Using the essential premise that fish must preferably be chilled at 0°C and at least to the practical level of 10°C (Anon, 1974) a minimal salt concentration of 3% ensures wholesomeness well in excess of a 7 day requirement for very short life foods and moreover allows a safety factor of at least 1 day at room temperature for mishandling which may take place between purchase and consumption of the product.

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Histidine metabolism in mackerel (*Scomber scombrus*). Studies on histidine decarboxylase activity and histamine formation during storage of flesh and liver under sterile and non-sterile conditions

JOSÉ FERNÁNDEZ-SALGUERO* AND IAN M. MACKIE

Summary

The concentrations of histidine and histamine in the flesh and liver of mackerel have been determined during storage at 0, 2, 10 and 23°C. Under sterile and non-sterile conditions little histamine was produced during storage of muscle even after 18 days at 0°C. At 10°C the levels of histamine exceeded 100 mg/100 g tissue in both liver and muscle after 5 days' storage. The concentration of histidine in muscle remained virtually constant throughout all storage periods but in liver it increased markedly. Histamine showed greatest increase in liver samples. Histidine decarboxylase activity as measured by release of $^{14}CO_2$ from ^{14}C-L-Histidine in crude extracts showed a decrease in muscle and an increase in liver. The significance of bacterial and tissue enzymes on the production of histamine is discussed.

Introduction

The major route of histidine metabolism in fish muscle is decarboxylation to histamine by enzymes of contaminating bacteria (Kimata, 1961; Ienistea, 1971) under conditions favourable to enzymic synthesis and activity. The histamine produced during storage of scombroid species such as mackerel and tuna is often implicated as the causative agent of 'scombroid poisoning' but as yet no direct link has been established (Halstead & Courville, 1967). The mechanism of its formation in the body and of its excretion are controversial (Ienistea, 1971). It has been shown, however, that pelagic fish, and particularly fish of the Scombrodei, have unusually high levels of free histidine in tissue extracts and it has been suggested that the potential for histamine production is related to this concentration of free histidine (Edmunds & Eitenmiller, 1975). Little information is, however, available on

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the relationship between the level of histidine decarboxylase activity in tissue and the conditions of storage on the production of histamine from histidine in scombroid species nor have the optimal conditions for the activities of tissue and bacterial enzymes been clearly established.

Edmunds & Eitenmiller (1975) showed for several species of fish including Spanish mackerel (*Scomberomorus maculatus*) that a build-up of histidine decarboxylase activity took place during the early part of storage at 4°C and at ambient temperature and that it tended to decrease with storage time. There was no correlation between build-up of enzymic activity and the production of histamine.

This paper attempts to identify more closely the factors which are important in the production of histamine during storage of mackerel (*Scomber scombrus*) under sterile and non-sterile conditions. This work was part of a study on the metabolism of histidine in tissue of mackerel after death; the complementary study on the formation of urocanic acid by deamination has been published (Mackie & Fernandez-Salguero, 1977). It was shown that urocanic acid was produced to the extent of about 1% of the concentration of histamine and that it was formed mainly by tissue enzymes.

Materials and methods

The mackerel (*Scomber scombrus*) were caught off Oban by hand-line in July 1976 and were transported by road to the laboratory in a container mixed with ice. Fish for the experiment at 2°C were caught off Aberdeen by the Station's research vessel. Sterile muscle portions and minces were prepared either immediately after catching on board the research vessel or in the Dunstaffnage Marine Research Laboratory (Oban). Each side of the fish was thoroughly washed with cotton wool impregnated with 10% formaldehyde solution. A U-shaped cut was made into the dorsal region of the lateral trunk muscles and a portion of the skin was aseptically cut loose from one side of the fish and pulled off in a sterile atmosphere. An interior cut parallel to the U-shaped one was made with a sterile scalpel and the muscle portion was quickly removed with sterile forceps and put into sterile jars. Using this procedure, 70% of the samples were obtained sterile.

Sterile mince was prepared from sterile fish flesh with a sterile mincer in a laminar air flow cabinet (Slee). The sterility of all samples was checked before analysis.

The sterile muscle portions and minces were placed in sterile jars and stored at 0 and 2°C; whole fish, fillets and non-sterile minces were sealed in polythene bags and stored at 0 and 2°C. Whole fish were also stored at 10 and 23°C and their livers were analysed for both histidine and histamine.

Fish silage was prepared as described by Tatterson & Windsor (1974). Whole fish of not more than 1 day in ice was minced in a small laboratory mincer and extruded through a disc with holes of 5 mm diameter. The minced material was mixed with 3.0% by weight of 98–100% formic acid.

Proteus morganii *inoculated muscle*

A sterile muscle sample, inoculated with *Proteus morganii* (NCMB 865 from Torry Research Station) was held for 12 days at 10°C. A sterile muscle sample from the same part of the fish was used as a control.

Reagent

^{14}C-L-Histidine uniformly labelled with specific activity of 330 μCi per μmol was obtained from the Radiochemical Centre, Amersham.

Preparation of perchloric acid and trichloroacetic acid extracts

Perchloric acid extracts of tissue were prepared as described previously (Mackie & Fernandez-Salguero, 1977). Five per cent trichloroacetic acid was used instead of 0.6 M perchloric acid when histidine and histamine were determined. (Perchloric acid interferes with elution of amino acids from the amino acid analyser.)

Determination of histidine

The concentration of histidine in trichloroacetic acid extracts was determined with an automated Locarte Amino Acid Analyser as described previously (Mackie & Ritchie, 1974).

Determination of histamine

The trichloroacetic acid extracts (10 ml) were eluted through a column of 3.0 g Amberlite CG 50 resin according to the procedure of Kawabata, Uchida & Akano (1960) to separate histamine from histidine. The concentration of histamine in the histidine-free eluate was determined by fluorimetry after reaction with o-phthalaldehyde using Shore's procedure (1971).

Histidine decarboxylase assay

Histidine decarboxylase activity in the muscle and liver was determined in crude extracts by an isotopic decarboxylase assay method of Hakanson (1963) as modified by Levine & Watts (1966). The sample tissue (10 g) was homogenized in 90 ml of ice cold distilled water in an Ultra-Turrax homogenizer and centrifuged at 20 000*g* for 15 min. To 0.6 ml of the supernatant solution was added 2.4 ml of 0.1 M sodium acetate buffer (pH 5.5) (Levine & Watts, 1966),

0.2 ml of 0.025% tetracycline, 0.2 ml of 0.02% pyridoxyl-5′-phosphate and after allowing to stand for 10 min, 0.2 ml of uniformly labelled ^{14}C-L-Histidine was added. After incubation at ambient temperature (23° ± 2°C) for 5 h in an airtight reaction flask, 2.0 ml of 1.2 M perchloric acid was injected throt.gh the rubber cap into the flask to stop the reaction and release dissolved $^{14}CO_2$. The liberated $^{14}CO_2$ was trapped in 0.5 ml of 2 M sodium hydroxide placed in a small vessel fixed to the bottom of the flask. After standing overnight to ensure complete liberation of $^{14}CO_2$, the sodium hydroxide was transferred to a scintillation vial and 10 ml of toluene scintillation fluid was added. The isotopic activity was measured with a Liquid Scintillation Counter (UNILUX-1 Nuclear Chicago). For the blank the labelled histidine was added after the addition of perchloric acid. The activity was expressed as nmol histamine/min/g protein.

Protein determinations were done by the procedure of Folin-Ciocalteau (Bailey, 1967).

Results and discussion

Tables 1 and 2 show the concentrations of free histidine during storage of various mackerel preparations at 0, 2, 10 and 23°C. Such levels are within the range expected for mackerel (Lukton & Olcott, 1958; Hardy & Smith, 1976); values in excess of 1000 mg/100 g flesh have been reported for some species of tuna (Lukton & Olcott, 1958). In such fish free histidine is the major component of the nitrogenous extractives, its concentration being as much as 100 times higher than in non-scombroid species such as cod and saithe (Mackie & Ritchie, 1974). During storage at 0 and 2°C the concentration of histidine in samples stored under sterile and non-sterile conditions remained constant within the limits of biological variation for up to 18 days in ice which is well beyond the period when mackerel is acceptable for eating; after 10 days in ice, or after 1 day at about 20°C, mackerel is generally considered to be inedible.

Table 1. Concentration of histidine in various preparations of mackerel held at 0°C (mg/100 g tissue)

Time of storage (days)	Sterile muscle	Fillet	Sterile mince	Mince	Muscle (whole fish)	Liver
1	551.0	567.4	524.1	441.8	571.0	35.3
4	386.8	508.1	573.6	541.5	441.7	83.8
7	474.9	577.9	497.5	593.9	543.3	107.7
10	398.8	504.5	498.8	496.4	499.5	163.6
14	445.1	490.2	508.3	499.3	451.4	176.3
18	513.5	357.2	522.1	–	395.8	210.5

Table 2. Concentration of histidine in various preparations of mackerel held at 2, 10 and 23°C (mg/100 g tissue)

	2°C				10°C		23°C	
Time of storage (days)	Sterile muscle	Muscle (whole fish)	Liver	Time of storage (h)	Muscle (whole fish)	Liver	Muscle (whole fish)	Liver
1	413.2	452.6	44.3	6	–	–	417.1	22.3
3	491.9	365.3	53.0	15	–	–	–	77.7
5	378.7	329.3	69.0	24	388.7	24.4	–	99.0
8	352.3	383.8	108.6	36	–	–	327.2	183.2
12	399.0	301.6	203.9	72	–	–	–	–
20	481.4	–	–	120	324.5	299.4	–	–
25	409.4	–	–	–	–	–	–	–

There is, however, some evidence for a decrease in the concentration of histidine in the whole muscle stored at all storage temperatures as well as in fillets stored at 0°C. It can be inferred that a dynamic equilibrium exists between histidine released from muscle proteins and in its metabolism. It must further be inferred that the catabolic process is very weak and that it is only of importance at the end of the various periods of storage.

An examination of the concentration of histidine in the liver during the same storage periods shows that a very different situation exists. While in muscle the initial concentration of histidine was in the range 388.7–571.0 mg%, in liver it was very much lower, viz. 22.3 mg % after 6 h of incubation at 23°C and 44.3 mg % after 1 day at 2°C. On the other hand, histidine was produced steadily in liver during storage rising to levels greater than 183.2 mg % in all the storage experiments. The activity of proteolytic enzymes, either autolytic proteases of the liver cells or proteolytic enzymes of contaminant microflora, apparently release more histidine from proteins than is converted by the catabolic processes.

The corresponding histamine concentrations in the same samples are given in Tables 4 and 5. As expected the concentrations of histamine in very fresh

Table 3. Concentration of histidine in various preparations of mackerel held at 2, 10, 23 and –30°C (mg/100 g tissue)

Time of storage (days)	Fish silage		Time of storage (days)	10°C		Time of storage (months)	–30°C
	2°C	23°C		Control	*P.morganii*		Frozen mackerel
3	406.0	424.6	12	255.2	5.7	8	292.3
22	322.6	333.2	–	–	–	–	–

Table 4. Concentration of histamine in various preparations of mackerel held at 0°C (μg/g tissue)

Time of storage (days)	Sterile muscle	Fillet	Sterile mince	Mince	Muscle (whole fish)	Liver
1	0.08	0.08	0.19	0.10	0.07	0.25
4	0.05	0.11	0.40	0.16	0.13	0.38
7	0.12	0.23	0.15	0.17	0.43	4.87
10	0.11	0.17	0.28	0.20	0.92	25.41
14	0.08	0.86	0.20	0.62	1.14	59.85
18	0.06	114.00	0.21	79.52	1.86	546.25
25	0.24	–	–	–	–	–

fish are low (0.07–1.24 μg/g of tissue) and during storage of sterile muscle and sterile mince the levels remain steady or show only a slight increase even after 25 days at 0°C. In non-sterile samples of fillet, mince and muscle from whole fish, there was little increase even after 14 days' storage but after 18 days, when obvious bacterial spoilage had taken place, the concentration of histamine rose sharply. In liver, on the other hand, the concentration of histamine rose steadily and quickly during the 18 days' storage, reaching a level of 546.3 μg/g of tissue. At all storage temperatures the histamine content of the liver was higher than that of the corresponding muscle; samples of concentrations of 224.7 and 14.06 μg/g respectively were found after 12 days' storage at 2°C. The values reported here for muscle are higher than values given for Spanish mackerel by Edmunds & Eitenmiller (1975) who found less than 2.0 μg/g of tissue after 14 days at 4°C. Hardy & Smith (1976) found no histamine in *Scomber scombrus* even after 15 days in ice. At the higher temperatures of 10 and 23°C the build-up of histamine was correspondingly more rapid and after 5 days at 10°C the concentration in both muscle and liver

Table 5. Concentration of histamine in various preparations of mackerel held at 2, 10 and 23°C (μg/g tissue)

	2°C				10°C		23°C	
Time of storage (days)	Sterile muscle	Muscle (whole fish)	Liver	Time of storage (h)	Muscle (whole fish)	Liver	Muscle (whole fish)	Liver
1	0.07	0.09	0.41	6	–	–	0.12	0.25
3	0.08	0.12	0.47	15	–	–	0.51	1.36
5	0.12	0.17	1.74	24	0.20	0.58	2.14	56.37
8	0.10	2.19	104.17	36	–	–	50.00	186.83
12	0.13	14.06	224.73	72	2.00	180.50	–	–
20	0.11	–	–	120	1820.83	1330.00	–	–

Table 6. Concentration of histamine in various preparations of mackerel held at 2, 10, 23 and −30°C (μg/g tissue)

Time of storage (days)	Fish silage		Time of storage (days)	10°C		Time of storage (months)	−30°C
	2°C	23°C		Control	*P.morganii*		Frozen mackerel
3	0.11	0.50	12	3.56	2424.9	8	0.52
22	10.45	11.40	–	–	–	–	–

exceeded 1000 μg/g, a level which has been suggested by some workers as being high enough to cause poisoning (Kimata, 1961; Halstead & Courville, 1967).

It was of interest to examine the concentration of histidine and histamine in silage during storage at 2 and 23°C respectively because of the possible production of histamine by non-bacterial enzymes during prolonged storage at relatively high temperatures. The data given in Tables 3 and 6 show that the content of histidine decreases to the same extent at both temperatures and that some of it at least is catabolized to histamine. As this system is likely to be nearly or fully sterile the increase in histamine presumably by some enzyme process is somewhat surprising, but it does indicate that attention should be given to the development of toxicity in silage made from mackerel.

The effect of inoculating the sterile sample of muscle with *Proteus morganii* was to convert nearly all of the free histidine to histamine during 12 days at 10°C, the amount produced again being above the suggested toxic level. Not surprisingly little change in either the level of histidine or histamine was observed in a sample of muscle frozen and stored at −30°C.

The figures for the activities of histidine decarboxylase in muscle and liver are given in Table 7. During storage at 0°C the activity in the muscle fell but,

Table 7. Histidine decarboxylase activities for the examined samples held at 0°C (nmoles histamine/min/g protein)*

Time of storage (days)	Samples	
	Muscle (whole fish)	Liver
1	7.90	0.12
4	3.82	0.21
7	4.13	0.23
10	4.44	0.95
14	4.35	2.53

*Means of duplicate analyses.

on the other hand, it increased in the liver. The value in the liver was, however, much lower than in the muscle. A trend of decreasing decarboxylase activity in flesh has also been observed by Edmunds & Eitenmiller (1975).

It is evident from these results that catabolism of histidine is predominantly a bacterial enzymic process and that all of the free histidine of muscle can be converted to histamine by bacteria such as *Proteus morganii* when present in sufficient amounts and if suitable conditions of storage prevail for the histidine decarboxylase activity. It appears, however, that the bacteria which develop on mackerel during storage at 0°C do not readily decarboxylate free histidine and that levels reached in spoiled mackerel are little higher than in sterile muscle stored at the same temperature. At high temperatures conditions exist for the growth of bacteria capable of producing relatively large amounts of histamine. These results reinforce previous findings (Kimata, 1961; Edmunds & Eitenmiller, 1975; Hardy & Smith, 1976) that if high concentrations of histamine are to be avoided in fish and fishery products, storage at chill temperatures after processing should be adopted.

Tissue enzymes are of little importance in the decarboxylation process when fish are stored under normal commercial conditions but possibly under low pH conditions as in silage there are more favourable conditions for their involvement. Further work would have to be done to establish optimal conditions of pH and temperature for their activity.

It is also clear that high levels of free histidine and relatively high levels of histidine decarboxylase do not necessarily lead to a build-up of histamine. Indeed the converse has been found in liver which has relatively low levels of free histidine and low levels of histidine decarboxylase, yet the greatest increase in histamine was found in this tissue during storage at all temperatures.

Acknowledgments

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The effect of heat processing on the structure and rheological properties of carrageenan gels

P. A. AINSWORTH AND J. M. V. BLANSHARD

Summary

Rheological investigations of carrageenan and heat processed carrageenan gels show that the strengths of the gels are greatly reduced on processing whereas very little change takes place in the modulus of rigidity. The results are discussed in terms of the crosslink density and network chains in the gels, and these indicate that the rigidity modulus appears to be strongly dependent on crosslink density whereas the gel strength, as well as being influenced by crosslink density, is also dependent on the gel network chains.

Introduction

Carrageenan is the name given to the mixture of sulphated polysaccharides found in certain species of red marine algae (*Rhodophyceae*). This polymer has found widespread use in the food industry because of its ability at low concentrations to form viscous solutions and gels, which are extremely useful in the texturing of foodstuffs.

The tendency for carrageenan samples to gel depends to a large extent on their monosaccharide composition (Anderson *et al.*, 1968). The gelling fractions of carrageenan are very similar in their monosaccharide composition, consisting of alternating structures of 1,3-linked D-galactose 4-sulphate and 1,4-linked 3,6-anhydro-D-galactose units (Anderson, Dolan & Rees, 1968).

On gelation, disordered polymer networks are formed, together with double helix crosslinks (Anderson *et al.*, 1969), giving regions of local order. These ordered regions are disrupted by the presence of D-galactose sulphate units in place of the 3,6-anhydro sugar units. A decrease in the strength of carrageenan gels is observed as the crosslinks are disrupted (Anderson *et al.*, 1968), showing that crosslink density is an important contribution to the strength of the gels. When certain cations are added to carrageenan samples stronger gels are produced by the formation of electrostatic forces bridging the charged sulphate

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groups on adjacent double helix crosslinks resulting in aggregation (Anderson *et al.*, 1969). The work in this present study was carried out with a view to understand more of the relationship between the structure and rheological properties of carrageenan gels.

Materials and methods

Samples

The carrageenan samples used in this study were extracted from a variety of species of *Rhodophycea* located at different habitats (Table 1). Different species and locations were chosen to obtain carrageenan samples of varying molecular structures.

Extraction and purification of carrageenan

Each seaweed sample was stirred for 1.5–2 h in water at 85–95°C and pH 8–9. The extract was filtered, the filtrate evaporated, roller dried and then milled.

The extracted carrageenan samples were purified by the procedure of Smith, O'Neill & Perlin (1955). This resulted in the separation of the gelling and non-gelling fractions of carrageenan. Part of the carrageenan extract from sample 1 was heated for 3 h at 2.07×10^5 Nm^{-2} with 15% (w/v) potassium hydroxide followed by neutralization with hydrochloric acid. This produced alkali modified carrageenan (sample 3).

To minimize deterioration, the pure samples were freeze dried and stored in air-tight containers at 5°C until use.

Estimation of 3, 6-anhydro-D-galactose

Ree's modification (Rees, 1961) of Yaphe's resorcinol method (Yaphe, 1960) was used for the analysis. The amount of 3,6-anhydro-D-galactose

Table 1. Carrageenan samples

Sample	Seaweed species	Seaweed habitat
1	*Eucheuma cottonnii*	Tanzania
2	*Chondrus crispus*	Ireland
	Gigartina stellata	Canada
3	*Eucheuma cottonnii*	Tanzania
4	Unknown	Japan

present was calculated by reference to a standard curve obtained using known concentrations of methyl 3, 6-anhydro-D-galactoside.

Heat processing

The required concentrations of the pure samples were dissolved in deionized water and placed in Kilner jars for processing in a Milwall vertical stationary retort. A retort temperature of 129°C was maintained for 59 min. The sample containers were removed from the retort and placed in hot water (80°C) and the samples used at this temperature or cooled in running cold water depending on the experiment.

Measurement of intrinsic viscosity

The intrinsic viscosities of the carrageenan samples before and after heat processing were measured at a temperature of 25°C, using an Ubbelohde viscometer. The viscometer had a flow time for 0.1N sodium chloride of 265 seconds.

Solutions of the carrageenans below 0.1% (w/v) concentration were prepared in 0.1N sodium chloride in order to obtain a linear relationship between η_{sp}/c and c, where η_{sp} is the specific viscosity and c is the concentration of the polymer in g per 100 ml of solution.

Measurement of the modulus of rigidity

A parallel plate viscolastometer was used to study the variation of the strain in response to a constant, shear stress with time of the polysaccharide gels. The ratio of the strain to a constant shear stress is known as the creep compliance of the system. The viscoelastometer was based on the one described by Shama & Sherman (1968).

Gels were required with sufficient strength to stand without support and to resist compression by the apparatus. It was found that gels containing 2% (w/v) polysaccharide and 2% (w/v) potassium chloride were ideal. The carrageenan samples were dispersed in deionized water and heated to 80°C, in a water bath, followed by the addition of potassium chloride. Immediately the potassium chloride dissolved, the hot solution was poured into perspex moulds and allowed to gel. The moulds were designed to produce gel samples ideal in shape for the viscoelastometer. Heat processed samples were prepared in a similar manner. The hot solutions were removed from the retort and placed in the moulds to gel. The gels were aged for 24 h at 25°C in a high humidity atmosphere prior to measuring the rigidity modulus, to eliminate any volume change during the course of the experiment. The temperature and humidity were maintained throughout the experiment.

The stress applied to the gels was in the range 100–200 N m^{-2}. Within this range the stress was linearly related to strain showing linear viscoelastic behaviour.

Measurement of gel strength

The gel strengths of the pure carrageenan samples were measured using a FIRA jelly tester. The unprocessed and heat processed carrageenan gels were prepared in the same way as those for the rigidity modulus experiment except that the solutions were allowed to gel in standard FIRA jelly moulds. The gels were aged for 24 h at 25°C in a high humidity atmosphere prior to measuring the gel strength.

Results and discussion

Two components were obtained on fractionation of samples 1 and 2. Only the kappa fractions were used. Fractionation of samples 3 and 4 into kappa and lambda components did not occur on the addition of potassium chloride. This behaviour is explicable in terms of their origin or the processing to which they

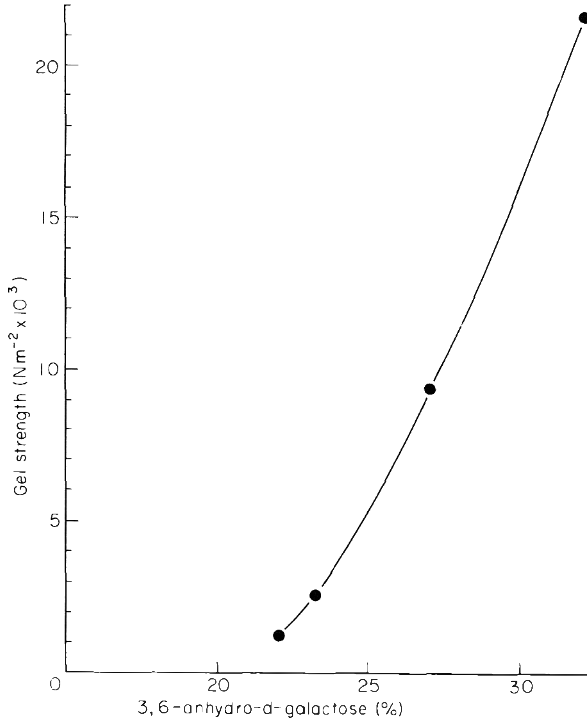

Figure 1. Comparison of gel strength and 3,6-anhydro-D-galactose content of carrageenan samples.

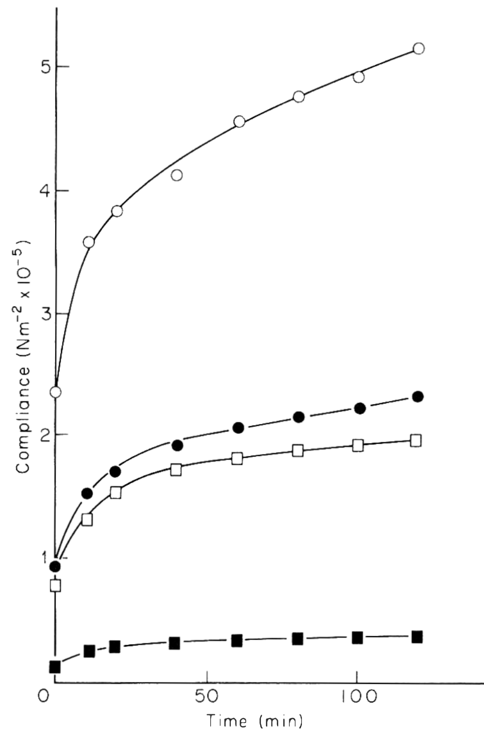

Figure 2. Creep compliance–time curves of carrageenan samples. ○ sample 1; ● sample 2; □ sample 3; ■ sample 4.

have been subjected. Sample 3 is alkali modified sample 1, and since alkali treatment increases the kappa component at the expense of the lambda (Stanley, 1963), it appears that sample 3 is mainly composed of kappa carrageenan. From the apparent strength of the gel of sample 4 on the addition of potassium ions and no fractionation occurring it seems that this sample is mainly composed of kappa carrageenan.

It is evident (Fig. 1) that increasing the 3, 6-anhydro-D-galactose content of the samples results in an increase of the gel strengths. A larger number of 3,6-anhydro-D-galactose unit would allow longer lengths of the polymer to be available for double helix crosslink formation resulting in stronger junctions. Junction zone size therefore appears to be an important contributing factor to gel strength. Information on the sulphate content of the samples is also available. (Ainsworth & Blanshard, 1978).

The shapes of the creep compliance–time curves for all the carrageenan samples are indicative of viscoelastic behaviour (Fig. 2). However, the creep compliance at any time depends on the carrageenan sample, decreasing in magnitude as the strength of the gel increases.

When the gels were subject to a stress an instantaneous deformation was recorded in all cases. If this deformation be represented as a compliance then its reciprocal value is the modulus of instantaneous elasticity. The theory of

Table 2. Relationship between the modulus of rigidity and gel strength of unprocessed carrageenan samples

Sample	Modulus of rigidity ($Nm^{-2} \times 10^3$)	Gel strength ($Nm^{-2} \times 10^3$)
1	0.43	1.20
2	1.09	2.70
3	1.18	9.50
4	7.40	21.40

rubber elasticity predicts that the rigidity modulus of a cross-linked network depends upon the density of the crosslinks and not on the intervening chains (Treloar, 1949). If the theory of rubber elasticity which was derived primarily for random polymer systems is applicable to carrageenan gels, the rigidity will therefore give an indication of the number of crosslinks in the gel. It can be seen in Table 2 that the modulus of rigidity increases with the strength of the gel. However, there is increasing evidence in gels that the rigidity modulus is not an exact indication of strength since gel strength must also depend to some extent on the number and nature of the network polymer chains between the crosslinks. Heat processed carrageenan samples behave in a similar manner to unprocessed samples in that they show creep compliance–time curves for viscoelastic behaviour. Very little change in the rigidity modulus was observed, however, a dramatic fall in gel strength took place (Table 3). If the theory of rubber elasticity holds for carrageenan gels then the density of the crosslinks is unaffected by heat processing, and therefore cannot give an overall indication of gel strength. A significant contribution to strength must be due to the polymer chains connecting the crosslinks.

Intrinsic viscosity experiments (Table 4) showed that hydrolysis of the polymers occurred on heat processing and therefore it is expected that depolymerization of the intervening chains takes place.

It has been suggested that the rigidity modulus of gels is independent of molecular weight above a certain critical value while the breakstrength continues to increase with molecular weight (Mitchell, 1976). The results obtained from heat processed carrageenan samples are consistent with this since the molecular weight of each carrageenan sample is reduced by heat degradation,

Table 3. Relationship between the modulus of rigidity and gel strength of heat processed carrageenan samples

Sample	Modulus of rigidity ($Nm^{-2} \times 10^3$)	Gel strength ($Nm^{-2} \times 10^3$)
1	0.41	0.80
2	1.00	1.30
3	1.16	4.10
4	7.20	10.30

Table 4. Intrinsic viscosities of unprocessed and heat processed carrageenan samples

Sample	Unprocessed	Heat processed
1	6.1	0.5
2	8.6	1.8
3	1.6	0.2
4	3.0	1.0

causing a reduction in gel strength but having very little effect on the rigidity modulus.

On gelation of carrageenan solutions it is expected that crosslinking occurs at random producing network chains of varying lengths, and the difference in the structure of gels formed from unprocessed and heat processed samples will be mainly in the greater number of longer network chains existing in gels of the former type, and these chains influence the gel strength.

From our observations it therefore appears that the density of crosslinks is an important factor contributing especially to the rigidity modulus, but that the network chains also have an extremely important role in giving strength to the gel.

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Measurement of the permeability of chilled meat packaging film under conditions of high humidity

W. J. RIGG

Summary

Modifications to the American Society for Testing and Materials standard method (D1434) for the determination of film oxygen permeability are described. The method gives results consistent with published data and allows the measurement of permeability under likely food storage humidity conditions.

It is recommended that the permeability of hydrophilic materials used for meat packaging should be measured at 100% relative humidity (RH) to permit adequate comparison with other films.

Introduction

The film material for vacuum packaging chilled meat is selected largely by consideration of its permeability to oxygen. Oxygen permeating into packaged meat will allow the growth of aerobic putrefactive bacteria, consequently shortening storage life.

The high RH conditions that occur in meat storage can increase the permeability of hydrophilic materials such as nylon and cellophane, while hydrophobic materials such as polyvinylidene chloride and polyethylene are unaffected. The importance of differing permeability at various humidities has prompted a number of workers to develop a variety of methods for measurement.

Davis (1964) developed a volumetric procedure in which the permeation of water vapour through the film was negligible. This was achieved by maintaining similar water vapour partial pressures on each side of the film. A similar method was used by Pilar (1960) who manometrically measured the gas which permeated through the film. Meyer *et al.* (1957) and Notley (1963) developed methods based on condensing the water vapour that had permeated through to the measuring side of the cell. The quantity of permeating gas was measured by Notley, using thermal conductivity and by Meyer *et al.* using a McLeod gauge.

In this study, modifications to the standard method of the American Society

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for Testing and Materials (ASTM D1434) are described which give reproducible results consistent with published data where this is available.

Materials and methods

The measurement of gas permeability of films in the apparatus used by this laboratory is based on the volumetric method described by Stern, Sinclair & Gareis (1964). In this method the test film is clampled in a stainless steel cell and a constant high pressure is applied to the lower half. Gas permeates through the film into the top side of the cell where it displaces a slug of liquid in a vertical capillary tube of known cross sectional area. The rate of ascent of the slug is monitored and the gas permeability calculated using this data. A detailed description of the apparatus including sources of error and reproducibility has previously been reported, Rigg & Mawson (1976).

The approach in modifying this apparatus was to establish and maintain a microclimate of the desired RH around the film.

To obtain this microclimate one glass fibre filter paper of the same diameter as the test film that is exposed to permeating gas was placed on either side of the test film. The required RH on each side of the film was obtained by soaking each of these filter papers in an appropriate saturated salt solution. The salt solutions used and the RH established by them are shown in Table 1.

Excess solution was removed by pressing the glass filter paper with paper tissues. Before installation in the measuring cell each filter paper was allowed to dry slightly to ensure that the solution within the filter paper was saturated.

The micro climate established in this manner was maintained by sandwiching the test film and filter paper between polyethylene discs of similar size to the test film. Polyethylene has a high oxygen and low water vapour permeability. The 'sandwich' of film and filter paper is shown in Fig. 1.

The additional layers of polyethylene decrease the measured oxygen permeability of the test film to some extent. The sample film and enveloping films can be considered to function as a laminate in which the combined permeability is related to the permeability of the individual layers. Knowing the permeability of the enveloping polyethylene and the combined 'sandwich,' the permeability of the sample film can be calculated using the following

Table 1. Saturated salt solutions used for establishing relative humidity

Saturated salt solution	Relative humidity (25°C)
$Mg(NO_3)_2\ 6H_2O$	53%
NaCl	75%
K_2CrO_4	86%
(H_2O)	(100%)

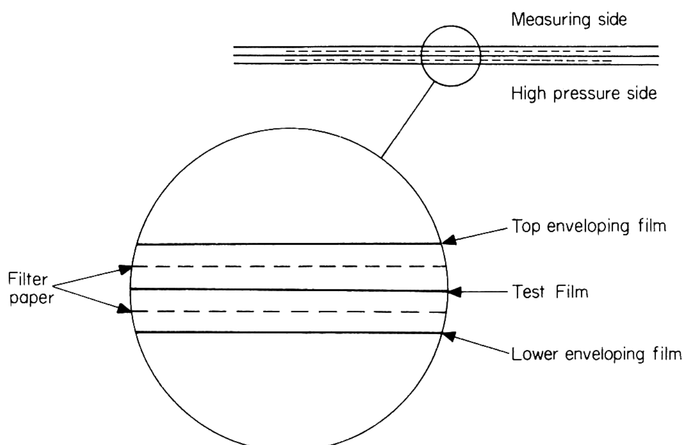

Figure 1. Installation of test film to obtain required relative humidity.

formula.

$$\frac{1}{P_1} = \frac{1}{P_2} + \frac{1}{P_3} + \frac{1}{P_4}$$

where P_1 = measured permeability of test film and enveloping film combined; P_2 = permeability of test film; P_3 + P_4 = permeability of each enveloping film (7000 $cm^3/m^2/24$ hr/Atmos at 25°C).

All results have been corrected by this method.

In considering the migration of water vapour in the 'laminate' of film and filter paper, three distinct water vapour pressure gradients can be distinguished.

Transfer of water vapour across the test film will occur if the RH differs across it. This will simulate the movement of water vapour in the practical situation.

The high pressure side of the enveloping film is at 0% RH therefore loss of water vapour from the bottom paper will occur in that direction. However this does not affect the determination of oxygen permeability of the test film.

Loss of water will also occur through the enveloping film on the measuring side thereby contributing to the vapour pressure as measured by ascent of the capillary liquid. This contribution would be negligible if the gas enclosed by the measuring system were allowed to equilibrate to the same water vapour pressure as that below the top enveloping film. The calculated equilibration time was approximately 10 hr and in practice over 16 hr was allowed.

Results and discussion

The determination of the accuracy of gas permeability measurements is difficult since unlike most analytical procedures no method of standardization is available. Reliance therefore has to be placed on comparing results with published

data for similar film. This can be impracticable since published data are rare and where they do exist they often do not include a complete specification of the film under test. This is further complicated by the variety of test methods and units used.

The reproducibility of permeability measurements under ASTM conditions was found to be dependent upon the time taken for each determination. A long determination time could allow environmental factors such as atmospheric pressure and variations in temperature of $\pm 0.1°C$ allowed by the ASTM method to have effect, so that film of low permeability and consequent long determination time can give the poorest reproducibility. Table 2 shows typical reproducibility of measurements by the modified method under various humidity conditions.

Published data on the effect of humidity on packaging film have concentrated on cellophane (Davis, 1964; Pilar, 1960; Notley, 1963; Kunz & Cornwell, 1962). The results obtained by these workers for nitrocellulose coated cellophane are recorded in Table 3, together with results obtained with the modified method for a film of similar specification. To allow direct comparison between results all have been converted to oxygen transmission rates using $cm^3/m^2/24$ hr/Atmos. units, for a film of 20 μthickness.

In calculating results, Pilar (1969) used a relative permeability constant defined as the ratio of the permeability at a particular RH divided by the permeability at 0% RH. The permeability constant at 0% RH was however not reported, so on the basis of his results this figure was assumed to be 0.25×10^{-16} mole-cm-cm^{-2} sec^{-1} (cm Hg)$^{-1}$. While this figure may not give exact oxygen transmission values any error will be uniform throughout the range of measurements and will indicate the actual trend.

To show the similarity in trends the results from Table 3 are shown graphically in Fig. 2 over the range 40–100% RH.

Table 2. Reproducibility of permeability measurements

Film	Humidity (%)	Permeability*	Standard deviation
Nitro cellulose cellophane	ASTM	33	1
	53	210	7
	75	440	16
	100	2740	9
Nylon 6	ASTM	25	1
	53	34	2
	75	55	1
	100	165	4
Nylon 6/PE	100	44	1
Nylon/Ionomer/PE	100	340	2

*$cm^3/m^2/24$ hr/Atmos at 25°C.

Table 3. Effect of relative humidity on nitrocellulose coated cellophane

Davis		Davis		Notley†		Kunz		Pilar		Rigg	
RH	OTR*	RH	OTR	RH	OTR	RH	OTR	RH	OTR	RH	OTR
0	0.76	0	0.87	20	0.72	43	27	0	15.8	0	13
33	5.6	33	14.9	36	7.2	51	76	40	78.8	53	235
53	19.1	53	34.7	63	72.2	81	259	60	126.1	75	500
75	155	75	192	87	722	93	1075	70	204.9	100	2620
92	1019	92	726	100	2890	98	2061	80	457		
								90	1626		
								100	3057		

*OTR = Oxygen transmission rate in $cm^3/m^2/24$ hr/Atmos at 25°C.
† Film laminated to polyethylene.

The large differences in permeability between the samples observed under standard conditions and higher humidity could possibly result from differences in film composition. For example Davis obtained different values for films A & B which were the same specification but supplied by different manufacturers.

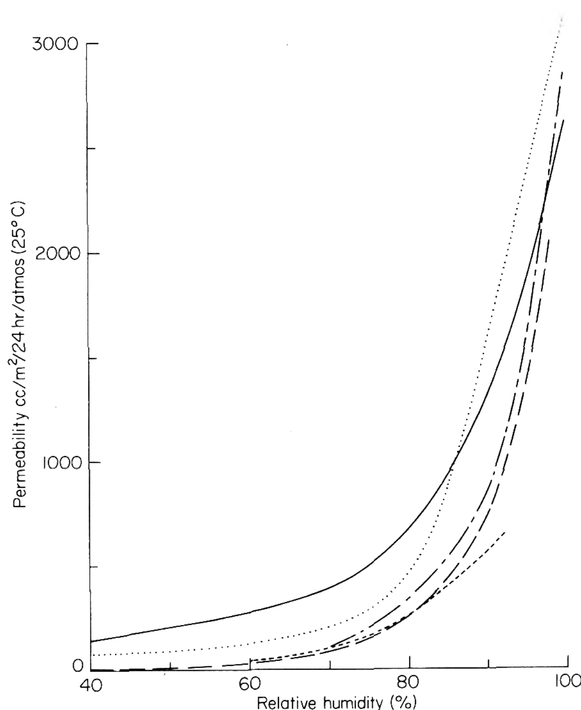

Figure 2. The effect of humidity on the oxygen permeability of cellophane. Modified method (—); Pilar (. . . .); Notley (– · –); Kunz, Davis–film A (– – –); Davis – film B (- - - -).

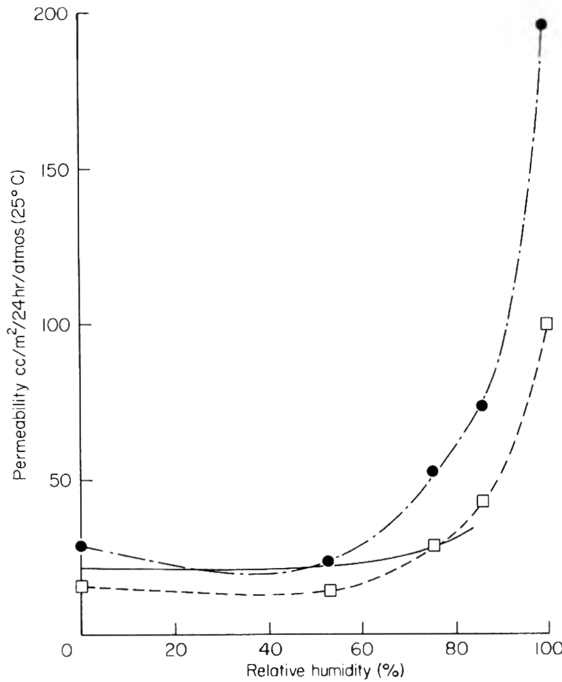

Figure 3. The effect of relative humidity on the oxygen permeability of nylon. Manufacturers results (——); modified method – 28 μm (– – –); modified method – 18 μm (– · –).

No published data on the effect of RH on oxygen permeability were available to confirm the results for nylon. However Meyer *et al.* (1957) found that the permeability of nylon film to carbon dioxide increased 2–3 times when the RH increased from 0 to 100%, while Simril & Herschberger (1950) reported a ten fold permeability increase over the same range. These results may not be directly comparable with the situation for oxygen, since the higher solubility of carbon dioxide in water may have an effect, but they do serve to indicate the range of increases likely to be observed.

The only data available on nylon film with which results from the modified method could be directly compared were supplied by a film manufacturer. The permeability of a film of 25 μm thickness was reported over a range of RH from 0 to approximately 83%.

Permeability measurements of two samples of this film of 28 μm and 18 μm thickness were carried out over a range of humidities. These results are shown in Fig. 3. Although the manufacturer's results terminate at the point of rapid increase in permeability, a similar trend is evident in the three curves.

The rapid increase in permeability observed with cellophane at conditions above 70% RH is also apparent with nylon film.

Conclusions

The modification to the standard method to enable permeability measurement at various RH gives reproducible results.

The difficulties in comparing the results from this method with results from other methods are considerable. However as far as can be ascertained the results obtained give trends consistent with published data.

There is a marked increase in the permeability of nylon and cellophane film over 70% RH. This is the minimum RH likely to be found in meat storage conditions, and 100% is possible. It is recommended that the permeability of hydrophilic materials used for meat packaging should be measured at 100% RH to permit adequate comparison with other films.

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Studies on the vitamin C content of developing pea seeds

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Summary

The effect of maturity on fresh pea weight, dry solids content and vitamin C content has been studied over several years with particular emphasis being placed on the cultivar Dark Skinned Perfection. Regarding the vitamin C content special attention was paid to the partition of the vitamin between the cotyledons and the testa of the developing pea, and also to the contribution made by dehydroascorbic acid to the total vitamin C content. Changes in the fresh pea weight and the level of constituents were found to correlate well with maturity expressed in terms of tenderometer reading. Cultivar has been shown to affect the vitamin C content of the pea and the proportion present as dehydroascorbic acid.

It was postulated that the observed changes in vitamin C content could arise from structural changes occurring in the pea seed during development.

Introduction

The content of a nutrient in a processed vegetable will be influenced by its concentration in the fresh vegetable. The latter will be dependent upon the stage of maturity, which is also an important consideration when selecting vegetables for processing.

The variation in ascorbic acid (AA) content of fresh peas has been shown to be associated with cultivar and stage of maturity at harvest. The term pea is used to refer to a whole seed as removed from the pod, consisting of the testa and enclosed cotyledons, and irrespective of the stage of maturity.

Influence of cultivar

Mack, Tressler & King (1936) compared the AA contents of peas from seventeen cultivars and found variations ranging from 19 mg/100 g fresh weight

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(F.Wt) in large seeded, to 40 mg for small seeded cultivars as harvested. Heinze, Hayden & Wade (1947) reported significant variation in the AA content of peas according to cultivar, and also in the AA content of peas from the same cultivar grown in three successive years. Hybrids produced by crossing cultivars yielded peas with AA contents in the same range as the parent plants in all cases.

Alexander & Feaster (1947) observed that AA contents of peas from cultivars Alaska and Profusion exceeded 60 mg/100 g F.Wt although Lynch, Mitchell & Casimer (1959) stated that the AA contents of peas from cultivars commonly used for food ranged from 19–31 mg/100 g F.Wt. Robertson & Sissons (1966) noted that Dark Skinned Perfection Peas contained more AA than peas from the other cultivars studied, but concluded that more work was necessary to establish whether the differences were entirely due to cultivar. Cultivar studies in India by Lal, Pande & Khanna (1967) showed a similar range of AA contents (32–60 mg/100 g F.Wt) but no maturity criteria were given.

Influence of maturity

It has been noted that the AA content per unit weight decreased as peas matured, and that in a given cultivar the content was inversely proportional to the sieve size of the peas (Mack *et al.*, 1936). Lee & Whitcombe (1945) also reported that AA levels decreased from 38 to 23 mg/100 g F.Wt in cultivar Alderman between tenderometer readings (TR) of 62 and 140 respectively. With cultivar Alaska, Kramer *et al.* (1950) also noted that the AA content decreased as the TR increased from 100 to 200, but with cultivar Thomas Laxton, the content increased up to TR 150 and then declined. As seeds mature, AA may still accumulate although the amount per unit weight declines (McKee, Robertson & Lee, 1955).

Information relating to the distribution of AA between the cotyledons and testa or seed coat is sparse. Todhunter & Sparling (1938) removed the seed coats of blanched, frozen peas for separate analysis and found that the seed coats contained twice as much AA per unit weight as the cotyledons. They concluded that the higher proportion of AA in smaller seeds was due to the higher seed coat/cotyledon ratio. According to Morrison (1972), in cultivar Puget this ratio decreased from 0.53 to 0.35 between TR 87.5 and 114 respectively. It should be noted that the results of Todhunter & Sparling (1938) have been incorrectly quoted in some papers, inferring that the data were for fresh peas.

Early data have not included the dehydroascorbic acid (DHA) present in peas, but both forms of AA were measured by Morrison (1972, 1974) and reported for cultivar Sparkle as 'total' vitamin C. Its content declined from 42 to 29 mg/100 g F.Wt between TR 77.5 and 123.5. Values for the DHA contents were not given, but since the growing seed consists of rapidly metabolizing tissue, it was considered probable that significant amounts might be

present. It was evident that wide variations may occur between cultivars due to effects of season and location.

This paper describes studies involving the determination of both the AA and DHA contents of whole peas and the component testa and cotyledons at various stages of maturity up to and beyond the optimum harvest time for freezing. This work preceded studies made under known and controlled conditions into the mechanism of loss of Vitamin C from peas of one cultivar at a known stage of maturity, during water blanching.

Materials and methods

Growing and sampling

Twenty-one field plots were laid out on a sandy loam soil (pH 5.9–6.3) near Chertsey in Surrey. Dark Skinned Perfection peas were used as the main-crop in 1973, but in 1974 and 1975, two plots were sown with Swan peas, and two with Swift peas. Each plot (3.7 x 9.1 m) was dressed with a low nitrogen fertilizer (N/P/K = 13/13/20) at the rate of 37.7 g m^{-2} in early March. Plots were sown at intervals with enough seed to provide about 2000 plants per plot. Pods at various stages of maturity were collected during the period 29th June to 8th August. These were removed by hand from the first two nodes of randomly selected vines, shelled in the laboratory and graded to provide uniform fresh weight samples of about 20 g, which during the course of the work covered eight stages of maturity. Pea samples at each maturity were subjected to chemical analysis within three hours of picking. Each pea sample was placed in a covered 100 ml beaker, and although no change in the vitamin C content was observed prior to analysis, a 1.5% fresh weight loss was recorded due to water loss from the respiring peas at room temperature.

Determination of vitamin C

The reagents used in the analysis of vitamin C were prepared in the standard manner described by the A.O.A.C. (1970). The vitamin C was extracted from 20 g samples of whole peas by macerating with 50 ml ice cooled 3% meta-phosphoric 8% acetic acid extracting solution for 20 sec using an ultra homogenizer prior to centrifuging at 270 *g* for 4 minutes. Centrifuging was repeated after resuspending the solids in an additional 60 ml extracting solution. The supernatent was filtered through muslin and made up to 200 ml with extracting solution. The AA was then determined by titration with 2, 6-dichlorophenolindophenol dye and the total vitamin C (AA plus DHA) by the fluorimetric method as described by A.O.A.C. (1970). The DHA present was then calculated by subtracting the AA content from the total vitamin C content.

All analyses for the vitamin C content of the component cotyledons and testa were carried out using further 20 g samples of peas. Cotyledons and

testas were separated with the aid of a scalpel (dipped in extracting solution), and extraction carried out as above, but resuspending in an additional 20 ml extracting solution and finally making up each extract to 100 ml. At each maturity stage two replicate samples were analysed for vitamin C content, and the mean value has been recorded.

The timespan during which peas could be usefully harvested was 6–8 weeks in July and August. Thus the amount of experimental work that could be carried out was limited. Therefore, to ensure fulfilment of the proposed study, replication of experiments was kept to a minimum. Variation between pea samples was controlled by careful sample preparation and an estimate was obtained of the compositional variation between samples of peas of similar maturity. Typical variation of the AA and DHA contents between replicate pea samples is shown in Table 1.

Maturity index

For a given cultivar of pea, it has been shown that the alcohol insoluble solids content correlates well with the commercially used maturity index, the TR (Ottosson, 1958). The alcohol insoluble solids were determined on 20 g samples of whole peas using the basic method of Moyer & Holgate (1948). Pea samples were blended with 50 ml 85% ethanol for 30 sec using an ultra turrax homogeniser. The final residue was dried to constant weight in a circulated-air oven at the elevated temperature of 103–105°C, in order to complete the determination on the same day. Correlation of the alcohol insoluble solids content with TR for the cultivar Dark Skinned Perfection, is reported elsewhere (Selman, 1977). At each maturity two replicate samples were analysed for alcohol insoluble solids, and the mean value has been recorded. The typical variation of the alcohol insoluble solids content between replicate pea samples is shown in Table 1.

Determination of dry solids content

The dry solids content was determined by drying 20 g pea samples to constant weight at 103–105°C in a circulated-air oven. In order to reduce the drying time to 5–6 hr, each individual pea was slit with a scalpel. In a second 20 g sample, cotyledons and testas were separated with the aid of a scalpel and the dry solids content of the two component parts was determined after drying by the same procedure.

The typical variation of the dry solids content between replicate samples of whole peas is shown in Table 1. At each maturity two replicate samples were analysed for dry solids, and the mean value has been recorded.

Table 1. Variation of percentage dry solids, percentage alcohol Insoluble solids (AIS) and vitamin C content (mg/100 g fresh weight) between 10 pea samples of approximately uniform maturity, harvested from randomly selected vines from one plot on one day in 1973 and 1974. Pea cultivar – Dark Skinned Perfection

	1973					1974				
			Vitamin C mg/100 g fresh weight					Vitamin C mg/100 g fresh weight		
Sample	Dry solids (%)	AIS (%)	AA	DHA	Total vitamin C	Dry solids (%)	AIS (%)	AA	DHA	Total vitamin C
1	21.01	10.89	32.5	3.3	35.8	21.57	10.82	36.9	3.1	40.0
2	21.29	11.69	30.9	1.5	32.4	21.60	10.92	36.9	2.6	39.5
3	20.97	10.81	31.9	2.2	34.1	21.33	10.88	33.9	1.9	35.8
4	20.95	11.05	30.5	2.4	32.9	21.53	10.92	35.4	2.0	37.4
5	20.85	11.95	30.5	3.0	33.5	21.14	10.93	32.9	3.4	36.3
6	21.35	11.27	31.5	1.8	33.3	21.42	11.01	35.4	3.5	38.9
7	20.94	10.35	30.5	2.5	33.0	21.36	10.82	34.4	3.0	37.4
8	21.02	10.84	30.3	0.7	31.0	21.34	11.01	33.4	3.0	36.4
9	21.37	10.81	30.7	3.8	34.5	21.12	11.17	34.4	2.5	36.9
10	20.69	10.71	32.5	2.7	35.2	20.92	10.69	33.4	5.0	38.4
Mean	21.04	11.04	31.2	2.4	33.6	21.33	10.92	34.7	3.0	37.7
Standard error of mean	0.14	0.11	0.2	–	0.2	0.13	0.07	0.6	–	0.6
Standard deviation	0.44	0.35	0.7	–	0.5	0.41	0.22	1.8	–	1.9
Coefficient of variation	2.09	3.17	2.2	–	1.5	1.92	2.01	5.2	–	5.2

Results and discussion

The objectives of this work were twofold: (i) to follow the changes which take place in the pea as it develops towards botanical maturation, and (ii), to follow the changes in amount and distribution of vitamin C in the developing pea. This included the development of the pea from 0.28 g to 0.69 g F.Wt and corresponding TR 84 to 135. The usual maturity for harvesting peas for freezing is about TR 100, and this is considerably higher for canning.

Data obtained relating to changes in F.Wt and dry solids content is summed up in Table 2. By observation it is apparent that as the pea matures there is a steady increase in its F.Wt, the increase being due largely to the growth of the cotyledons. Consequently the proportion of the testa as part of the whole pea falls from 47 to 25% during development from TR 84 to 135. Such a result is to be expected (McKee *et al.*, 1955). The cotyledons represent the energy reserves for the growth of the embryonic pea plant, and increase in size as sugar and starch are progressively added. The testa largely serves as a protection for the cotyledons and the only increase required is to cover the expanding cotyledons. Though the testa becomes somewhat larger to enclose the growing cotyledons, it does this by changing its composition in terms of a net increase in water content, the dry solids of the testa remaining constant.

During maturation the cotyledons grow in size but the moisture content gradually falls. This may be related to the osmotic pressure within the cells, as sugar is steadily converted to starch during this period. The overall moisture content of the pea did not change so markedly with maturation as the effect was offset by the increasing hydration of the testa. Structural changes in the testa cells have been observed during this period. It has been shown that the inner layer of the testa parenchyma (Fig. 1) undergoes resorption (Reeve, 1946). At the same time secondary thickening of the sclereid cell walls also occurs. The fall in overall moisture content particularly if associated with a

Table 2. Relationship between pea maturity, fresh weight and weight of dry solids. Pea cultivar – Dark Skinned Perfection (1974). (Means from two replicate samples)

Stage of maturity		Fresh weight (g)				Weight of dry solids (g)		
% Alcohol insoluble solids	Tenderometer reading	Whole pea	Cotyledons	Testa	Proportion of testa (%)	Whole pea	Cotyledons	Testa
8.93	84	0.280	0.147	0.133	47.4	0.054	0.025	0.030
9.23	87	0.350	0.217	0.133	38.0	0.068	0.040	0.032
9.56	91	0.430	0.285	0.145	33.8	0.086	0.054	0.032
10.58	100	0.460	0.316	0.144	31.4	0.097	0.065	0.033
11.01	103	0.490	0.346	0.144	29.3	0.105	0.073	0.031
11.54	108	0.510	0.366	0.144	28.3	0.109	0.082	0.029
12.25	115	0.570	0.413	0.157	27.6	0.125	0.094	0.032
14.52	135	0.690	0.517	0.173	25.1	0.159	0.125	0.033

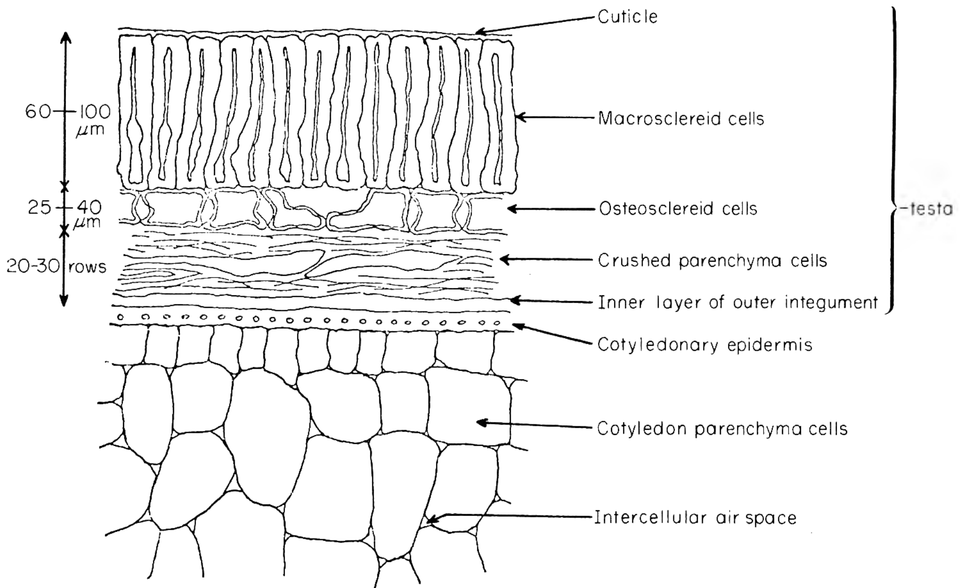

Figure 1. Section through seed coat and portion of cotyledon of a pea, *Pisum sativum*. (After Hayward, 1938.)

concurrent fall in the soluble solids content, suggests that losses of solubles during a process such as blanching will be less the more mature the pea.

Linear regression lines may be fitted to the data in Table 2 to relate F.Wt of the pea to TR in the range TR 84–135 as follows:

F.Wt of whole pea (mg) $a = 7.38x - 287, r = +0.974$
F.Wt of cotyledons (mg) $b = 6.63x - 357, r = +0.970$
F.Wt of testa (mg) $c = 0.74x + 70.1, r = +0.952$

The weight of dry solids may be expressed similarly:

Weight of dry solids in whole pea (mg) $d = 1.93x - 98.6, r = +0.986$
Weight of dry solids in cotyledons (mg) $e = 1.86x - 122, r = +0.988$

Where x = TR and r = product moment correlation coefficient.

Plotting the results in Figs 2 and 3 shows a point of inflexion in the graphs at TR 91. The data indicate that the F.Wt and dry solids content of the cotyledons increase regularly and more rapidly when maturing beyond TR 91, and the effect is reflected in the F.Wt and dry solids content of the whole pea. Regression lines are as follows:

Maturity range: TR 84–91

F.Wt of whole pea (mg) $f = 21.4x - 1510, r = +0.999$
F.Wt of cotyledons (mg) $g = 19.6x - 1490, r = +0.996$
Weight of dry solids in whole pea (mg) $h = 4.57x - 330, r = +0.999$
Weight of dry solids in cotyledons (mg) $i = 4.11x - 319, r = +0.995$

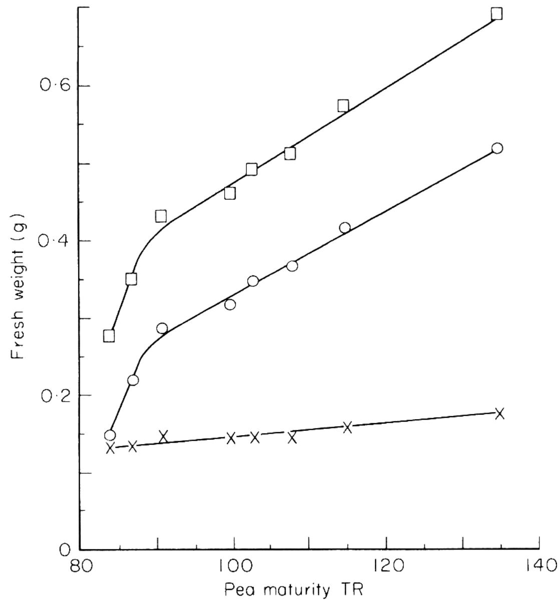

Figure 2. Relationship between pea maturity (TR) and pea fresh weight (g). ⊡ whole pea; ⊙ cotyledons; × testa.

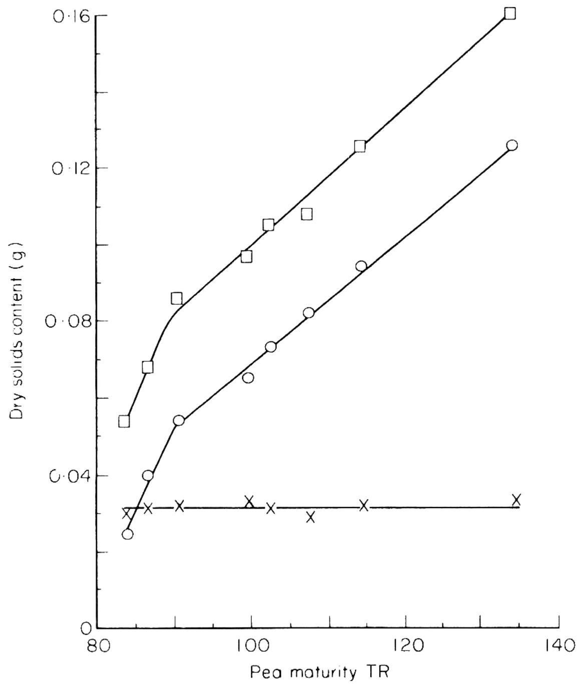

Figure 3. Relationship between pea maturity (TR) and pea dry solids content (g).

Maturity range: TR 91–135

F.Wt of whole pea (mg) $j = 6.14x - 142, r = +0.994$
F.Wt of cotyledons (mg) $k = 5.42x - 215, r = +0.997$
Weight of dry solids in whole pea (mg) $l = 1.69x - 70, r = +0.997$
Weight of dry solids in cotyledons (mg) $m = 1.65x - 97, r = +0.998$

Where x = TR and r = product moment correlation coefficient.

Sample tenderometer readings might thus be used to identify the stage of crop development and its potential yield. It is appreciated that the amount of data on which to postulate two rates of growth is limited and further confirmatory work is needed. However, it could parallel the onset of starch and protein deposition in the cotyledon cells which serve as a food reserve for use in the subsequent germination of the pea seed.

The distribution of the vitamin C content of peas is expressed on a F.Wt basis in Table 3. The concentration of total vitamin C in the fresh tissue of the cotyledons appears to be reasonably constant over the range TR 87–135 (mean value = 28.8 mg total vitamin C/100 g fresh weight, and standard deviation = ± 1.6 mg total vitamin C). The concentration of total vitamin C in the testa falls steadily and progressively with increasing TR, falling from a value approximately double the concentration in the cotyledons, to a value little more than half the original. The net effect on the concentration of total vitamin C in the whole pea is a sum of the above two effects. This resulted in a gradual and regular decrease in total vitamin C of about 30% over the range TR 87–135. There is a very notable difference in the composition of the total vitamin C between the cotyledons and testa. The former contained a small and constant amount of DHA representing less than 10% of the total vitamin C. However

Table 3. Distribution of vitamin C content of peas (mg Vitamin C/100 g fresh weight) between cotyledons and testa at different maturities. Cultivar–Dark Skinned Perfection (1974). (Means from two replicate samples)

	Vitamin C mg/100 g fresh weight								
	Whole pea			Cotyledons			Testa		
Tenderometer reading	AA	DHA	Total vitamin C	AA	DHA	Total vitamin C	AA	DHA	Total vitamin C
84	–	–	56.3	–	–	37.6	–	–	77.0
87	29.7	13.1	42.8	26.0	1.8	27.8	35.7	31.5	67.2
91	30.0	10.1	40.1	28.7	2.2	30.9	32.5	25.7	58.2
100	26.7	11.3	38.0	25.0	3.6	28.6	30.5	27.9	58.4
103	31.0	3.5	34.5	27.3	0	26.5	39.9	13.8	53.7
108	28.6	8.3	36.9	26.1	3.2	29.3	34.8	21.5	56.3
115	29.0	4.5	33.5	28.5	2.5	31.0	30.2	10.0	40.2
135	27.5	2.9	30.4	26.2	1.3	27.5	31.3	7.6	38.9

Table 4. Distribution of vitamin C content of peas (mg vitamin C/pea) between cotyledons and testa, at different maturities. Cultivar–Dark Skinned Perfection (1974). (Means from two replicate samples)

	Vitamin C mg/pea								
	Whole pea			Cotyledons			Testa		
Tenderometer reading	AA	DHA	Total vitamin C	AA	DHA	Total vitamin C	AA	DHA	Total vitamin C
84	–	–	0.158	–	–	0.055	–	–	0.102
87	0.104	0.046	0.150	0.056	0.004	0.060	0.047	0.042	0.089
91	0.129	0.043	0.172	0.082	0.006	0.088	0.047	0.037	0.084
100	0.123	0.052	0.175	0.079	0.011	0.090	0.044	0.040	0.084
103	0.152	0.017	0.169	0.094	0	0.092	0.057	0.020	0.077
108	0.146	0.042	0.188	0.096	0.011	0.107	0.050	0.031	0.081
115	0.165	0.026	0.191	0.118	0.010	0.128	0.047	0.016	0.063
135	0.190	0.026	0.210	0.135	0.007	0.142	0.054	0.013	0.067

Data refer to estimations on 20 g samples of peas. Results from these have been divided by the number of peas in each sample to give the above figures per pea.

the testa tissues contained DHA amounting to nearly 50% of the total vitamin C in the less mature peas (TR 87), but thereafter the proportion of DHA falls to about 20% of the total vitamin C over this maturation period.

In Table 4 data on the distribution and amount of vitamin C are reported on a 'per pea' basis. The results reflect both the change in concentration of vitamin C in the component tissues and the enlargement with development. The amount of total vitamin C in the cotyledons increases about threefold during this period, the amount of DHA present being variable and comprising no more than 10% of the total vitamin C. The total vitamin C present in the testa was initially double that found in the cotyledons, but this progressively decreased with increasing maturity to about 50% of that found in the cotyledons at TR 135. The high proportion of DHA in the testa is reflected in the proportion of DHA present in the whole pea. Linear regression lines may be fitted to the data in Table 4 to relate the total vitamin C content of the pea to TR in the range TR 84–135 as follows:

Total vitamin C in whole pea (mg) $n = 0.00109x + 0.0646, r = +0.948$
Total vitamin C in cotyledons (mg) $p = 0.00172x - 0.0819, r = +0.957$
Total vitamin C in testa (mg) $q = 0.00063x + 0.1457, r = -0.859$

Where x = TR and r = product moment correlation coefficient.

The presence of relatively high concentrations of AA in tissues, such as those in developing seeds which are undergoing rapid metabolism, has previously been noted but has not been fully explained (Sebrell & Harris 1967). The

presence of or association of the enzyme ascorbic acid oxidase with the cell wall has suggested that the oxidation of AA to DHA and the resulting electron transfer may be necessary to the metabolic pathways associated with cell wall elongation and cell division (Hallaway, Phethean & Taggart, 1970). At the time of optimum maturity for freezing, rapid growth of the cotyledon parenchyma cells is occurring largely by cell expansion. The results thus appear to be in agreement with such a postulated function of AA in the growth and development of the cell wall. At the same time, the sclereid cells of the testa (Fig. 1) continue to enlarge, thus accommodating the growth of the cotyledons, the sclereid cell walls being in a plastic condition. However, the AA content of the testa remained nearly constant whilst the DHA tended to decrease from a relatively high value. The presence of relatively higher quantities of DHA in the testa particularly during the early development of the pea, suggests that there is greater metabolic activity in the testa and/or that some at least of the metabolic pathways are different in the testa. It would appear desirable to seek some explanation based on the metabolism and known development of the pea.

Two major changes in the structure of the testa appear to occur during the development of the pea seed. Firstly, the cells of the inner layers of the parenchyma tissue of the testa undergo a degree of resorption during development towards the optimum freezing maturity, and secondly, secondary development of the sclereids in the form of deposition of hemicelluloses on the cell walls takes place, the rate of deposition increasing after the optimum freezing maturity. Such changes are those already identified by Reeve (1946) to occur in peas, although Dark Skinned Perfection peas may exhibit these changes at slightly different maturities to the peas studied by Reeve. The high content of DHA in the testa could be associated with either or both of these processes. Both the processes are concerned with changes in the cell wall, either with the breakdown of parenchyma cells, or the build-up of secondary thickening. The content of DHA is, however, highest in the most immature peas, and therefore it is more probable that the DHA observed in the testa tissues is associated with the breakdown of testa parenchyma cells during the resorption process. An example of a metabolic pathway associated with cell wall breakdown and involving AA, is the catabolism of the amino acid tyrosine (Dagley & Nicholson, 1970) which is known to be present in the cell wall (Bonner & Varner, 1965). The high DHA content in the testa could be explained in part if such catabolism was occurring during cell resorption. If this was the case, then either the rate of oxidation of AA in a given reaction must be greater in the testa cells, or there are more metabolic pathways involving the oxidation of AA.

In view of the higher DHA content in the testa tissues, it was anticipated that the activity of the enzyme ascorbic acid oxidase would be greater in the testa tissue. However, simple tests showed that in 1973 peas, the ascorbic acid oxidase activity was 1.6 times greater in the cotyledons, whereas the activity of peroxidase enzymes was approximately twice as great in the testa tissue (Selman, 1977). It is most probable that the higher peroxidase activity in the

Table 5. Comparison of the distribution of vitamin C between cotyledons and testa of three pea cultivars (fresh weight basis). (Means from two replicate samples)

Cultivar	Tissue	Alcohol insoluble solids (AIS) (%)	Tenderometer reading (TR)	Proportion of testa as % fresh pea weight	Dry solids content of whole seed (%)	Vitamin C mg/100 g fresh weight		
						AA	DHA	Total vitamin C
Dark Skinned Perfection (1973)	Whole pea	11.13	105		21.3	30.6	2.3	32.9
	Cotyledons					22.4	2.2	24.2
	Testa			29.9		49.9	3.3	53.2
Dark Skinned Perfection (1974)	Whole pea	11.01	103		21.3	31.0	3.5	34.5
	Cotyledons					27.3	0	26.5
	Testa			29.3		39.9	13.8	53.7
Swan (1975)	Whole pea	11.34	106*		22.5	26.7	24.0	50.7
	Cotyledons					25.7	15.3	41.0
	Testa			25.2		28.5	41.2	69.7
Swift (1975)	Whole pea	12.68	118*		23.1	29.1	14.0	43.1
	Cotyledons					24.7	9.7	34.4
	Testa			27.6		26.5	36.4	62.9

*Based on AIS/TR correlation for Dark Skinned Perfection peas.

testa tissue may be directly linked with the higher DHA content observed in the testa, as the presence of peroxidase is known to be a requirement for the continued function of ascorbic acid oxidase *in vivo,* which may be inhibited eventually by the hydrogen peroxide produced during the oxidation of AA. It has also been shown that the structural changes occurring in the development of the testa appear to reduce the oxygen content of the cotyledon tissues (Wager, 1957). The observed changes in the amount of DHA in the testa suggest that oxidative reactions that might give rise to DHA can proceed more readily in the testa. This could be due to the availability of oxygen, lack of protective substances or different enzyme systems.

Table 5 shows the concentrations of vitamin C in the cotyledons and testa of Dark Skinned Perfection peas harvested at the optimum harvest time for freezing in 1973 and 1974. Although the fresh weight proportions of cotyledon and testa were similar in both years, due to differences in concentration, distribution of vitamin C between the cotyledons and testa was different. Peas harvested in 1974 had a relatively greater amount of AA in the cotyledons, the amount being 62.3% of the AA in the whole pea as compared to 51.3% in 1973. However, examination of the amount of total vitamin C in the peas for both years shows that the distribution of total vitamin C between the testa and cotyledons was very similar in both years. The anomaly is the difference in the amount of DHA present in the testa of the peas from the two years. It is difficult to provide an explanation for this anomaly (such as the effect of growth environment) because although the peas were of the same cultivar, the seed for each year came from a different source.

The AA concentrations in the whole pea of the cultivars Swan & Swift were similar to those of Dark Skinned Perfection, but the DHA concentrations were significantly higher. Consequently, the concentrations of total vitamin C were also higher. The high concentrations of DHA observed in Swan and Swift peas, are so much greater than those observed between Dark Skinned Perfection peas grown in 1973 and 1974, that it would seem that environment is only exerting a relatively minor effect, and therefore it is most probable that the cultivar accounts for these differences.

Conclusions

It has been shown that the changes in F.Wt and dry solids content of Dark Skinned Perfection peas, observed over two consecutive years were related directly to the maturation of the pea seed as expressed by TR. There is an indication of a point of inflexion in this relationship at about TR 91, at higher values both factors appear to increase more rapidly with TR. This may be explained perhaps by the onset of starch and protein deposition in the cotyledons.

Peas of one cultivar, i.e. Dark Skinned Perfection, picked at an approximately uniform standard of maturity and harvested at random from the plots of pea vines, showed a remarkable uniformity for dry solids content, AA and

total vitamin C content in any one year, but when comparing peas grown in 1973 with those in 1974, the latter contained a somewhat higher content of AA. The DHA content was similar for both years and in general represented less than 10% of the AA content.

The contribution made by DHA to the total vitamin C content (AA + DHA) in peas is apparently very dependent on cultivar, and for Swan and Swift peas, both grown in 1975, the contribution was approximately 50% and 30% respectively.

Cultivar therefore has a very marked effect on total vitamin C content of peas and the proportion contributed by DHA can be substantial. When considering the vitamin C content of peas it is essential that due attention is paid to the estimation of DHA. The effect of the environmental conditions experienced over the years during which this work was carried out on pea composition was small. This is perhaps not surprising as the crops of peas were grown in the same area so that environmental changes e.g. climate and cultivation, were minimal. The results may well have been different had similar experiments been carried out on different soil in a region with a different climate.

Assuming mechanical harvesting of peas, the yield per vine (and therefore also per unit area) will obviously increase as the peas are allowed to advance in maturity. The data reported here linking maturity with fresh pea weight, dry solids content and total vitamin C provide a means for calculating an indication of the increased yield of peas, dry solids and of vitamin C to be obtained by deferring harvesting to a particular TR. Unfortunately only limited advantage can be taken of this information, as the major concern of the consumer, and consequently of industry, is the quality of the pea expressed in terms of tenderness, and not in the total yield of nutrients.

Marked changes in the AA and DHA contents occurred during the development of Dark Skinned Perfection peas, the concentration of total vitamin C falling to one half its value as the peas mature from TR 84 to 135. Against this with the increase in size of the pea as it matured, the amount of total vitamin C per pea increases. A substantial proportion of the vitamin C activity resides in the testa, and it is possible that this fact is significant during processing, e.g. blanching in hot water, where the vitamin C in the testa can be expected to be lost more readily through extraction and oxidation as compared with the vitamin C in the cotyledons.

To sum up, this study has demonstrated the importance of cultivar and maturity in relation to the nutrients in the pea and has indicated their relevance to pea processing operations. DHA can provide a substantial proportion of the total vitamin C in the pea and it should not be over-looked when estimating total vitamin C content.

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Energy consumption during cooking

ROGER COLLISON

Summary

An analysis has been made of the energy expended in the cooking of a number of different foods in an electric forced-convection oven. Out of the total energy used of 1.4 to 1.7 MJ per kg of food, 0.43 to 0.72 MJ was actually absorbed by the food. Most of the balance was lost to the environment as heat. These results are discussed in terms of energy conservation.

A feature of the results is the close similarity between the energy absorbed by the food, and the sum of the sensible and latent heats.

Introduction

Since the oil crisis of 1973, there has been a sharp rise in the cost of energy, plus an increased awareness of the implications of the accelerating depletion of the world's fossil fuel resources. This has stimulated a series of investigations into the utilization of energy in many industries, including those concerned with the production, processing and culinary preparation of food.

It has been estimated (Leach, 1976) that the total input of energy into agriculture in the United Kingdom amounts to 378 PJ or about 5% of our national energy expenditure. As well as direct inputs such as tractor fuels, this figure also includes indirect inputs such as the energy used to manufacture fertilizers. A further 6.4% of the national energy bill is used in the food processing industry; this includes direct inputs for freezing, dehydrating and canning food as well as a host of indirect inputs such as transport and the manufacture of packaging materials (Leach, 1976).

Less emphasis has been given to measuring the expenditure of energy in kitchens. Singer, Smart & Hunt (1976) have estimated that the direct energy inputs into domestic kitchens and catering establishments in the U.K. amounts to 448.6 PJ. This includes cooking, transport, kettles, refrigerators, freezers

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and dish washers. Out of this figure, 250 PJ or 3.3% of the total U.K. consumption is actually used for cooking purposes. These authors have estimated that an electric cooker is on average about twice as successful in utilizing supplied energy as its gas counterpart. They also show, by considering the data for gas cooking alone and ignoring electricity, that cooking in catering establishments consumes about 6.5 times the gas per meal compared with home cooking.

In considering the efficiency of the cooking process, it is important to know the minimum energy needed for cooking under different conditions. Taylor (1977) with electrical equipment, has used as little as 0.7 to 2.2 MJ per kg of food employing continuous batch cooking with optimum food loads for good efficiency. Collison & Wilson (1977) have taken this a stage further by measuring the heat absorbed by potatoes during cooking; they found that to a first approximation the heat absorbed was equal to the sum of the sensible and latent heats and was about 0.6 MJ per kg of potatoes for baking and about 1.2 MJ per kg during frying when more water is lost. In the present paper, the experimental technique has been refined and the method is extended to a wider range of foods. Comparison of the heat absorbed by the food with the energy supplied to the cooker provides a means of assessing the efficiency of utilizing energy during cooking.

Materials and methods

Cooking methods

All the food was cooked in a forced-convection oven (Blodgett Zephaire) with a rated power of 5.6 kW. Provided it had been switched on for an hour or so, this oven was effective at maintaining a state of near-thermal-equilibrium with the surroundings; this was essential for making the measurements necessary for this work.

Details, including cooking times and oven temperature settings, are as follows:

Small sponge cakes, average weight 36 g were baked in bun cases on a flat metal baking tray at 193°C for 7 min 32 sec.

Pork sausages were cooked on metal trays at 177°C for 16 min 28 sec.

Individual Yorkshire pudding, average weight 33 g, were baked on special metal trays at 204°C for 21 min 32 sec.

Pieces of cod fillet were cooked in 320 g water on a porcelain dish at 177°C for 8 min 22 sec.

Potatoes were baked unpeeled at 232°C for 4 min 23 sec.

The conditions were similar to those pertaining in practical situations, although the finer points of culinary detail were sometimes sacrificed for accuracy of measurement. After the food was introduced to the oven, the

doors were left closed until the food was removed after cooking, to prevent complicating the heat losses.

Calculation of energy consumption

The oven temperature was controlled by a 'snap-action' thermostat. During cooking, the thermostat light was alternatively on and off, corresponding respectively to the consumptions of full or zero heating power. The energy consumed was measured by a kilowatt-hour meter, and also by multiplying the measured power loading of the oven by the time the thermostat light was on. (Corrections were made for the electricity used by the fan.) The two methods gave results which were in close agreement.

In order to obtain a balance between the times when the thermostat was 'on' and 'off', cooking times were adjusted so that the food was introduced and withdrawn from the oven at the same stage of the thermostat 'on-off' cycle; for convenience this was taken as the point where the light went on.

Calculation of sensible and latent heats

The sensible heat is the product of the total load, the average temperature rise, and the specific heat. The average temperature rise was measured by taking thermocouple readings at representative positions in the food immediately before and after cooking. Specific heats were taken from the literature (Charm, 1963; Morley, 1972) or by using the formula (Elson, 1977).

$$Cp = 4.18\,(1.0\,W + 0.5\,F + 0.3\,S)$$

This gives the specific heat in $Jg^{-1}\,^{\circ}C^{-1}$ where W, F and S are respectively the weight fractions of water, fat, and solid.

The latent heat is the product of the weight loss during cooking (in kg) and the latent heat of water. Calculations were made using steam tables.

Results and discussion

Efficiency of cooking process

The data in Table 1 refer to the energy consumption while the food was in the oven, and does not include the preheat energy.

The total energy used during cooking is broken down into four parts:

(1) Environmental losses through the oven walls and door. This is the energy which is required to maintain the oven at its temperature setting without a food load.

(2) 'Open-door' losses which occur while the oven is being loaded with food. These are measured in separate experiments with no food load; the energy required to restore the oven to its temperature setting is measured after the door is opened for the same length of time as in loading.

(3) The heat absorbed by the food tray or dish is calculated from a knowledge of their specific heats and assuming that they are heated to the oven temperature.

(4) The remaining heat which is absorbed by the food.

The results show that the heat absorbed per kg of food varies between 0.43 and 0.72 MJ. This represents between 31 and 44% of the total energy used during cooking. Rather more energy, between 43 and 62% of the total energy was lost to the environment as waste heat.

The efficiency of cooking in terms of energy utilization also depends on the preheat energy. Table 2 gives the preheat energies and environmental losses for two ovens of similar internal volume, but differing in that oven A is of very light construction and has double-glazed glass windows in the two doors, whereas oven B is of very heavy construction and has no windows. Not surprisingly the heavier oven (B) requires more preheat energy, but the subsequent rate of loss of heat to the environment is lower. It is not possible to generalize from these figures as to which oven is more efficient, because the two factors concerned are in opposition. The ideal oven, in terms of energy efficiency, would combine good thermal efficiency with a low preheat energy. It is interesting to note that the lighter oven (A) with the glass windows had its environmental losses at 121°C reduced from 7.92 to 4.15 MJ per hour, by lagging the outside of the windows with asbestos cloth.

Heat changes during cooking

During cooking, three basic changes occur in the food: the temperature is raised, a complex series of physico-chemical reactions take place, and very often water is lost. The overall heat absorbed by the food during cooking consists of three corresponding terms:

sensible heat + net heat of reaction + latent heat.

In practice, sensible heat determinations are derived from specific heat measurements, which in the case of food will include the heat of any reactions which may take place. It therefore follows that:

heat absorbed during cooking = sensible heat + latent heat.

The results presented in Table 1 substantiate this relationship remarkably well in view of the nature of these experiments, and the fact that specific heat data do not always apply accurately over a wide range of temperatures. Provided that specific heat data are available, it is a simple matter to measure the

Table 1. Analysis of energy utilization during cooking. Energy values are expressed in MJ per kg of initial food load

Commodity	Initial load (g)	Total energy used during cooking	Environmental losses	'Open door' losses	Energy absorbed by tray or dish	Energy absorbed by food	Energy absorbed by food (%)	Environmental losses (%)	Sensible and latent heat
Small sponge cakes	1825	1.38	0.62	0.09	0.15	0.52	38	45	0.47
Yorkshire pudding	3930	1.24	0.59	0.06	0.06	0.53	43	48	0.53
Pork sausages	2060	1.59	0.75	0.06	0.20	0.58	36	47	0.68
Cod	2000	1.40	0.87	0.07	0.03	0.43	31	62	0.38
Potatoes	3000	1.70	0.87	0.21	0	0.72	42	51	0.70

Table 2. Thermal characteristics of two ovens as a function of temperature setting

Characteristic	Oven A	Oven B
Internal capacity (m^3)	0.19	0.20
Rated electricity power (kW)	11	6.4
Temperature setting (121°C)		
Preheat energy (MJ)	3.96	6.14
Environmental losses (MJ/hr)	7.92*	3.41
Temperature setting (177°C)		
Preheat energy (MJ)	6.71	9.41
Environmental losses (MJ/hr)	12.3	10.9
Temperature setting (232°C)		
Preheat energy (MJ)	9.90	12.8
Environmental losses (MJ/hr)	16.8	11.0
Temperature setting (288°C)		
Preheat energy (MJ)	14.2	17.7
Environmental losses (MH/hr)	19.5	14.9

*This was reduced to 4.15 MJ/hr by lagging the outside of the door windows with asbestos cloth.

efficiency of energy utilization for any cooking process by comparing the sum of the sensible and latent heat changes with the total energy supplied to the cooker.

Conclusions

It has been estimated that the heat absorbed by a variety of foods when cooked in an electric forced-convection oven lies in the range of 0.43 to 0.72 MJ per kg of food. This represents between 31% and 44% of the electrical energy supplied to the oven during cooking. Most of the remaining energy is lost to the environment as heat, and clearly there is scope for increasing the efficiency of energy utilization during cooking by improvements in thermal insulation.

A good relationship was found between the heat absorbed by the food and the sum of the sensible and latent heats. This relationship makes it possible to measure the efficiency of energy utilization for any cooking process, provided reliable specific heat data are available.

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Air thawing of lamb carcasses

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Summary

The effect of air temperatures, velocities and wrapping on the thawing time, weight change, appearance and change in bacterial numbers was studied on 20 kg lamb carcasses. The optimal condition for thawing wrapped lambs in 24 h was to use air at 10°C and 0.75 m/s and for unwrapped lambs 7.5°C and 0.75 m/s. Changes in bacterial numbers for these conditions were insignificant.

Introduction

The majority of frozen lamb sold in the United Kingdom is imported, principally from New Zealand, but the marketing requirements of the European Economic Community have led to a reduction in those supplies and it is estimated that imports will fall by 10 000 tonnes (5%) during 1978 (Meat and Livestock Commission, 1977). Replacement of this shortfall by home-produced chilled lamb is limited by the seasonal nature of production, and the period of availability can only be extended by frozen storage. Joints from frozen lambs are prepared either by band-sawing, which produces crudely cut pieces, or by thawing followed by conventional butchery. Since the latter is generally preferred by both the meat trade and the consumer, particularly for the sale of home produced frozen meat, there is a demand for data on thawing methods that will produce lamb comparable in appearance and quality to that of chilled meat.

Available thawing methods depend either on the thermal properties of the foodstuff and heat conduction from the surface, or on their dielectric or resistive properties. Electrical methods such as resistance, dielectric and microwave thawing are generally unsuitable for carcass meat because the irregular shape of the product creates problems in the application of parallel electrodes, and inhomogeneities in composition and initial meat temperature produce

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uneven heating (Bailey, 1975). Heat conduction methods which use convection from air, water or steam at sub-atmospheric pressure are not constrained by carcass shape and are therefore more often used. Earlier work on thawing pork by vacuum steam-heat and water (Bailey *et al.*, 1974) and beef by vacuum steam-heat (James & Creed, 1977 unpublished data) has demonstrated that the poor surface appearance produced by wet thawing methods would not satisfy the basic requirement of appearance equivalent to the chilled product. In addition the capital and running costs of such plants are high and operation difficult when compared with thawing in air, which can often utilize existing refrigeration equipment.

It is important that bacterial growth during thawing be minimized to ensure an acceptable shelf life when the thawed product is subsequently retailed, and the multiplication of food poisoning bacteria which may have been present on the carcass before freezing is prevented. There are no regulations in the United Kingdom which relate to the thawing of frozen meat, but legislation has been implemented in France (Ministère de l'Agriculture, 1974) and applies to all thawing systems using a medium temperature above 4°C, and therefore includes the majority of existing systems. It requires that there must be no more than a tenfold increase in the bacterial count, and the meat surface not rise above 10°C for longer than 10 h, during the thawing process.

In the only published work on thawing lamb in large joints or carcass form, Vanichseni (1971) used air, and a combination of water and air to process lamb shoulders for subsequent boning. In air at 20°C and 0.2 m/s the thawing time of unwrapped shoulders was 8 h, and increased to 60 h in air at 2°C and 0.1 m/s. A high relative humidity was maintained to minimize weight loss and no significant changes in bacterial numbers were recorded on the small number of samples tested. Insufficient experimental data were given for other air temperatures or velocities and little information was provided on carcass appearance. The effect of carcass wrapping was not investigated.

This investigation has established the environmental conditions for thawing wrapped and unwrapped lamb carcasses in air which produce meat of sufficiently good appearance and bacteriological quality to be sold in the thawed state. A relationship has also been derived which allows thawing time to be predicted for a range of air temperatures.

Materials and methods

Meat

Seventy-two lamb carcasses of 17 to 22 kg (medium commercial weight range) were obtained on or the day after slaughter. The carcasses were frozen, either wrapped in stockinette cloth or unwrapped, in air at −30°C and 1 m/s and stored at −30°C for an average of 16 days before thawing.

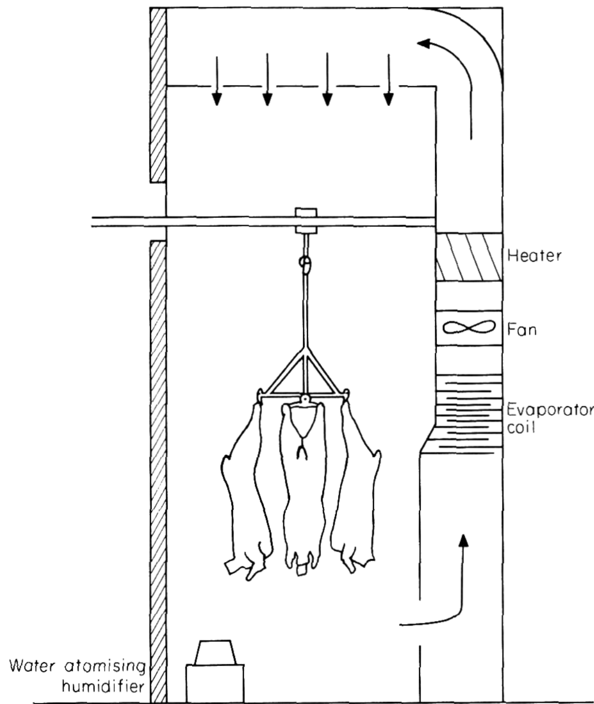

Figure 1. Experimental room for lamb carcass thawing. Arrows indicate direction of air flow.

Equipment

The lambs were thawed in a specially designed room (Fig. 1) in which the air velocity was set at 0.75 m/s ±0.25 m/s over the whole cross-section or 2.25 m/s ±0.25 m/s over half of the room. At the low velocity four lambs were thawed at the same time, but only two at the high velocity. Air temperature was controlled at 5, 10 or 20°C ±0.5°C using a mercury-contact thermometer, and relative humidity maintained at 85% ± 5% using a water atomizer and a 'Danfoss' humidity sensor.

Measurements

The measurements described under the headings 'Temperature' and 'Environmental conditions' were recorded as voltage outputs on a 'Solartron' computer-controlled data-logging system.

Temperature

Multi-junction thermocouple probes, consisting of five copper–constantan thermocouples accurate to ±0.5°C, bonded at 1 cm intervals to a composite rod

(5 mm in diameter) were inserted into the leg and shoulder of the lamb before freezing. The probe position locating the thermal centre of the leg was found by preliminary experiments to be at right angles to the thickest part of the leg, inserting to a depth of 9 cm. The thermal centre of the shoulder was found by inserting the probe under the foreleg downwards towards the neck to a depth of 12 cm. Towards the end of each experiment the meat temperature in the leg and shoulder was checked with a hypodermic thermocouple probe to ensure that the thermal centre had been located. If a lower temperature was found measurements were continued until this temperature had risen to 0°C. The surface temperature of a 7 cm^2 area of the carcass surface was recorded to ±2°C using a 'Heimann' radiation thermometer. These areas were either on the leg, breast or shoulder of one lamb during each run so that typical surface temperature curves for each area were obtained for each of the twelve thawing conditions.

Environmental conditions

The air velocity was measured to ±0.1 m/s using 'Wallac' hot-wire anemometers located halfway down the carcass at 10 cm from the surface of the meat and at 15 cm above the carcass. Relative humidity was measured to ±2% r.h. using a 'Hygrodynamics' lithium chloride sensor 1 m above the carcasses, and air temperature to ±0.5°C using copper–constantan thermocouples located 1 m above the carcasses.

Weight

The lambs were weighed to ±20 g before and after thawing.

Bacteriology

An area of 50 cm^2 on the breast of the left-hand side of the lamb was sampled immediately before freezing and the right-hand side treated similarly after thawing. The area, defined by a sterile metal template was swabbed with wet and then with dry cotton wool swabs which were bulked to form a composite sample, shaken with a saline/peptone diluent (peptone 0.1%/NaCl 0.9%) and dilutions prepared for plating.

Total viable counts were obtained by spreading duplicate 0.02 ml drops of decimal dilutions on the surface of Plate Count Agar (Oxoid) + 1% NaCl and incubating at 25°C for 4 days. A presumptive coliform count was made by spreading 0.1 ml of the swab suspension on to MacConkey Agar without salt (Oxoid CM7B) and incubating at 35°C for 24 h. All plates were previously dried at 60°C for 20 min.

Appearance

The appearance and colour of the fat and lean surfaces of the carcasses was noted immediately after thawing and after subsequent chill-room storage.

Results

The effect of air temperature, air velocity and wrapping on carcass thawing time

Thawing time was defined as the time taken for the thermal centre of the leg or shoulder to reach 0°C from the initial frozen temperature of –30°C. The shoulders thawed more slowly than the legs in forty-eight of the seventy-two runs because they were slightly thicker than the legs.

Air temperature had the greatest effect on thawing time (Table 1); an increase from 5 to 10°C produced almost a 50% reduction in thawing time. Wrapping the carcass in stockinette significantly increased ($P<.05$) the thawing time under the same conditions by an average of 26%. Increasing the air velocity by a factor of three produced small reductions in thawing time which were only significant ($P<.01$) at 5 and 10°C for unwrapped and at 5°C for wrapped lambs.

Table 1. Mean weights and thawing times for groups of six lambs thawed at 5, 10 and 20°C, wrapped and unwrapped at low and high air velocities

Thawing temperature (°C)		Air velocity	Mean weight (kg)	Mean thawing time (–30–0°C) (h)		
				Leg	Shoulder	Longest*
5	Unwrapped	Low	19.88	30.64	30.15	32.10
		High	19.97	27.93	25.43	29.01
	Wrapped	Low	19.89	37.62	39.97	40.37
		High	19.69	33.80	36.18	37.00
10	Unwrapped	Low	19.99	16.53	18.04	18.47
		High	19.82	14.77	14.32	15.28
	Wrapped	Low	19.01	21.67	17.54	22.05
		High	19.92	18.25	20.06	20.08
20	Unwrapped	Low	20.10	10.46†	12.11†	11.84
		High	19.90	8.91	10.70	10.88
	Wrapped	Low	19.97	12.64	14.81	14.87
		High	20.01	10.62	13.43	13.96
Least significant difference between means in each column (P = 0.05)			1.78	2.76	3.30	2.14

*Values in this column are the mean of the longest individual thawing times for each group of carcasses.

†Mean of five samples.

Table 2. The mean percentage weight changes of groups of six lambs thawed at 5, 10, 20°C, 0.75 or 2.25 m/s, wrapped or unwrapped

Thawing temperature (°C)	Low air velocity		High air velocity	
	Wrapped	Unwrapped	Wrapped	Unwrapped
5	0.2	−0.6	0.2	−0.6
10	1.1	0.3	1.0	0.1
20	1.2	0.3	0.6	−0.6

Least significant difference between means ($P = .01$) is 0.57.

The relationship between the longest mean thawing time (Table 1) and the thawing temperature was obtained by using a least-squares curve fit technique (Fig. 2 and appendix).

The effect of air temperature, air velocity and wrapping on weight change

Wrapped lambs gained between 0.2 and 1.2% in weight during thawing (Table 2), the gain being significantly higher ($P<.01$) at 10°C than at 5°C with no further increase when thawing at 20°C. Unwrapped lambs gained between 0.1 and 0.3% at 10°C, 0.75 m/s, 20°C, 0.75 m/s and at 10°C, 2.25 m/s, and lost 0.6% under all other conditions. Increasing the air velocity had little effect on weight change.

The effect of air temperature, air velocity and wrapping on bacterial numbers

Mean bacterial counts before freezing and after thawing and their significance are listed in Table 3. Since significant differences ($P<.05$) were recorded between the means of total viable counts on the fresh lambs, the difference between the thawed and fresh counts was used to determine the effect of thawing treatment. Counts of presumptive coliforms were rarely obtained and in view of these low numbers no statistical analyses were made. The presumptive coliform count exceeded $5/cm^2$ only once on fresh carcasses and was always within the range 1 to $3/cm^2$ on thawed carcasses.

Total viable counts on fresh carcasses ranged from 5.62×10^3 to $8.91 \times 10^4/cm^2$ and were within the range considered normal for commercially slaughtered lambs (Ingram & Roberts, 1976). The change in counts during thawing was small, ranging from a twofold decrease to a sevenfold increase with final counts of between 1.41×10^4 and $3.72 \times 10^5/cm^2$. At 10 and 20°C, 0.75 m/s the increases in counts on wrapped lambs were significantly higher ($P<.05$) than on those thawed at 5°C at the low velocity. Air temperature had no other significant effect. Wrapping or raising the air velocity had no

Table 3. Means of bacterial counts from the breasts of lambs before freezing and after thawing at 5, 10 and 20°C, wrapped and unwrapped at low and high air velocities (Expressed in $\log_{10}/cm^2$)

Thawing temperature (°C)		Air velocity	Bacterial counts Fresh	Thawed	Differences
5	Unwrapped	Low	4.32	4.17	−0.15
		High	4.37	4.30	−0.07
	Wrapped	Low	4.70	4.30	−0.40
		High	4.27	4.70	0.43
10	Unwrapped	Low	4.95	5.50	0.55
		High	4.48	4.40	−0.08
	Wrapped	Low	4.78	5.43	0.65
		High	4.38	4.80	0.42
20	Unwrapped	Low	4.05	4.57	0.52
		High	4.08	4.22	0.13
	Wrapped	Low	4.03	4.88	0.85
		High	3.75	4.15	0.40
Least significant difference between means in each column ($P = .05$)			0.76	0.72	0.85

significant effect on changes in bacterial numbers, although upon thawing at 10 and 20°C there was a tendency for numbers to increase more on wrapped than on unwrapped meat and this increase was greater at 0.75 m/s than at 2.25 m/s.

The effect of air temperature, air velocity and wrapping on appearance

The appearance of the lean and fat surfaces of the thawed carcasses was not greatly affected by thawing temperature or air velocity, except for those thawed unwrapped at 20°C, 2.25 m/s when the lean surface was considerably darker than those thawed at 5 and 10°C. Under all other conditions the lean surfaces were slightly moist and of normal colour. The colour of the fat surfaces of thawed wrapped carcasses was noticeably paler than those thawed unwrapped under the same conditions, except at 5°C, 2.25 m/s where there was no difference between wrapped and unwrapped; the fat in both cases was slightly darker than normal. This pale appearance of the fat became less apparent after overnight storage in a chill-room.

The effect of air temperature, air velocity and wrapping on surface temperature

The surface temperature results (Table 4) are based on one carcass for each thawing condition. In most cases the surface of the breast, the thinnest section,

Table 4. Period of time spent above 0 and 10°C for meat surface at the leg, breast and shoulder during single thawing runs

Thawing temperature (°C)		Air velocity	Time (h) above 0°C			Time (h) above 10°C		
			Leg	Breast	Shoulder	Leg	Breast	Shoulder
5	Unwrapped	Low	21.4 (3.0)	31.4 (2.3)	12.3 (2.1)	–	–	–
		High	16.3 (2.8)	28.3 (4.4)	11.1 (2.4)	–	–	–
	Wrapped	Low	20.8 (1.8)	31.5 (3.6)	9.1 (2.4)	–	–	–
		High	24.9 (2.1)	36.1 (3.2)	26.9 (2.4)	–	–	–
10	Unwrapped	Low	10.1 (6.2)	17.9 (8.5)	9.4 (4.2)	–	–	–
		High	13.5 (7.6)	20.0 (7.6)	11.9 (8.0)	–	–	–
	Wrapped	Low	16.6 (6.8)	22.4 (7.2)	21.3 (5.6)	–	–	–
		High	19.5 (6.8)	21.1 (8.0)	19.7 (6.2)	–	–	–
20	Unwrapped	Low	10.7	11.9	13.0	7.2 (15.7)	11.1 (18.5)	10.3 (16.8)
		High	10.7	9.2	11.5	7.9 (18.4)	8.8 (19.2)	8.3 (18.4)
	Wrapped	Low	11.2	13.3	15.1	7.4 (17.2)	12.8 (16.8)	12.1 (18.6)
		High	13.8	15.6	11.9	11.9 (18.8)	15.0 (18.0)	8.8 (17.0)

Figures in parentheses indicate highest temperature (°C) attained during thawing.

rose above 0°C well before the surface of the thicker sections of the leg and shoulder. This was most noticeable at lower air temperatures where the breast was above 0°C for up to three times as long as the surface of the leg and shoulder.

In almost all cases the surfaces of wrapped carcasses were at temperatures above 0°C longer than unwrapped carcasses thawed under the same conditions. Increasing air velocity had little effect on surface temperature. The highest temperatures reached by the surface (Table 4) were usually within 3°C of the thawing temperature.

Discussion

Selection of a commercial thawing system for the production of lamb carcasses with a quality suitable for retail sale will depend upon a number of related considerations. The appearance of the carcass must be comparable to that of a chilled carcass with respect to the colour and moistness of the fat and lean surfaces; the thawing time must be commercially convenient, either 24 hr or overnight (15 hr approximately); the storage life of the thawed meat must be sufficient for commercial trading requirements; weight loss must be kept to a minimum. The thawing time–temperature relationship shown in the appendix can be used to calculate the minimum air temperature for thawing in 15 or 24 hr (Table 5).

Increasing the thawing temperature from 5 to 20°C reduced thawing time, had little effect on bacterial numbers, but darkened the lean surfaces of unwrapped lambs thawed at 20°C, 2.25 m/s.

Increasing the air velocity from 0.75 to 2.25 m/s produced only a small reduction in thawing time and had little effect on weight changes, bacterial numbers or appearance. There is consequently little justification for using systems with air velocities greater than 0.75 m/s, particularly as such systems also require extra fan power and more sophisticated design. Systems operating

Table 5. Minimum air temperatures required to thaw 20 kg lamb carcasses from –30 to 0°C in 15 and 24 hr

Conditions	Minimum air temperature thaw to 0°C in: 15 hr	24 hr
2.25 m/s Unwrapped	11	6
0.75 m/s Unwrapped	13.5	7
2.25 m/s Wrapped	16	8.5
0.75 m/s Wrapped	18.5	9.5

at low velocities and a slightly higher temperature would produce comparable results with lower capital and running costs.

Wrapping carcasses increased thawing time but eliminated weight losses, and made no significant difference to the bacterial growth. The colour of the surface fat of wrapped carcasses was slightly pale after thawing but returned to normal after overnight chill-room storage.

The changes in the total viable counts of bacteria during thawing were small and were not greatly influenced by air temperature, air velocity or wrapping. Vanichseni (1971) made similar observations on bacterial growth during the thawing of lamb shoulders. Increases in growth on the breasts of lamb carcasses were small, despite the fact that these surfaces attained the highest temperatures over the longest periods during thawing. Even less growth is likely on the cooler surfaces of the leg and shoulder. The growth of coliforms, as an indicator of the possible growth of food-poisoning bacteria, was also insignificant.

The optimal conditions for thawing wrapped lambs are 20°C at an air velocity of 0.75 m/s for overnight schedules and 10°C and 0.75 m/s for 24 hr thawing. For unwrapped lambs the conditions would be 15°C and 0.75 m/s for overnight and 7.5°C and 0.75 m/s for 24 hr schedules. Increases in bacterial numbers during these thawing regimes never exceeded tenfold and are unlikely to have much effect on the shelf-life of a carcass with normal initial levels of bacterial contamination but might create problems in carcasses initially heavily contaminated.

If the suggested conditions are examined apropos the French legislation, thawing in a 24 hr cycle would be completely acceptable for both wrapped and unwrapped carcasses, but thawing in 15 hr would result in surface temperatures above 10°C for more than 10 hr and consequently contravene the regulations. If legislation of this type is adopted by other countries in the future, more exact process design data and closer monitoring of meat temperatures will be required than is at present the practice in the United Kingdom.

Acknowledgments

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Appendix

Thawing temperature–thawing time relationship

A method of calculating thawing times in commercial operation has been derived which applies to lambs of 20 kg weight, thawed either wrapped or unwrapped, at an air velocity of 0.75 m/s and 2.25 m/s over the temperature range from 5 to 20°C.

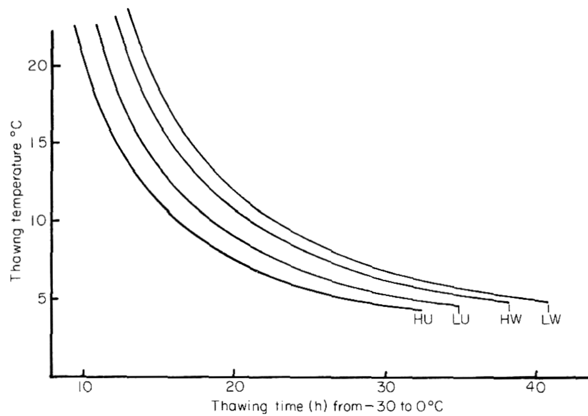

Figure 2. Relationship between thawing time and thawing temperature for lambs of 20 kg average weight, thawed unwrapped (U), wrapped (W) at 0.75 m/s (L) or 2.25 m/s (H).

The longest mean thawing times in Table 1 were used with the corresponding thawing temperatures to produce four curves for lambs thawed wrapped or unwrapped, at high and low velocities (Fig. 2). The curves of best fit produced by the least squares technique were hyperbolic, of the form

$Y = \mathrm{A} + \mathrm{B}/X$

where Y = thawing time in hours

X = thawing temperature in °C.

For the four curves the constants A and B had the following values:

	A	B
Unwrapped, 2.25 m/s	4.0	123
Unwrapped, 0.75 m/s	5.0	135
Wrapped, 2.25 m/s	5.5	156
Wrapped, 0.75 m/s	5.7	172

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Technical note: A study of the hydrolysis of polyphosphate additives in chicken flesh during frozen storage by ^{31}P-FTNMR spectroscopy

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Introduction

During the production of frozen chickens, it is a common commercial practice to inject an aqueous solution of polyphosphate salts into the carcase breast muscle immediately after slaughter and evisceration. The benefits claimed for this treatment include reduction in thaw-drip (Van Hoof & Daelman, 1975) and of cooking loss and an increase in oxidative stability of the cooked meat (Farr & May, 1970). The most effective salts are diphosphates and tripolyphosphates, although longer chain polyphosphates are also active (Iles, 1973). In pork and beef these salts are hydrolysed by muscle phosphatases to inactive orthophosphate (Mihályi-Kengyel & Körmendy, 1973; Sutton, 1973) and thus the benefits of polyphosphate treatment must depend on processes that occur before or during hydrolysis. It is therefore important to study the hydrolysis of polyphosphates in meat, and ^{31}P Fourier transform nuclear magnetic resonance (FTNMR) spectroscopy has been shown (Jozefowicz *et al.*, 1976; O'Neill & Richards, 1978) to be a rapid specific detection technique with advantages over chemical or chromatographic methods. The following work was conducted in order to ascertain if polyphosphates are hydrolysed during the frozen storage of treated chickens at −20°C.

Materials and methods

^{31}P-FTNMR spectroscopy has been used to identify phosphorus-containing metabolites within living tissue (Hoult *et al.*, 1974; Burt, Glonek & Bárány, 1977) and to observe the hydrolysis of diphosphate and tripolyphosphate salts injected into raw meat (O'Neill & Richards, 1975). In the present work, samples of approximately 2 cm^2 cross-section by 5 cm length (approximately 10 g weight) were cut from the same region of the *pectoralis major* muscle of

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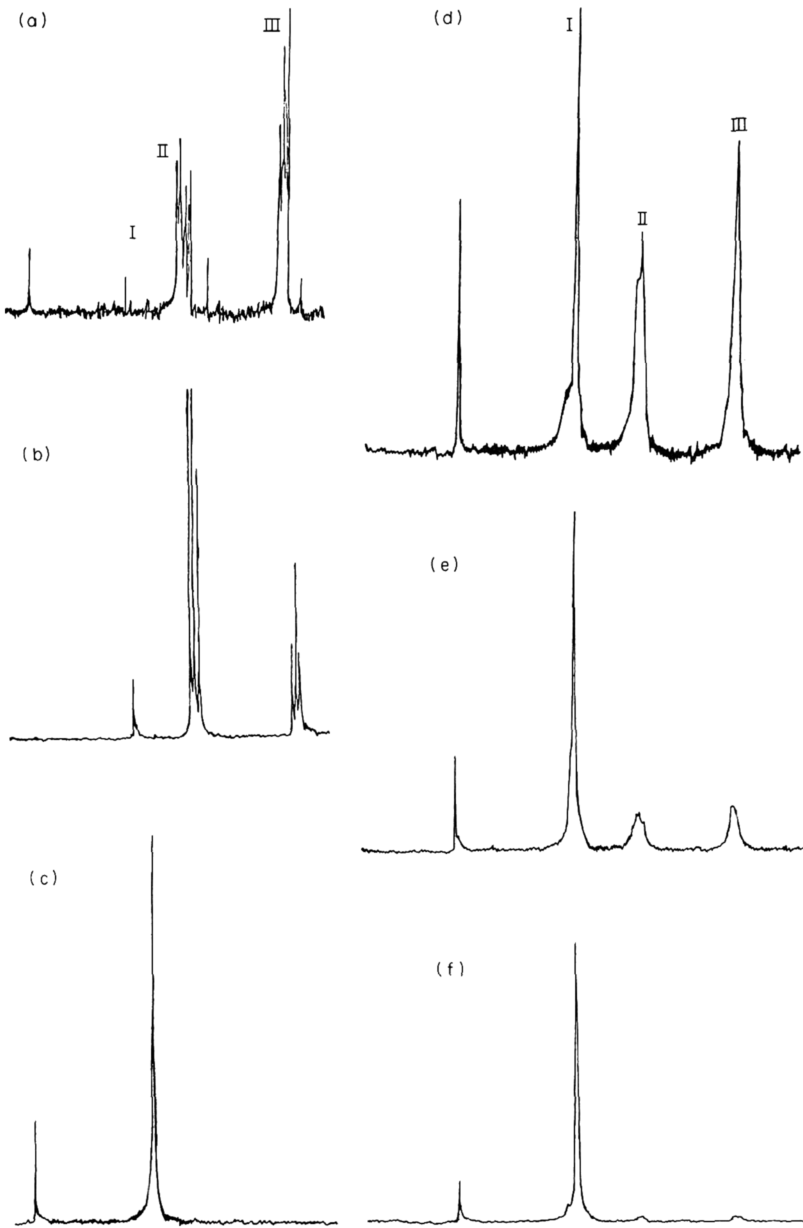

Figure 1. ^{31}P-FTNMR spectra at 40.3 MHz for (a) injection mixture; (b) mixture containing ortho-, di- and tripolyphosphates; (c) an untreated chicken after 5 months storage; (d, e, f) chicken samples after 5, 15, and 43 months storage respectively. The signals for orthophosphate, end and middle ^{31}P nuclei of higher phosphates are labelled I, II and III respectively. The left hand peak in each spectrum is the signal from sodium methylenediphosphonate which is at −16.8 p.p.m. from external 85% H_3PO_4. Each spectrum is the accumulated result of 400 × 8 μs pulses (flip angle 36°), repetition time 2.25 sec, sampled as 4095 data points with a spectral observation and frequency filter settings of 5000 Hz.

frozen chickens and immediately macerated with approximately 10% w/w powdered disodium ethylenediamine tetraacetic acid to prevent hydrolysis of polyphosphate during subsequent thawing (O'Neill & Richards, 1978). The thawed sample ($\simeq$3 g) was then tamped into a 10 mm o.d. NMR tube and a capillary containing a D_2O solution of sodium methylenediphosphonate inserted coaxially through the centre of the meat. ^{31}P-FTNMR spectra were obtained with a JEOL PFT-100P spectrometer operating at 40.3 MHz with deuterium field frequency lock. Spectroscopic observations were conducted so as to minimize the effects of saturation and other possible causes of non-linear spectrometer response (O'Neill & Pringuer, 1977). Spectra were also obtained of the injection solutions, a typical example of which is shown in Fig. 1 and from one of the 5-month chicken carcases that had not been treated with polyphosphate.

Results and discussion

The chickens, which had been injected with a commercial polyphosphate solution, had been in frozen storage for periods of 5, 15 and 43 months. The commercial polyphosphate and injection procedure were similar in each case. No signals from adenosine triphosphate or creatine phosphate were detected, showing that these naturally occurring phosphates had been hydrolysed, presumably during rigor, and thus did not hinder observation of the synthetic phosphates. The relative amounts of orthophosphate and higher phosphates are given by the integrated peak areas of the orthophosphate and the end/middle

Table 1. Effect of storage on polyphosphates in frozen chicken carcases

Storage time (months)	Integrals*		Relative total added phosphate*†	Fraction of polyphosphate hydrolysed (%)§
	Ortho-phosphates	Higher phosphates		
5	7.2	6.7	7.0	4
	6.7	13.5	13.5	0
	7.7	1.2	2.0	40
	7.1	24.0	24.2	1
15	7.7	7.8	8.6	9
	8.3	3.8	5.2	27
	10.2	7.1	10.4	32
43	11.0	0	4.1	100
	16.4	4.9	14.4	66
	12.5	1.0	6.6	85
Untreated control	6.9	–	–	–

*Normalized relative to the capillary solution of sodium methylenediphosphonate with an integral of 1.0 arbitrary units.

†Excess abundance of phosphorus nuclei over that in untreated sample.

§Fraction of polyphosphate phosphorus nuclei appearing as orthophosphate.

phosphorus signals that appear in separate parts of ^{31}P-NMR spectrum. Inter-spectrum comparisons were made by normalizing the integrals with respect to that for the sodium methylenediphosphonate contained in the capillary, in the same manner as has been used previously for quantitative ^{31}P-FTNMR analysis (Burt, Glonek & Bárány, 1976). These normalized results are presented in Table 1.

The total added phosphate was calculated as the sum of the integrals for the end/middle phosphorus nuclei of the higher phosphates together with excess orthophosphate arising from hydrolysis of the higher phosphates. In each case, the natural orthophosphate level was taken to be that found in the untreated sample. The fraction of polyphosphate hydrolysed was then calculated from the excess orthophosphate and the total added phosphate. Only minor errors are likely to arise from variation in packing of the meat in the NMR tube. More significant causes of scatter in the results in each group are probably the variations between individual injections and differences in the actual sample site relative to the injection site which was not known exactly. In previous experiments, ice crystals were noticed around the injection site, and it may be supposed that the injected polyphosphate is not evenly distributed throughout the breast muscle. The exceptionally high degree of hydrolysis found for the notable exception in the 5-month-old samples (Table 1) may be explained in terms of a relatively small amount of polyphosphate in contact with a normal level of muscle phosphatase enzymes. Another important cause of variation in the results is probably the time lapse between injection of the carcase and completion of freezing, which would greatly affect the degree of diffusion and hydrolysis of the polyphosphate prior to freezing.

Data in Table 1 show a marked variation in total added phosphate in the excised samples, thereby confirming that the injection solutions remain localized prior to thawing. There is nevertheless a consistency within each group and a consistent trend for hydrolysis with increased storage. Thus this ^{31}P-FTNMR spectroscopic method demonstrates that there is substantial hydrolysis of added polyphosphates in frozen chickens during long-term storage at $-20°$C.

Acknowledgments

The chickens were obtained from J. P. Wood & Sons, Craven Arms, Shropshire, by arrangements with Albright & Wilson Ltd., Oldbury, who also supplied the polyphosphate solutions.

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Technical note: Detection of incubator rejects in whole eggs after normal commercial processing

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Introduction

Following the suggestion of Salwin, Staruszkiewicz & Bond (1972) that the presence of β-hydroxybutyric acid (βHBA) in liquid egg could be used to confirm adulteration with 'incubator rejects', i.e. those eggs that have failed to develop into a chick, Robinson, Barnes & Taylor (1975) showed that the presence of approximately 16 mg of βHBA per 100 g in a sample of liquid egg would indicate that the material had been prepared from incubator rejects.

In a subsequent study Heaney & Curtis (1976) modified the assay method of Robinson and showed that individual incubator rejects contained widely varying quantities of βHBA. On the basis of their studies, these authors suggested that adulteration of fresh liquid whole egg with incubator rejects could be precluded by limiting the concentration in liquid whole egg to not more than 0.2 mg/100 g.

Although a test for detecting incubator rejects was now readily available, it was necessary to test its validity under conditions of commercial processing. For instance, in the United Kingdom, it is mandatory to pasteurize liquid egg which is to be sold for human consumption and therefore it was necessary to establish whether βHBA could be detected after pasteurization of incubator rejects or whether compounds were produced in unadulterated egg that interfered with the analysis. This paper describes the study, together with an examination of the effects of spray-drying and frozen storage on the detection of βHBA.

Materials and methods

Materials

Incubator rejects which had been 'candled' at 18 days were obtained from local hatcheries. All the incubator rejects used in a particular experiment came

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from the same hatchery and were held at 4°C until they were broken out. The maximum delay between removal from the incubator and breaking-out was 6 days.

Liquid whole 'fresh' egg was purchased from a commercial egg-processing plant.

Preparation of pasteurized and frozen egg samples

12 x 0.5 kg samples of 'fresh' unpasteurized egg (FNP) were canned and blast-frozen as controls. 4500 incubator rejects were broken-out and the resulting liquid egg mixed with an equal weight of FNP egg to give a total weight of approximately 320 kg of the mixture non pasteurized (MNP). 12 samples of MNP were canned and frozen as above.

FNP egg and the bulk of the above mixture were heated at 64.4°C for 2.5 min in a plate contact pasteurizer (APV Co. Ltd., Crawley, England). 12 x 0.5 kg samples of 'fresh' pasteurized (FP) and mixture pasteurized (MP) were canned and blast-frozen.

Frozen samples were stored at –20°C for subsequent analysis at 3-monthly intervals.

Preparation of spray-dried egg samples

18 kg samples of FNP, FP, MNP and MP egg were spray-dried in a NIRO atomizer (NIRO Atomiser Ltd., Soeborg, Denmark) using inlet and outlet temperatures of 200 and 80°C respectively. The resulting powders contained approximately 3% water. The powders were stored in the dark at 4°C until analysed.

Analysis

For the analysis of liquid egg 2 × 0.5 kg samples were thawed overnight at ambient temperature and 20 g of egg taken from each for βHBA estimation using the method of Heaney & Curtis (1976).

In the case of the spray-dried material 25 g of egg were made up to 100 g with water. Duplicate 20 g samples of this preparation were analysed as above. Results are expressed as mg βHBA/100 g egg.

The entire preparation and analytical procedure was carried out on three separate occasions.

Results and discussion

Originally it had been intended that samples of incubator rejects used in this study would consist only of rejects. Calculations and subsequent

breaking-out trials showed, however, that in order to have sufficient material to fill the pasteurizer, thus ensuring proper heat treatment, and to have 'raw' material available for spray-drying, it was necessary to have at least 8000 incubator rejects per experiment. However, since the maximum number of incubator rejects guaranteed from a common source at a specified time did not exceed 5000, it was decided to carry out the work using a 1:1 (w/w) mixture of 'fresh' liquid egg and incubator rejects.

The quantities of βHBA found in 'fresh' non-pasteurized eggs were always below the limit of 0.2 mg/100 g egg suggested for infertile eggs by Heaney & Curtis (1976). As shown in Table 1, pasteurization did not raise the level beyond that figure. Likewise pasteurization had no significant effect on the βHBA content of the mixture (Table 1). In additional to βHBA, lactic and, occasionally, succinic acids were found before and after pasteurization. It was concluded therefore that the heating process did not produce compounds which interfered with the detection of incubator reject eggs in liquid whole egg by estimation of βHBA.

The initial concentrations of βHBA in the incubator rejects used in this study were calculated from the data in Table 1 by doubling the levels found in MNP (to account for dilution) and substracting from these figures the levels found in FNP. The values ranging from 6.3 to 11.1 mg/100 g egg, were lower than the 15.9 mg/100 g found by Robinson *et al.* (1975). The most likely explanation for this difference was that the rejects used in the current study contained larger proportions of infertile eggs with a low βHBA content, than did the bulked samples used by Robinson *et al.* Such variability between batches of incubator rejects means that, while a level of βHBA in liquid egg above 0.2 mg/100 g can be assumed to indicate adulteration with incubator rejects, the concentration of βHBA cannot readily be used to estimate the degree of adulteration.

βHBA was readily detected in the mixtures after spray drying although the concentrations were generally lower than in the liquid egg from which the powder was prepared (Table 2). There are two possible explanations for this discrepancy. First, a genuine loss of βHBA during spray-drying or, second the

Table 1. Effect of pasteurization on βHBA in liquid egg

	Sample			
Experiment No.	FNP	FP	MNP	MP
I	0.06, 0.05	0.04, 0.05	5.26, 5.33	5.22, 5.17
II	0.08, 0.17	0.14, 0.13	5.74, 5.45	5.42, 5.57
III	0.04, 0.05	0.14, 0.13	3.20, 3.19	2.85, 2.92

Fresh non-pasteurized (FNP), fresh pasteurized (FP), mixture non-pasteurized (MNP), and mixture pasteurized (MP). Results are expressed as mg βHBA/100 g egg.

Table 2. Effect of spray drying on the βHBA in egg samples

Experiment No.		Before spray drying	After spray drying
I	FP	0.05, 0.04	0.08, 0.06
	MP	5.22, 5.17	5.10, 4.98
II	FP	0.14, 0.13	0.07, 0.10
	MP	5.42, 5.57	4.27, 4.31

Results are expressed as mg βHBA/100 g liquid egg (containing 25 g solids).

powder was less homogenous than the liquid and thus did not have the same composition on reconstitution. Whatever the reason, the differences between the liquid and spray-dried egg were not considered to be practically significant. As with pasteurization, spray-drying did not produce compounds which interfere with the detection of βHBA. The same was true for a mixture which was desugared prior to spray drying.

The βHBA concentrations remained relatively stable during 12 months of frozen storage at −20°C as is illustrated by the 'fresh' pasteurized and mixture pasteurized samples from Experiment II (Table 3).

Table 3. Effect of frozen storage at −20°C on the βHBA in liquid egg

Storage time (months)	Sample	
	Fresh pasteurized	Mixture pasteurized
0	0.14, 0.13	5.42, 5.57
3	0.08, 0.10	5.45, 5.81
6	0.05, 0.05	5.21, 5.14
12	0.13, 0.13	5.71, 6.12

Results are expressed as mg βHBA/100 g egg.

These studies have clearly demonstrated that βHBA is stable to a variety of commercial processing techniques and, as such, is a reliable indicator of adulteration of liquid whole egg with incubator rejects.

Acknowledgments

The authors are grateful to Mrs C. C. L. Wilkinson for carrying out some of the analyses. They also wish to express their appreciation to Mr D. Buckley

(Ross Poultry Hatchery, Ely), Mr M. Youngs (John Rannoch, Ltd., Stowmarket), Mr R. Ogden (Spillers Technical Research Station, Cambridge) and their staff for supplying and processing the eggs. The work was financed by the Eggs Authority.

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Book reviews

Physical, Chemical and Biological Changes in Food Caused by Thermal Processing. Ed. by T. Hoyem and O. Kvale. London: Applied Science Publishers 1977. Pp. xi + 398. £25.00.

The book records the proceedings of an international symposium organized by the Norwegian Food Research Institute in September 1975.

After an introductory chapter by Anton Skulberg (NFRI) in which some pertinent political, economic and social implications of food and the food industry are discussed, one is introduced to the main subject matter of the symposium under four main section headings whose titles are unfortunately rather awkward and verbose.

Section one deals with the theoretical mathematical fundamentals related to the thermal processing of foodstuffs. Five chapters written by distinguished authors, cover a range of topics including drying, sterilization, continuous pasteurization, evaporation concentration and industrial scale frying operations. The thermal sterilization of hermetic food containers is expertly dealt with by W. F. Hermans and includes a detailed description of the batch and continuous processing of flexible food containers including plastic milk bottles and retort pouches.

The second part of the book consists of eight chapters, each of which deals specifically with the physical, chemical and biological significance of heat on particular groups of food constituents including muscle proteins, fats and oils, carbohydrates, vitamins, and enzymes. The nutritional implications of these thermal changes are particulars well emphasized as in chapter 11 which deals specifically with the losses of vitamins in processed foodstuffs. The apparent lack of knowledge in this area for one of the more sensitive vitamins, folic acid is reflected in its sparse coverage when compared to that of vitamin C (12 lines and 4 pages respectively).

The title of the third section 'Applications within Industry' is self explanatory. A further nine eminent authors cover a range of topics as varied as 'heat penetration studies on canned fish' to the 'thermal release of bound nicotinic acid in cereals' and the 'thermal processing and technology of wine'.

The fourth and final section of the book is again orientated towards the nutritional aspects of the topic and includes a chapter by Professor Bender which expertly puts the whole symposium and book into some sort of perspective by a clever analysis of the errors and incorrect assumptions that can and have resulted from poorly designed experimentation.

To sum up, the book is very well presented with excellent illustrations on almost every page and the subject matter is both extensive and comprehensive with a generous list of topical references to complement every chapter.

The price at £25.00 for 388 pages is probably too prohibitive as a general textbook but for food scientists, technologists, and nutritionists closely involved in the thermal processing of food this book should be an ideal reference volume.

J. D. Malin

Fish Protein Concentrate: Panacea for Protein Malnutrition. By E. R. Pariser, Mitchel B. Wallerstein, Christopher J. Corkery and Norman L. Brown. Cambridge, Mass.: MIT Press, 1978. Pp. xix + 296. £12.25.

This book is the third published under the Nutrition Policy Series of the MIT International Nutrition Planning and Policy Program and Cornell University Program on International Nutrition and Development Policy. The aim of the series of monographs is to make information available systematically on alternative approaches to nutrition planning and on actual nutrition intervention programmes that have been attempted.

The authors, two of whom are food chemists (E.R.P. and N.B.), one a political scientist (M.W.) and the fourth a writer (C.J.C.) with an overview of nutrition planning have given a very comprehensive and detailed analysis of the FPC story as seen through American eyes. They have pooled resources admirably to give an extremely wide ranging account of the interaction of the various interests – technical, political, cultural, bureaucratic and industrial and of the differing goals among developers and conflicting priorities within and between institutions, all of which lead to the conclusion with the benefit of hindsight that FPC was doomed to failure as a concept and as a product in relieving urgent malnutrition problems in low income countries.

The book has 296 pages and is divided into four parts. Part I is a review of the 'History and cultural significance of fish as food' in which is included accounts of the early attempts to manufacture fish protein concentrate or fish flour for human consumption. The major part of the book however is Part II (pp. 19–113) – the history of policy issues pertaining to the growth and development of FPC in the United States described in great detail from the initiation of the project in 1961 to the final termination in 1972. The amount of detail touches on the irrelevant at times but what comes through clearly are the problems stemming from the decision by the Kennedy Administration to develop the potential of the oceans and to alleviate as part of this exercise, world-wide protein malnutrition. Pressures to meet political objectives and conflict of interests among different bodies are all documented here; they are of interest at this level of detail from a historical point of view but of marginal interest to food scientists and nutritionists at large. The conflict, long drawn out, between the Food and Drug Adminsitration (FDA) and the Bureau of Commercial Fisheries (BCF) is also documented in great detail and gives the reader an insight into the attitude and working of these different Public bodies in the U.S.A. The description on p. 70 of the about turn by the FDA over the packaging problem is unfortunately repeated on p. 94 as is part of p. 44 on p. 87 – small points but slightly annoying to the reader as is the need to refer

to the list of acronyms at the beginning of the book to determine what for example is an EDP (Experimental Demonstration Plant).

Part III brings together the work done on FPC in other countries, Canada and Sweden, Morocco and Chile, and the world at large. The account of the Moroccan project is of interest because of various national and international bodies involved in one way or another, and the apparent inevitability of problems over policy and objectives leading to the eventual termination of the projects. (At the time of publication a Swedish company was still producing FPC but I understand that this is no longer so.) In their conclusions in Part IV 'An Analysis of the International Experience', the authors state that there is little hope that 'technological fixes' such as FPC can ever obviate the need to confront their fundamental concerns for social equity' – a lesson that has been learned at great cost and after much time spent by many individuals and committees.

The last 53 pages are annotated technical references to what must be all the relevant publications both technical and political on FPC at least in the U.S.A.

This book should be prescribed reading for all individuals concerned with food or nutrition programmes in developing countries. At £12.25 it is good value for the food science/nutrition library.

Ian M. Mackie

Water Activity and Food. By John A. Troller and J. H. B. Christian. New York: Academic Press, 1978. Pp. xiv + 235. £12.65.

This book is essentially a very timely review of the literature related to the concept of water activity and its application to food. It is primarily intended for food scientists and those engaged in food research. The book starts with two very useful chapters on the basic concepts and methods of measuring water activity and equilibrium relative humidity. In the following two chapters the following topics are discussed, enzyme reactions, non-enzymatic browning, lipid oxidation, food texture, food pigments and nutrients in relation to the effect of water activity. The next four chapters are concerned with microbiological aspects viz. microbial growth, food preservation and spoilage, microbial spoilage and food-borne pathogens. These are followed by a chapter on the control of water activity and moisture which deals with dehydration, concentration and intermediate moisture foods. The important topics of packaging, storage and transport of food products in relation to the environmental conditions are also dealt with in a separate chapter. The final chapter deals briefly with food plant sanitation. Each chapter concludes with a selected list of references, many of which are additional reading to supplement the information given in the text. There are two appendices, the first giving approximate water activities for some foods together with corresponding water activities of solutions of sodium chloride and sucrose where applicable, and the second giving approximate minimum levels of water activity permitting growth of microorganisms.

This book is excellently written and should become the standard text. The authors and publishers are to be congratulated on producing a book which will be of great usefulness to food scientists and technologists as well as biochemists and microbiologists.

S. D. Holdsworth

Microbiology of Foods. By D. A. Mossel. Utrecht: University of Utrecht, 1977. Pp. 165. US$9.00.

This book is an essential reference book for all food microbiologists. It is written by one of the world's acknowledged experts on the subject and is unique in that it concentrates on basic principles underlying the behaviour of micro-organisms in foods in general, rather than on the subject in the context of individual commodities.

It consists of 88 pages of text, tables and figures and is a concentrate of well documented information supported by 2310 references so that the reader can readily consult the evidence given to support any of the author's statements. He is well known for his mastery of a number of languages and has produced a comprehensive collection of references drawn from many countries. No doubt the occasional errors of English idiom or spelling will be corrected in subsequent editions. After a brief introduction the book divides into four main parts: (a) Diseases of microbial origin transmitted by food (b) The mechanism of microbial deterioration of foods, (c) The control of microbiological quality of foods and (d) The microbiological monitoring of foods.

In the section on diseases the coverage is wide and includes discussion of some of the less familiar diseases spread by food and the food environment, including those caused by viruses, protozoa and helminths. Microbial deterioration is considered in relation to the intrinsic and extrinsic factors governing food spoilage and includes nine valuable and detailed tables. If any criticism can be levelled at this section, it is that little reference has been made to frozen storage and its influence on microbial growth or survival.

In the section on control of microbiological quality of foods the author draws on his extensive experience in this field and discusses the methods available; in a relatively short space he summarizes the arguments which are so relevant at the present time. In the final section on microbiological monitoring he outlines the difficulties of sampling and considers the principles underlying the choice of various organisms for assessment of quality and the creation of reference values. Although in individual instances one might wish to argue on matters of detail, this book is invaluable for its wide coverage of the subject.

Ella M. Barnes

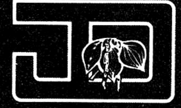
Books Received

Basic Biochemistry. By J. Edelman and J. M. Chapman. London: Heinemann Educational Books, 1978. Pp. vii + 133. Paperback. £2.90.

This is an unusual and interesting introduction into biochemistry for college and university students, which relies almost entirely on diagrams and flow charts to explain basic principles.

Practical Food Inspection. By C. R. A. Martin. London: H. K. Lewis, 1978. Pp. vii + 836. £15.00.

The ninth edition of a well established text addressed to those concerned with food inspection. It now covers many changes brought about by Britain's accession to the Treaty of Rome.



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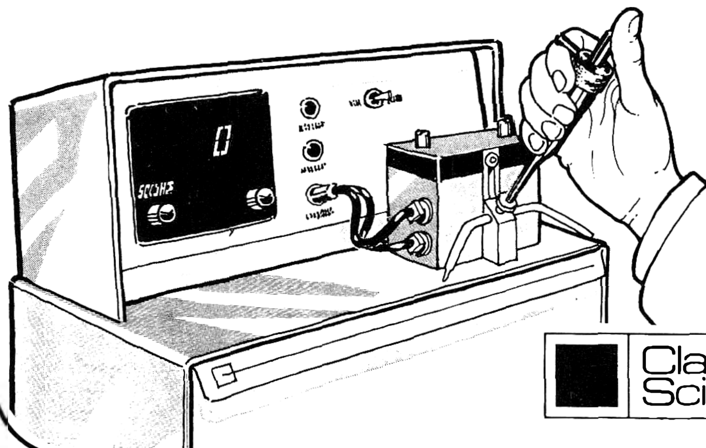

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Abbreviations. Abbreviations for some commoner units are given below. The abbreviation for the plural of a unit is the same as that for the singular. Wherever possible the metric SI units should be used unless they conflict with generally accepted 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	μm = 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^3
cubic foot	ft^3	= 0·028317 m^3
gallon	gal	= 4·5461 l
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'.

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

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