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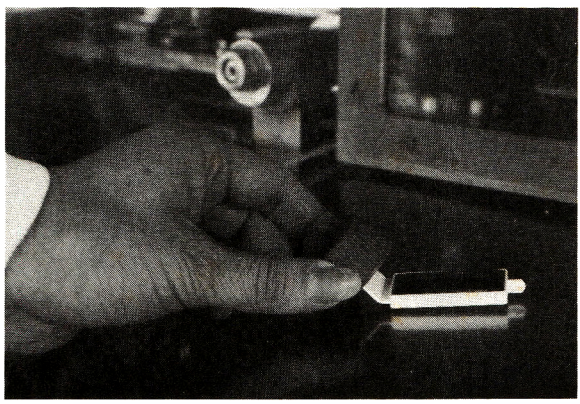

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Microwave and infra-red drying versus conventional oven drying methods for moisture determination in fish flesh

R. CHRISTIE, M. KENT AND A. LEES

Summary

A comparison is made between moisture determination by conventional oven drying techniques and the newer, rapid methods of microwave and infra-red drying. There are many significant differences observed between the techniques and these are explained in terms of temperature, oxidation and evaporation of other volatiles. No method appeared capable of achieving an accuracy of ±0.1% moisture content in an industrial situation. The microwave method was the best rapid substitute for conventional oven drying.

Introduction

In many branches of the food industry it is required to carry out moisture determination as quickly and precisely as possible. With high moisture content foods this generally involves drying the product in an air or vacuum oven. For reasonable precision and reproducibility, in the case of meat and meat products, a drying time of 16–18 hr in an air oven at 100–102°C has been specified (AOAC, 1980a). The comparable conditions in the vacuum oven are 2–4 hr at 125°C (AOAC, 1980b). Suggested drying times for other food products are listed elsewhere (Karmas, 1980). Such information is not available for fish and fish products but drying times in an air oven of 18–24 hr at temperatures between 101 and 105°C have been commonly used.

In many applications such long process times are inconvenient but although rapid methods exist (e.g., capacitance, conductivity, microwave attenuation, nuclear magnetic resonance (Harbert, 1971)) none of these are primary standards and all require calibration against some absolute method. Two methods exploited in recent years are: (a) the use of a microwave oven, and (b) an infra-red lamp, to heat and evaporate the water, replacing the more conventional air or vacuum ovens and considerably enhancing the speed of measurement.

The purpose of the work described here was to see how well such new methods performed on a high moisture food such as fish when compared to the more established and time consuming procedures.

Materials and methods

Based on previous experience of the sample-to-sample variation encountered in moisture determination of fish muscle using both air and vacuum ovens, eight samples were deemed necessary to provide data with a satisfactory level of precision. This would

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allow reliable comparison to be made between the various drying methods under investigation. To provide a reasonable load for the microwave oven the size of each sample needed to be approximately 30 g. To standardize the procedure similar weights were used in both air and vacuum ovens. In the infra-red (IR) dryer it was desirable that the material to be dried should be spread as uniformly and as thinly as possible to expose the maximum surface area to the IR radiation. To satisfy this requirement it was necessary to work with a sample size of about 10 g.

Three species of fish were used in the investigation, cod (*Gadus morhua*), herring (*Clupea harengus*) and scad (*Trachurus trachurus*). Batches of cod and scad were filleted, skinned and minced in a Baader 694 deboner. The minces were packed in cardboard boxes containing 7.5 kg in the case of cod and 2.6 kg in the case of scad. These packs were frozen in an air blast freezer at −30°C and subsequently stored at −30°C until required for experimental purposes. Storage at this temperature eliminated the possibility of moisture loss or deterioration of the product during the period of the experiment (about 3 weeks). One pack of each species was used in the present work.

The herring mince was produced from a much smaller batch of fresh fish with a fat content of approximately 17%. Each fish was filleted, the fillets skinned and minced in a hand mincer, the resulting mince mixed and then minced again. After further mixing it was frozen in sealed plastic bags each containing enough mince for any of the planned experiments.

In preparation for an experiment sufficient material was taken from each of the minces and thawed overnight at 0°C. Once thawed the cod, haddock and scad minces were again minced in a hand mincer and thoroughly mixed. The herring mince, after thawing, required further mixing immediately before the removal of samples, because the large amount of oil in the thawed samples tended to separate from the solid part during storage. After thawing all minces were kept at 0°C in sealed containers, in most cases samples being taken within 1 hr, the exception being material used in measurements made with the IR dryer. For reasons explained later, some of this material had to be stored for up to 7 hrs.

The methods investigated used a standard laboratory oven, a vacuum oven (Gallenkamp size 1), a microwave oven (Dysona 951), and an IR drying system specifically designed to obtain quickly the moisture content of a small sample of material (Mettler LP15 oven with top-pan balance type PC2000). Temperatures in the air and vacuum ovens were thermostatically controlled to an accuracy of ±0.5°C and ±1.5°C respectively. In both ovens the temperature was measured close to the samples under investigation. The vacuum oven was exhausted by a single stage gas ballast pump having an ultimate vacuum of 66 Pa. Samples for both air and vacuum ovens were contained in pre-dried aluminium basins approximately 63 mm in diameter and 44 mm deep having loosely fitting lids. These basins were weighed and about 30 g of mince was evenly spread, with the minimum of compaction, on the bottom of each basin. After reweighing the basins were then placed, with lids off, in the ovens. After the required drying time the lids were replaced and the basins transferred to sealed dessicators. Having cooled to ambient temperature the basins were weighed, and from the measured weight loss the moisture contents were calculated. All samples were weighed on a Sartorius top-pan balance having a discrimination of 0.001 g. The microwave oven was a standard laboratory unit fitted with a rotating table. This allowed a number of samples to be heated simultaneously as uniformly as possible. Power levels within the oven could be increased in steps up to 1.5 kW. It was found by experiment that setting 3

(nominally providing 175 W) achieved maximum drying rate in the samples with negligible charring. Power input however was checked after each experiment using the method recommended by the manufacturers.

Samples for the microwave oven were contained in weighed dry glass crystallizing dishes approximately 65 mm in diameter and 38 mm deep. Each dish was loaded with about 30 g of mince in the manner previously described. The dishes were placed on microwave absorbant material on the rotating table. This material was used to provide a continuing load as the moisture evaporated with consequent reduction in dielectric loss of the samples. At set time intervals the samples were removed, weighed and immediately returned to the microwave oven. The drying continued until either constant weight was achieved or, as in a few cases, charring occurred. The samples were then placed in the air oven so that a measurement could be made of any remaining moisture. Difficulties arose in drying herring and scad minces due to oil exuding from the samples. This oil, when heated, occasionally erupted in droplets with sufficient velocity to escape from the dishes. This false weight loss was prevented by inserting tightly fitting filter paper discs into the dishes above the samples. These discs were included in the tare weight of the dishes.

The IR drying system comprised a small oven and integral electronic top-pan balance with a discrimination of 0.01 g. The sample on the balance pan was exposed to the source of IR radiation, the intensity of which could be varied. This system was such that only one sample could be processed at any time. The minces were contained in aluminium foil trays 95 mm in diameter and 8 mm deep as supplied by the manufacturer. About 10 g of the mince was spread evenly in the bottom of each tray and then subjected to the IR radiation. The weight of the sample was monitored as the drying progressed. It was found by experiment that power setting 10 (12 was maximum) achieved the maximum drying rate with negligible charring for the cod mince. The problem of oil erupting from the samples of scad and herring minces, accentuated by the shallowness of the trays, could only be minimized by reducing the power setting to 8. This unavoidably increased the drying time.

Results and discussion

Equilibration of drying

The principal problem in determining the moisture content of food materials using an oven method is in deciding at what stage the procedure should be terminated. If dried to constant weight the total weight loss inevitably includes contributions from, for example, the loss of other volatiles as well as weight changes due to the effects of oxidation (Stitt, 1958). Standard methods, therefore, are generally a compromise which by specifying drying times and temperatures allow an acceptable degree of accuracy and reproducibility. For instance oven drying procedures often specify a 24 hr drying period but as Tables 1 and 2 show, for cod this gives far from the possible total weight loss. The time intervals in Table 1 are not equal but it appears from the data at 216 and 288 hr that, for cod at least, equilibrium has been reached.

The significance of the change in a time interval was determined by the paired *t*-test which enables a comparison to be made while eliminating sample-to-sample variation. This showed significant change in all other time intervals. As these changes become smaller with increasing time their significance obviously also decreases until between 216 and 288 hr no significant weight loss takes place. In comparison the data from the other species were not so consistant. Herring began to show evidence of oxidation

Table 1. Air drying of fish minces at 101°C; percentage moisture contents on a wet basis ± standard deviation

	Time (hr)									
Sample	24	48	72	96	120	144	168	216	288	336
Cod	80.88±0.07	80.97±0.08	—	—	81.00±0.08	81.02±0.08	81.03±0.08	81.05±0.08	81.05±0.08	—
Herring	62.36±1.10	63.20±0.33	63.24±0.30	63.29±0.29	—	—	63.32±0.36	63.26±0.28	—	63.25±0.29
Scad	77.07±0.10	77.17±0.10	—	—	77.18±0.10	—	77.20±0.10	—	77.25±0.10	—

Table 2. Significance for differences between pairs of observations for drying cod

Time (hr)	Time (hr)					
	48	120	144	168	216	288
24	*					
48		*				
120			*			
144				†		
168					†	
216						NS

*Significant at 0.1% level.
†Significant at 1.0% level.
NS = not significant.

(i.e. weight gain) and scad did not reach steady-state even after 288 hr. The larger standard deviation observed with herring is probably due to greater sample heterogeneity arising from the aforementioned problems with the oil content. In the vacuum oven the behaviour was altogether different (Table 3). All samples appear to reach or approach equilibrium at around 100 to 200 hr but this was followed by an increasing weight loss which probably indicates loss of volatiles other than water due to chemical decomposition.

Whatever the reasons for this slow approach, or even non-approach, to equilibrium it is clear that a drying time of 24 hr can only be an arbitrary choice based on consideration of convenience and accuracy. It is however the minimum time that must be allowed for drying. Thus oven methods cannot give definitive values for moisture contents but nevertheless have been adopted as standards, and will be the type of method against which other methods will be compared.

Microwave equilibration

The apparently long drying time in conventional oven drying is also encountered, albeit on a shorter time scale, in microwave drying. Because of this it was first necessary to find the minimum time required in the microwave oven to achieve no further significant weight loss, as well as the limitation imposed on the power level by the onset of charring. Presented in Table 4 are the data for increasing the time of drying. As can be seen, even up to 90 min drying, for cod and herring, significant changes still occur. The experiments were terminated when charring became evident, but at 90 min for cod the significance of differences between successive samples had diminished considerably. In the case of scad charring was apparent after only 72 min, probably due to variations in the power level within the oven. For cod and herring the power levels were 151 and 159 W respectively, in the case of scad the value rose to 180 W.

It should be noted that even after drying in the microwave oven, some samples subjected to further conventional air oven drying continued to lose weight. This is demonstrated in Table 5 where the moisture contents of the cod and herring samples determined by microwave oven drying, followed by up to 168 hr in an air oven at 101°C were still increasing significantly. Scad showed some apparent decreases in moisture content, presumably due to oxidation. It is interesting to note that transfer from microwave drying to air oven drying does not appear to change the between-samples

Table 3. Vacuum oven drying of fish minces at 101°C; percentage moisture contents on wet basis ± standard deviation

	Time (hr)									
Sample	24	48	72	96	120	144	168	216	288	336
Cod	81.17±0.16	81.23±0.16	—	—	81.30±0.15	81.31±0.15	81.31±0.11	81.36±0.11	81.37±0.11	—
Herring	63.39±0.32	63.46±0.33	63.48±0.32	63.54±0.31	—	—	63.54±0.32	63.58±0.32	—	63.62±0.32
Scad	77.12±0.13	77.20±0.14	—	—	77.28±0.13	—	77.30±0.11	—	77.38±0.10	—

Table 4. Percentage moisture contents on wet basis±standard deviations as determined by microwave oven drying for various times

	Time (min)					
Sample	36	54	63	72	81	90
Cod	69.36±0.05*	81.16±0.3 NS	81.21±0.29†	81.26±0.27‡	81.29±0.27 NS	81.31±0.27
Herring	55.49±0.55*	63.15±0.42†	—	63.44±0.52‡	—	63.55±0.50
Scad	60.70±2.64*	—	—	77.58±0.35	—	—

Level of significance of the differences between a mean and the mean in the next column:
*Significant at 0.1% level.
†Significant at 1.0% level.
‡Significant at 5.0% level.
NS = not significant.

Table 5. Percentage moisture contents on a wet basis±standard deviation as determined by microwave oven drying for 90 min followed by up to 168 hr of air oven drying at 101°C

	Time of microwave drying (min)	Time of additional air oven drying at 101°C (hr)			
Sample	90	24	48	120	168
Cod	81.31±0.27†	81.42±0.23*	81.45±0.48‡	81.46±0.23†	81.48±0.23
Herring	63.55±0.50 NS	63.62±0.50‡	63.63±0.50†	—	63.66±0.48
Scad	§77.58±0.35‡	77.59±0.29†	77.61±0.28 NS	77.59±0.28 NS	77.58±0.27

Significance of the differences between a mean and the mean in the next column:
*Significant at 0.1% level.
†Significant at 1.0% level.
‡Significant at 5.0% level.
§72 min microwave drying.
NS = not significant.

Table 6. Comparison of the percentage moisture contents obtained by various methods of drying ± standard deviation

	Microwave	IR	Air oven 101°C	Vacuum oven 101°C
Sample	(90 min)	(90 min)	(24 hr)	(24 hr)
Cod	81.31±0.27	81.46±0.19	80.88±0.07	81.17±0.16
Herring	63.55±0.50	63.76±0.54	62.36±1.1	63.39±0.32
Scad	§77.58±0.35	77.68±0.34	77.07±0.10	77.12±0.13

§72 min microwave drying.

variance, indicating that this is due principally to sample variation and to variation in the initial microwave drying. The standard deviation observed is somewhat higher than that encountered for cod, say, in Table 1 where only conventional drying had been used. Table 5 shows the significance levels of differences of successive measurements on the same samples. In contrast, IR drying for 90 min followed by up to 120 hr in an air oven at

Table 7. Significance of differences between various methods

	Significance		
	B	C	D
Cod			
A	NS	*	NS
B		*	‡
C			*
Herring			
A	NS	‡	NS
B		‡	NS
C			†
Scad			
A	NS	†	†
B		†	†
C			NS

*Significance at 0.1% level.
†Significance at 0.1% level.
‡Significance at 5.0% level.
NS = not significant.
Methods:
A. Microwave oven (90 min). (Scad 72 min).
B. Infra-red (90 min).
C. Air oven 101°C 24 hr).
D. Vacuum oven 101°C (24 hr).

101°C showed no further significant weight changes for any of the samples so it will not be discussed in this section.

Comparison of microwave and IR dried samples with conventionally dried samples (Tables 6 and 7) from the same batch reveals an interesting picture. Except in the case of scad, for the standard 24 hr in air at 101°C the weight loss is significantly less than in either the vacuum, IR, or microwave oven. IR and microwave give equivalent results for all samples differing from vacuum oven results only in the case of scad.

Temperature dependence

For cod mince it was readily shown on another set of samples from the same batch that the results are temperature dependent. Table 8 shows these data and it can be seen that if the air oven temperature is raised from 101°C to 105°C then the measured weight loss after 24 hr is increased and approaches that for the microwave oven (compare with Table 6). Similarly if the vacuum oven temperature is reduced to 70°C then this gives a result comparable to the air oven at 101°C. All these differences are statistically highly significant and are summarized in Table 9. No change in significance is found by repeating the comparison after 312 hr. Remembering that all the samples in this work were taken from the same well mixed batch of material then comparisons are acceptable between separate experiments, though sample-to-sample variation cannot be eliminated. The repeatability of the measurements for some of the methods is high. Clearly, the microwave and IR methods give results equivalent to long term and high

temperature exposure in a conventional air oven. The effects of such exposure to air are unknown but the effect of oxidation would be to decrease the apparent moisture content. This has been noted in some of the scad results. Some differences were also observed between behaviour in vacuum and in air but we must conclude that in the cod samples at least the effects of oxygen uptake or even volatile loss are inseparable from each other and moisture loss.

Table 8. Comparison of different temperatures of drying in conventional ovens for cod mince; percentage moisture contents on wet basis ± standard deviation

	Moisture content determined after drying for:	
Method	24 (hr)	312 (hr)
Air oven 101°C	80.98±0.03	81.16±0.03
Air oven 105°C	81.16±0.07	81.31±0.08
Vacuum oven 70°C	80.97±0.10	81.12±0.10
Vacuum oven 101°C (Table 3)	81.17±0.16	—
Microwave oven (Table 4)	81.31±0.27	—

Table 9. Comparison of significance of difference between various methods for cod

	Significance		
	B	C	D
A	*	NS	‡
B		†	NS
C			‡

*Significant at 0.1% level.
†Significant at 1.0% level.
‡Significant at 5.0% level.
NS = not significant.
Methods:
A, Air oven 101°C,
B, Air oven 105°C,
C, Vacuum oven 70°C,
D, Vacuum oven 101°C.

Precision and repeatability

A more fundamental and practical problem must now be addressed. To what precision does the food industry wish to measure moisture content of high moisture foods? A figure of ±0.1% moisture content is often referred to by fish processors but it is clear that only with a highly homogeneous sample could such a precision be achieved. All the IR and microwave oven data presented here are subject to standard deviation of typically ±0.3–±0.5% moisture. Conventional oven techniques seem capable of about ±0.1%–0.3% in general, reducing to about ±0.03% for extremely long equilibration times. It must be stressed again, however, that such results are only possible with well-mixed, homogeneous samples. In industrial situations such a quality of material would be highly unlikely. The repeatability of microwave and IR measurements is worse than for conventional oven methods, presumably due to the uncontrolled nature of the power input which can vary considerably. This is most serious in the case of IR drying where the main difficulty is that only one sample at a time may be used.

Conclusions

The requirements of the industry that moisture determinations be done rapidly and accurately seem to be largely incompatible. If the desired accuracy is ±0.1% moisture, then no oven drying method can achieve this accuracy with fish samples except under highly controlled laboratory conditions with very uniform samples and long drying times. No rapid method can compete with the accuracy of long term drying in air or in a vacuum. Since high accuracy seems unattainable then no great disadvantage accrues from using a microwave oven, which has the advantage of rapidity despite its larger errors. The results using a microwave oven, although less precise, were very close to those obtained by careful long-term drying in an air oven. At first sight, because of its speed, the IR method also appears comparable to microwave drying but it must be remembered that the drying time of 90 min refers to a single sample only and for equivalent precision eight such samples must be taken. This makes the duration of the determination almost as tediously long as that with conventional oven techniques.

In summary therefore we conclude that if time is no factor and accuracy is then long term (> 24 hr), standardized conventional oven drying methods must be used. If, on the other hand, a rapid answer is required with reasonable accuracy then a microwave system should be adopted. However, in this case extreme care must be taken to standardize both the power level and drying time, taking into account the possibility of charring. It is better to sacrifice some time by using less power than to risk reducing the accuracy by charring the samples. For the lowest possible accuracy and rapid results, a single sample used in the IR system would suffice, though again care must be exercised in adjusting power levels and drying time.

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Solubilization of pigskin and bovine tendon after pepsin and pancreatin treatment. Effect of incubation conditions and age of animal

ANITA LASER REUTERSWÄRD

Summary

Solubilization of pigskin, bovine tendon, insoluble collagen (bovine Achilles tendon), gelatin and meat was studied after treatment with pepsin and pancreatin, at 37°C for up to 4 hr. Pepsin at pH 1.5 solubilized 79–88% of the protein in unheated young pigskin as well as in calf and cow tendon. The young pigskin sample was more soluble in pepsin/pancreatin/TCA than the older one, 94 and 75%, respectively. No age effect was obtained for tendons, which were all more than 83% soluble after pepsin/pancreatin treatment. Pancreatin alone solubilized about 15% of young and old pigskin proteins. The solubility after pepsin and TCA precipitation was less than 10% for all pigskin and tendon samples but pepsin and pancreatin treatment resulted in TCA-soluble peptides. Heated samples had a bigger particle size than the corresponding unheated samples. The overall effect of heat treatment was statistically insignificant when considering both pepsin and pepsin/pancreatin/TCA solubilities. Solubilization of unheated old pigskin protein and insoluble collagen was highly influenced by pH during the pepsin incubation. Already at pH 2.0–2.5 the solubility decreased reaching about 50% at pH 3. Further treatment with pancreatin only slightly increased the solubility values. Gelatin and meat samples were highly solubilized by pepsin and pancreatin and not precipitated by TCA. The prominent pH effect was not observed for these samples. The solubilization data of unheated pigskin and raw tendon indicate that the digestibility of collagen could be high also *in vivo*. However, pH during the pepsin incubation seems to be critical for degradation of insoluble collagen.

Introduction

With the exception of collagenases, which are unique in their ability to degrade native collagen to small dialysable peptides, collagen is extremely resistant to proteolytic enzymes. Enzymic degradation of native collagen has been studied by several authors and reviewed extensively in connection with the leather industry (Gustavson, 1956), gelatin manufacture (Balian & Bowes, 1977; Johns & Courts, 1977), biochemistry and medicine (Mandl, 1961; Weiss, 1976; Bailey & Etherington, 1980) and tenderness of meat (Sørensen, 1976; 1981). In the literature data on the digestibility of collagenous food products are conflicting and have been under discussion since at least 1910 (Baumstark & Cohnheim, 1910). Studies performed are partially reviewed by Brüggeman *et al.* (1964), Loewit (1967; 1970) and Ashgar & Henrickson (1982). It is generally accepted that native collagen resists digestion (Cheftel, 1977; Prandl, 1980; Kies, 1981; Ashgar & Henrickson, 1982). In order to become digestible, wet heat treatment of the collagen is regarded necessary resulting in the formation of gelatin

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(Rogowski, 1980). According to this view denaturation by cooking makes collagen susceptible to enzymes (Paul, 1972; Ashgar & Henrickson, 1982) and thus digestible (Cheftel, 1979).

Only a few *in vitro* studies on enzymic degradation of highy collagenous materials using pepsin and hydrochloric acid (HCl) at 37°C have been performed. Neuman & Tytell (1950) treated Achilles tendon and solubilized about 95%. Franke (1964) found that both raw and heated pigskin were about 97% 'digestible'. On the other hand Loewit (1967), treating samples with pepsin/HCl and pancreatin, reported the 'digestibilities' of raw pigskin and tendon to be only 38% and 48% respectively. After cooking, the 'digestibilities' increased to 100% and 79%, respectively. Harkness, Harkness & Venn (1978) treated rat tail tendon and bovine tendon with pepsin/HCl and achieved a solubilization of 97% and 37% respectively. In these investigations solubility was used as the measurement of digestibility. However, it should be noted that the collagen helix can be solubilized intact after disruption of the telopeptide region by pepsin (Weiss, 1976). Thus, the solubility of collagen does not necessarily indicate the extent of degradation. The *in vitro* studies mentioned above have all been performed on collagenous materials from animals of unspecified ages. It is known that collagen from a growing animal shows increasing resistance to solubilizing agents, such as pepsin, with age (Weiss, 1976; Sims & Bailey, 1981).

In the present investigation solubilization of pigskin and bovine tendon protein after pepsin and pancreatin treatment, and simulated conditions in the stomach and small intestine were studied. The influence of: (a) pH during incubation with pepsin (b) the extent of trichloroacetic acid precipitation after solubilization (c) age of animal and (d) prior heating of pigskin and tendon were of special interest.

Materials and methods

Preparation of samples

Pigskins. Skins were removed immediately after slaughter from a 4-month-old pig and a 5-year-old sow. The pigskins were manually shaved with a razor. Another skin was taken from a 6-month-old pig which had been scalded according to slaughterhouse procedures. All the pigskins were freed of visible fat and meaty parts. Pigskin samples were finely minced in a Moulinex blender. Half of the samples were vacuum packed in thicknesses of less than 1 cm, heated in a waterbath at 74°±1°C for 30 min and immediately chilled. The heated samples were finely reminced in a Moulinex blender. All samples were freeze dried. The unheated samples were then minced in a knife homogenizer (Janke & Kunkel) and the heated samples in a Moulinex blender.

Tendons. Achilles tendons were collected from forty 7-month-old calves and from forty cows with an average age of 6.6 years (range 4–12.5). The calf tendons were collected 24 hr and the cow tendons 1–6 days after slaughter. Samples were freed of visible fat and meaty parts and frozen for not more than 2 months. After thawing, the tendons were cut into smaller pieces and mixed into one calf and one cow sample. The wet calf and cow samples were ground and passed through a screen of 3 mm before being immediately chilled. The temperature of the calf sample reached 32°C and the cow sample 44°C during grinding. Half of the cow sample was packed and heated as described for the pigskin samples above, and minced in a Moulinex blender. The

unheated calf and cow samples as well as the heated cow sample were freeze dried. Parts of the samples were homogenized in a hammer mill.

Meat. Meat of low collagen content was taken from the top round (*M. semimembranosus* and *M. adductor*), minced in a Moulinex blender, frozen, freeze dried and reminced in a Moulinex blender to a fine powder. Skin, with relatively high amounts of collagen, was freed of visible fat and meaty parts, minced in a Moulinex blender and frozen. The sample was thawed and used wet as the collagen fibres were difficult to cut after freeze drying.

Commercial samples. Insoluble collagen (bovine Achilles tendon) prepared according to Einbinder & Schubert (1951) and gelatin from swine skin (175 Bloom) were purchased from Sigma Chemical Company, U.S.A.

Determination of particle size

All freeze dried samples were surveyed under a low magnification microscope. The particle diameter was estimated and particles were photographed.

Enzymes

Porcine pepsin NF (Merck) and pancreatin from porcine pancreas 4 NF (Sigma Chemical Company, U.S.A.) were used. The enzymes were routinely checked for hydroxyproline-blank. The collagen content was 23.5% for pepsin and 3.9% for pancreatin expressed as collagenous nitrogen versus total nitrogen.

Pepsin incubation

Samples with 0.08 g nitrogen were mixed with 44 ml of 0.03 M HCl and the pH was measured before and after incubation. The initial pH values were 1.35–1.53 for all pigskin, tendon and gelatin and insoluble collagen samples and 1.62 for the meat samples. The pH values after incubation generally increased by 0.05 but in a few cases by 0.30 units. One ml of pepsin solution (50 mg) was added and incubation was performed in a shaking waterbath at 37°C for 2 hr. Samples were then chilled, the pH adjusted to 7.0 with 4 N NaOH and water was added up to 50 ml. Suction filtering through a glass filter with a porosity of 15–40 μm was used. An aliquot of the filtrate was precipitated with trichloroacetic acid (TCA) 1:1, usually to a final concentration of 25% w/v. After standing at +5°C overnight, samples were filtered through an OOH filter to obtain a clear solution.

Pepsin/pancreatin incubation

After the pepsin incubation, the pH was adjusted to 6.8 with 4 N NaOH and up to 50 ml of water was added up to 50 ml. Thereafter 25 ml of 0.1 M sodium phosphate buffer pH 6.8 was added as well as 50 mg of pancreatin. Incubation was performed at 37°C for 2 hr, samples were chilled in ice water and suction filtered as above. An aliquot of the solution was treated with TCA 1:1 usually to a final concentration of 6.5% w/v, left standing overnight at ±5°C and filtered as above.

Different incubation conditions

Young and old unheated pigskin samples were also studied with some other incubation conditions: (a) 0.03 M HCl for 2 hr followed by 0.1 M sodium phosphate buffer for 2 hr; (b) pancreatin for 2 hr or 4 hr; or (c) 0.03 M HCl for 2 hr followed by pancreatin for

2 hr. The TCA used was of a final concentration of 25% w/v. The effect of the HCl concentration (0.03 M–0.0001 M) was also studied during incubation with pepsin for 2 hr followed by pancreatin incubation for 2 hr and precipitation with TCA (final concentration 25% w/v). This was performed on old unheated pigskin and insoluble collagen, gelatin and non-collagenous meat samples.

Chemical analyses

Analyses were performed on each fraction for nitrogen and in some cases for hydroxyproline. Corrections were made for blank values obtained with enzymes only under the same conditions. All incubations were performed on five parallel samples unless otherwise indicated. Nitrogen was determined on powdered samples and on solutions using the Kjeldahl method. Hydroxyproline was determined using a Technicon Auto-Analyser on duplicate samples, according to Stegemann (1958) as modified by Weber (1973). Calculations of collagen and protein contents were made as described previously (Laser Reuterswärd *et al.*, 1982). Water content on all powdered samples was determined by drying overnight at 104°C.

Statistical evaluation

Means and standard deviations were calculated and analysed by Student's *t*-test for small samples and unknown variances assumed equal (Bailey, 1981).

Results

Composition and structure

The water content of the freeze dried samples varied between 1.1 and 7.8%. Protein and collagen contents are shown in Table 1. The variations in protein content were due mainly to the varying amounts of fat. Some representative microphotographs are shown in Fig. 1. Unheated pigskin samples had a more porous, crystalline-like, tight structure than the corresponding heated samples, which were aggregated and transparent. The particle sizes of unheated young and old pigskin samples were the same. The particles of the corresponding heated samples were much larger. The size of pigskin samples were 1–3 mm for the old heated pigskin, 3–4 mm for the young heated pigskin and 2–4 mm for both the young scalded and scalded/heated pigskins. Unheated, non hammer milled tendon samples looked spongy, the calf sample had a particle size of 2–3.5 mm and the cow sample 1–2.5 mm. Unheated, hammer milled calf and cow tendon samples were porous and crystalline-like (Fig. 1e) and the heated hammer milled cow tendon sample showed more tight particles (Fig. 1f). Insoluble collagen was porous but had smaller particles than pigskin and tendon samples. Gelatin and meat samples had extremely small particles, the gelatin being transparent.

TCA solubility

TCA is normally used for precipitating proteins from peptides (Oser, 1965). However, at a 5% TCA concentration collagen is still soluble and can be separated from other proteins (Harkness *et al.*, 1978). Therefore final concentrations of zero to 25% w/v of TCA were tested using samples from meat and old unheated pigskin.

After treatment with pepsin/HCl at pH 1.5 the concentration of TCA weakly influenced the precipitation of meat proteins with a linear regression equation of $y = -9.53x + 0.134$ and a linear regression coefficient significantly different from zero ($P < 0.01$). For pigskin, TCA concentrations equal to or more than 15%, gave sig-

Table 1. Composition and solubilization of pigskin, bovine Achilles tendon, insoluble collagen, gelatin and meat samples. Solubilities are expressed as a % of soluble nitrogen of initial nitrogen

Sample	Composition (%)		Solubility (%)			
	Protein/dry weight	Collagen/ protein	Pepsin	Pepsin/ TCA	Pepsin/ pancreatin	Pepsin/ pancreatin/ TCA
Pigskin						
Young, 4 month unheated	47.4	71.8	84.1 (3.9)	6.8 (0.6)	92.0 (3.5)	94.2 (4.0)
heated	52.1	71.7	86.3 (1.7)	5.9 (0.6)	98.4 (3.0)	97.0 (2.1)
Young, 6 month scalded	58.1	71.3	78.8 (2.3)	4.7 (0.4)	82.6 (4.0)	82.6 (3.5)
scalded/heated	63.5	73.7	88.6 (4.0)	6.2 (1.0)	94.5 (1.7)*	96.5 (2.9)*
Old, 5 year unheated	70.2	76.1	72.8 (1.8)	5.7 (0.8)	76.5 (2.3)	75.2 (3.5)
heated	70.8	77.6	60.8 (1.4)	3.6 (1.1)	79.4 (3.2)	81.4 (1.6)
Tendon						
Calf, 7 month unheated, not HM	93.5	92.5	78.9 (0.8)	5.2 (0.5)	86.6 (3.3)	86.8 (1.7)
Calf, 7 month unheated, HM	93.5	92.5	86.0 (3.1)	6.5 (0.6)	90.4 (1.6)	92.4 (8.9)
Cow, 6.6 year unheated, not HM	99.7	96.7	81.8 (3.8)	4.7 (1.3)	83.8 (3.5)	83.5 (3.7)
Cow, 6.6 year unheated, HM	99.7	96.7	87.5 (5.7)	10.3 (0.7)	92.7 (1.7)	91.9 (2.4)
Cow, 6.6 year heated, HM	97.2	94.8	88.0 (2.2)	5.6 (1.0)	91.9 (2.1)	92.8 (0.9)
Insoluble collagen	102.2	96.4	91.4 (2.2)	2.4 (0.5)	92.2 (3.6)	92.5 (3.0)
Gelatin	99.8	92.0	93.0 (0.9)	8.0 (0.7)	101.0 (2.1)	100.0 (2.3)
Meat, top round	94.2	1.8	94.9 (1.5)	72.4 (1.4)	100.6 (2.0)	100.5 (1.7)
Meat, shin	72.4	16.5	92.2 (2.3)	56.7 (2.3)	91.5 (1.8)	91.8 (2.4)

Incubation was performed with pepsin at pH 1.5 for 2 hr; or pepsin at pH 1.5 for 2 hr followed by pancreatin at pH 6.8 for 2 hr at 37°C. Precipitation was performed with 25% w/v or 6.5% w/v of Trichloroacetic acid (TCA). Heat treatment was performed at 74°C for 30 min, $n = 5$, standard deviations within brackets. HM = hammer milled.

*$n = 3$.

nificantly lower solubilities ($P < 0.001$) than the constant level found when using 0, 5 and 10% TCA. After pepsin/pancreatin treatment, six concentrations of TCA ranging from zero to 25% were tested, but the linear regression coefficient was not significantly different from zero ($P > 0.05$) for either the meat or the pigskin samples. For precipitating samples, treated with pepsin/HCl at pH 1.5 followed by pancreatin, a TCA concentration of 6.5% w/v was chosen but for all other samples a concentration of 25% w/v TCA was used.

The TCA-solubilities after only pepsin incubation were less than 10% for all the pigskin and tendon samples, likewise gelatin, and independent of age and heat treatment (Table 1). The TCA-solubilities after pepsin/pancreatin treatment (Table 1) showed no significant difference from values without TCA precipitation, when all values were tested with a paired *t*-test.

Effect of age and heat on solubilization

In Table 1 the results of solubilization with pepsin and pepsin/pancreatin are shown for pigskin, tendon, insoluble collagen, gelatin and meat samples. Since the particle sizes of the unheated pigskins were the same, this factor could not influence the comparison of various samples from animals of different ages. The young unheated pigskin sample was significantly ($P < 0.001$) more solubilized than the old unheated pigskin sample. This was true both for solubilities after pepsin treatment alone and after

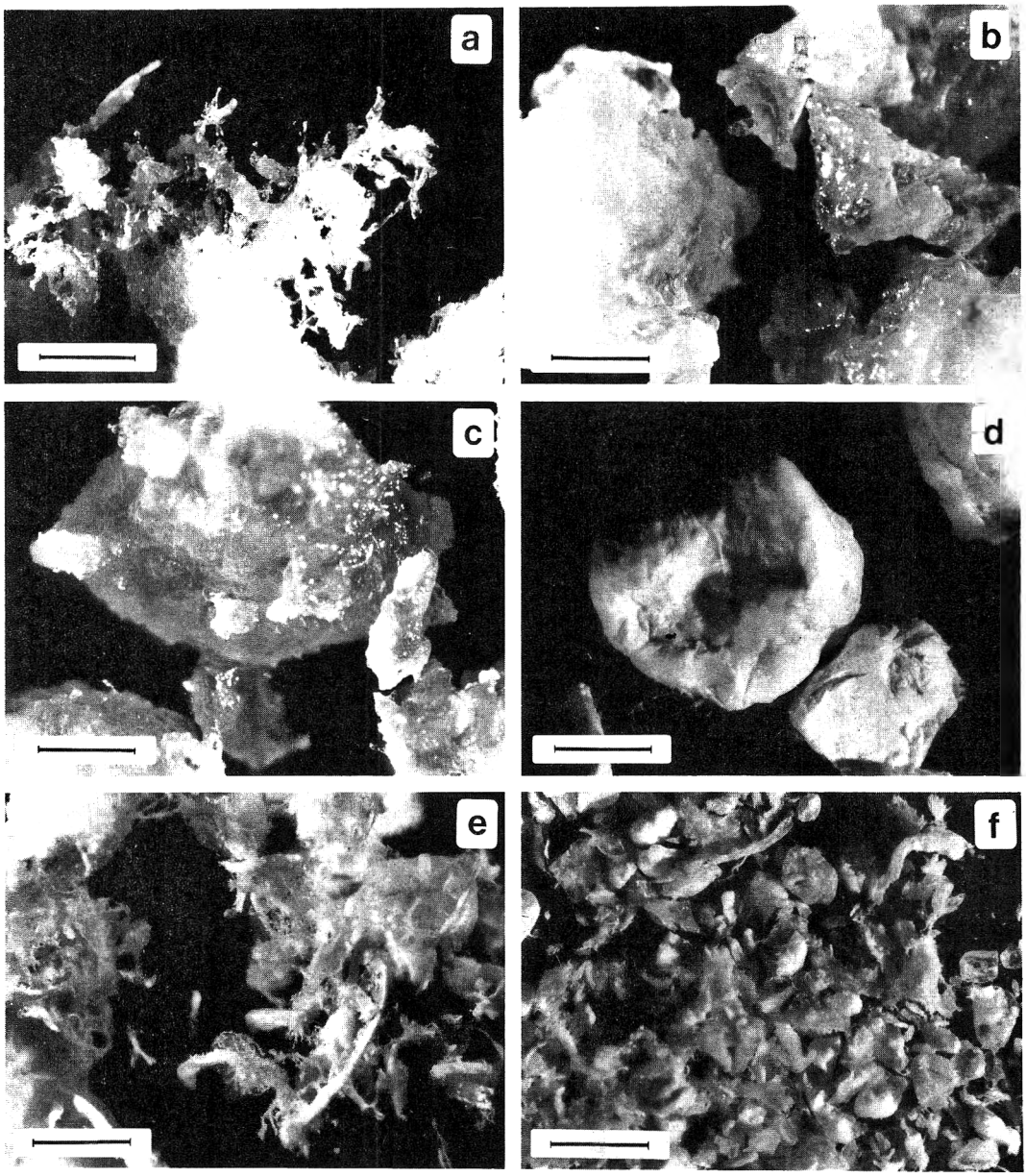

Figure 1. Microscope photographs of pigskin and bovine Achilles tendon samples, freeze-dried and minced: homogenized (Janke & Kunkel) = JK, Moulinex blender = MB and hammer milled = HM. Heat treatment was performed at 74°C for 30 min. The scale shown on the photographs is equivalent to 1 mm. (a) Young (4 month) unheated pigskin, JK; (b) young (4 month) heated pigskin, MB; (c) young (6 month) scalded pigskin, MB; (d) cow (6.6 year), MB; (e) cow (6.6 year), HM; (f) cow (6.6 year), heated, HM.

Table 2. Solubilization of unscalded, unheated young and old pigskins

In vitro design				Solubility (%)				TCA solubility (%)			
				Young		Old		Young		Old	
HCl	HCl/ pepsin	Buffer	Buffer/ pancreatin	Nitrogen*	HPRO†	Nitrogen	HPRO	Nitrogen	HPRO	Nitrogen	HPRO
2 hr	—	2 hr	—	29.7 (1.7)	20.2	10.3 (1.2)	5.2	4.0 ‡	—	2.7‡	—
—	—	—	2 hr	12.8 (0.8)	—	15.0 (0.8)	—	11.8 (1.8)	—	12.5 (0.7)	—
—	—	—	4 hr	15.3 (0.4)	—	16.1 (1.4)	—	—	—	—	—
2 hr	—	—	2 hr	62.5 (3.0)	—	28.3 (2.7)	—	60.6 (4.0)	64.2	28.1 (4.7)	26.6
—	2 hr	—	—	84.1 (3.9)	86.3	72.8 (1.8)	64.2	6.8 (3.9)	2.8	5.7 (0.8)	2.8
—	2 hr	—	2 hr	92.0 (3.5)	91.7	76.5 (2.3)	69.7	94.2 (4.0)	94.6	72.2 (3.5)	71.8

Samples were incubated in hydrochloric acid (HCl) at pH 1.5, sodium phosphate-buffer/pH 6.8, pepsin and pancreatin at 37°C. Solubilities are expressed as % soluble nitrogen of the initial nitrogen and % soluble hydroxyproline (HPRO) of the initial HPRO. Trichloroacetic acid (TCA) at 25% w/v was used (TCA-solubility).

*Nitrogen, $n = 5$ from incubation to Kjeldahl analysis, standard deviations within brackets.

†HPRO is duplicated on one sample with a nitrogen value close to average.

‡$n = 1$.

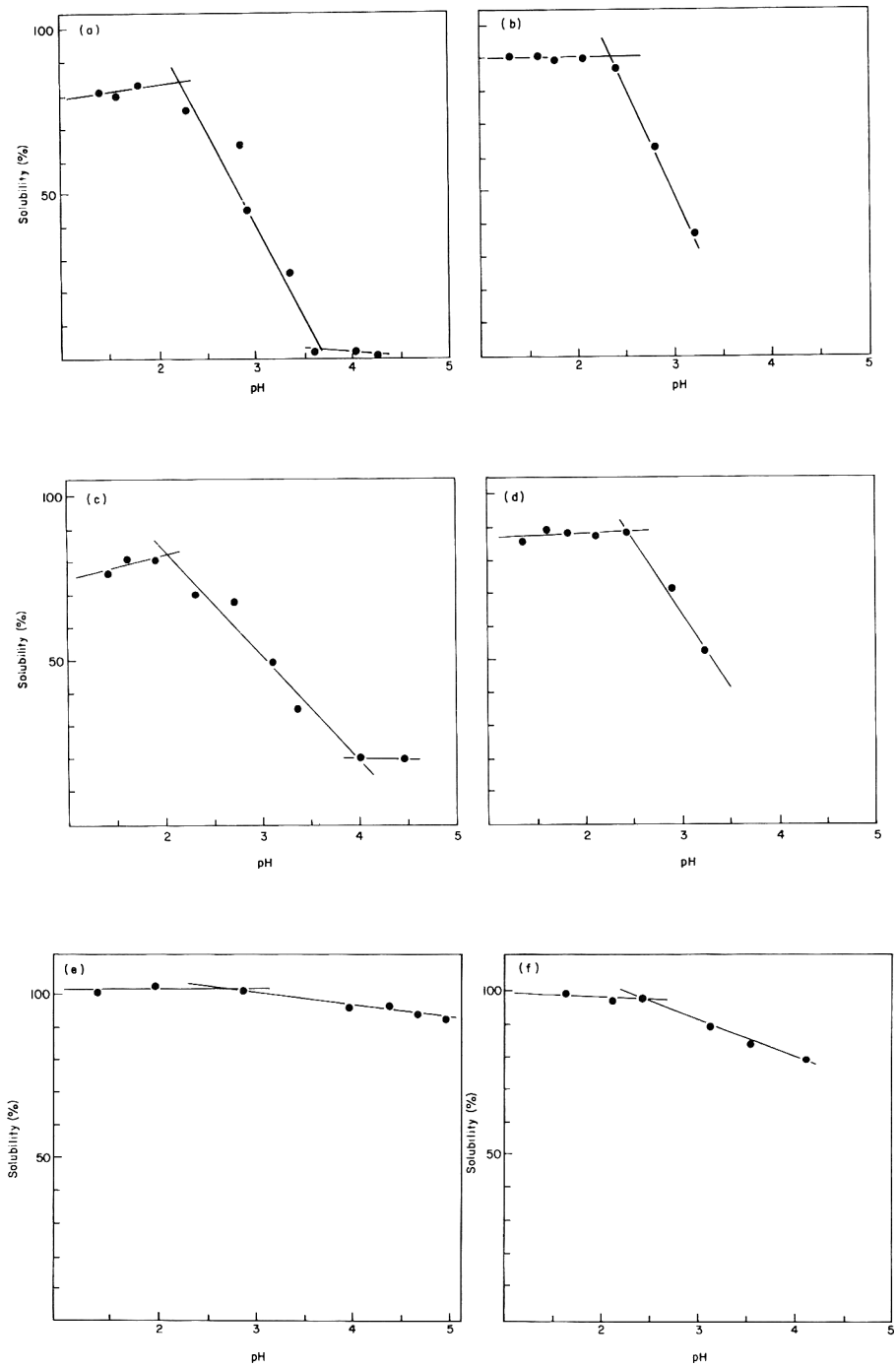

Figure 2. Nitrogen solubility, as a % of initial nitrogen, after incubation for 2 hr at 37°C with pepsin and hydrochloric acid at different pH, in (c), (d), (e), (f) followed by incubation for 2 hr at 37°C with pancreatin, pH 6.8. The pancreatin digests were precipitated with final concentration of 25% w/v of trichloroacetic acid. $n = 3$, standard deviations < 6.0. (a) Old (5 year) unheated pigskin; (b) insoluble collagen; (c) old (5 year) unheated pigskin; (d) insoluble collagen; (e) gelatin; (f) meat, top round.

pepsin/pancreatin/TCA treatment. In spite of the somewhat bigger particle size of the young heated pigskin sample this was significantly ($P < 0.001$) more soluble after pepsin, as well as after pepsin/pancreatin/TCA than the old heated one. Thus age had an influence on the solubilization of both unheated and heated pigskin samples.

The overall effect of heat treatment was insignificant according to the paired *t*-test, when including pepsin and pepsin/pancreatin/TCA solubilities. However, all heated samples had a bigger particle size than the corresponding unheated samples. This could have counteracted a heat effect. For the young pigskin sample no significant difference was found after heat treatment either for pepsin solubility or pepsin/pancreatin/TCA solubility. For the old sample a significantly lower ($P < 0.001$) value for the heated sample was obtained after pepsin treatment alone but a significantly higher ($P < 0.01$) value for the pepsin/pancreatin/TCA solubility. The young scalded sample had the same particle size as the scalded/heat sample and in this case a significant ($P < 0.01$) increase in solubility was obtained for both the pepsin solubility and the pepsin/pancreatin/TCA solubility due to heat treatment. The young scalded sample showed significantly lower solubility ($P < 0.05$) than the young unheated (unscalded) one, both as pepsin and pepsin/pancreatin/TCA solubility. This was probably due to the larger particles in the scalded sample.

For the tendon samples hammer milling significantly increased the solubility ($P < 0.01$) for the calf and cow samples, both after pepsin alone and after pepsin/pancreatin/TCA treatments. The particle size thus influenced the solubility of tendon samples. For the tendon samples no significant age effect was found either for the hammer milled calf and cow samples, or for the non-hammer milled calf and cow samples. This was true for both the pepsin and the pepsin/pancreatin/TCA solubilities.

Insoluble collagen, with its smaller particles, showed a significantly higher ($P < 0.05$) solubility in pepsin than the hammer milled calf sample, but an insignificant difference compared to the hammer milled cow tendon sample. The pepsin/pancreatin/TCA solubility was, for insoluble collagen, not significantly different from either the hammer milled calf or the cow samples. No significant difference in solubility (pepsin and pepsin/pancreatin/TCA) was found after heat treatment of the cow sample compared to the unheated hammer milled one. Significantly higher values (pepsin and pepsin/pancreatin/TCA) were found when comparing the heated cow sample to the non-hammer milled sample ($P < 0.05$). After pepsin treatment, the solubility of gelatin was not significantly different from that of insoluble collagen, but after treatment with pepsin/pancreatin/TCA a significantly higher ($P < 0.01$) solubility was obtained for gelatin than for insoluble collagen. The collagenous meat sample had a pepsin/pancreatin/TCA value significantly lower ($P < 0.01$) than the completely solubilized meat sample.

The effect of incubation conditions

Table 2 shows the impact of different combinations of HCl, buffer and enzyme incubations on the solubilities of unheated pigskins from young and old animals. Generally the older pigskin was much less solubilized than the young sample, except after the pancreatin treatment. The HCl/buffer solubilized only a small amount of nitrogen. Pancreatin treatment, for 2 hr or 4 hr, solubilized even less nitrogen. Pretreatment with HCl gave a significant ($P < 0.01$) increase in pancreatin solubilization for both the young and the old samples. TCA did not precipitate nitrogen solubilized by pancreatin treatment. The solubility of especially the old pigskin sample was much greater after incubation in HCl/pepsin than in HCl and pancreatin alone. Pancreatin

degraded all the HCl/pepsin solubilized nitrogen to TCA-soluble nitrogen, both in young and old samples. The hydroxyproline solubility showed the same pattern as the nitrogen solubility.

Figure 2 shows the effect of pH during incubation with pepsin alone or with pepsin/pancreatin/TCA treatment on the old unheated pigskin, insoluble collagen, gelatin and non-collagenous meat samples. At a critical pH the solubilities started decreasing with different slopes. For the old unheated pigskin and insoluble collagen this critical point, for the pepsin as well as for the pepsin/pancreatin/TCA solubilities, was in the range of pH 2.0–2.5. At pH 3.0 the solubilities were about 50%. For the gelatin sample a small decrease started around pH 3.0, but at pH 5.0 the solubility was still 92%. For the meat sample the critical pH was around 2.5, the solubility decreasing to 80% at pH 4.0.

Discussion

Preparation of samples

The samples used were prepared by grinding wet material and freeze drying and in some cases by hammer milling the freeze dried samples. The temperature of the wet tendons after grinding was less than 44°C. Gustavson (1956) reported that grinding of native collagen could result in somewhat increased solubility after trypsin treatment. However, the denaturation temperature is about 65°C for wet collagen fibres (Bailey, 1968) and much higher for dried fibres (Finch & Ledward, 1972). Hammer milling of the tendon samples resulted in smaller particles (Fig. 1e,f) and significantly increased solubilities. This is in accordance with Banga & Balo (1965) who also obtained a higher solubility of air dried hammer milled Achilles tendon than of fibres treated with collagen mucoproteinase. This was explained as being caused by physical disintegration.

Freeze drying is a procedure normally used when studying the chemical crosslinks of collagen (Light & Bailey, 1980). However, water undoubtedly has a fundamental role in maintaining the native collagen structure and when dehydrated collagen loses its regular structure (Privalov, 1982). This also occurs after freeze drying (Bailey, Robins & Balian, 1974). Adding water to dehydrated collagen again restores the structure (Privalov & Tiktopulo, 1970). It is unknown, however, if full structural restoration occurred during the relatively short incubation time used in the present study. The freeze drying would not have overestimated the solubility values since freeze drying is known to result in decreased solubility of fibres (Dutson, 1976).

Acid solubility

The amount of soluble material in young and old unheated pigskin, without the action of added enzymes, is shown in Table 2 as the acid soluble and neutral salt soluble collagen (Johns, 1977). The hydroxyproline solubility was 20.2% for young and 5.2% for old pigskin, values that are somewhat higher than those reported generally for skin by Johns (1977) and Ashgar & Henrickson (1982). The lower solubility of hydroxyproline in the old sample was expected since it is known that in collagen, while aging *in vivo*, the acid labile intermolecular crosslink aldimine is converted into a stable form (Bailey *et al.*, 1974; Sims & Bailey, 1981). The pigskin material used also included non-collagenous proteins; for young pigskin 28.2% and for old 23.9% of the total protein. The difference of solubility in acid/buffer between total protein (nitrogen) and

total collagen (hydroxyproline) (Table 2) is an estimate of solubilized non-collagenous proteins. For young pigskin the non-collagenous proteins amounted to 9.5% and for old pigskin to 5.1%. This showed that the non-collagenous proteins were not totally solubilized, although Johns (1977), indicated that these proteins could be extracted in acid.

The effect of pH on swelling and denaturation

Solubility after pepsin treatment at pH 1.5 showed quite high values; more than 84% for the unheated samples of young pigskin, tendon and insoluble collagen. These results are probably mainly due to the swelling effect of acid treatment. HCl has been shown to act with an osmotic swelling effect on collagen between pH 1–4, with a maximum at pH 2 (Gustavson, 1956). Apparently the interfibrillar space of collagen has to be sufficiently expanded for removing the steric hindrance of enzyme action (Bailey & Etherington, 1980). The lower values of pepsin solubility for the old unheated pigskin sample (Table 1) could be explained as a limited swelling capacity. A tighter structure is known to occur in mature collagen where the fibres are linked in register between pentafibrils (Bailey, 1982). The swelling of the freeze dried material used was obviously sufficient for the action of pepsin. Gustavson (1956) noted only 15% lower swelling values in HCl for dried hide when compared to a fresh sample.

Pepsin is known to solubilize collagen. However, it is generally accepted that the enzyme is not capable of disrupting the helical configuration of collagen but attacks only the telopeptides (Weiss, 1976). The way it acts on insoluble collagen is by cleaving one monomer close to its helix so that the intermolecular crosslink remains linked to the adjacent helix (Sims & Bailey, 1981). The lower solubilities obtained when using higher pH values in the pepsin/HCl step (Fig. 2) could be due to an insufficient swelling effect or perhaps to a lowered pepsin activity. It has been indicated that denaturation of insoluble collagen can occur only after solubilization of the monomer (Etherington, 1977) and denaturation is necessary for further degradation by pepsin or pancreatin. The unheated samples solubilized at pH 1.5 by pepsin at 37°C should have denatured to a large extent since the denaturation temperature for the monomer is pH-dependent, decreasing from 37°C at pH 3.8 down to 32°C at pH 1–2 for, for example, soluble calf skin (Dick & Nordwig, 1966). It is generally accepted that the acidic pH of the stomach causes protein denaturation (Cheftel, 1979). For insoluble collagen HCl seems to be necessary for three consecutive steps (i) to induce swelling (ii) to give an optimal pH for pepsin action and (iii) to lower the denaturation temperature of the helix.

Pepsin and pancreatin action in degrading the monomer

The low TCA solubility of nitrogen obtained after pepsin treatment of pigskin, tendon, insoluble collagen and gelatin samples (Table 1) indicated that only small amounts of the collagen monomer and gelatin had been degraded to peptides. These results could be explained by the fact that pepsin is known to cleave only at sites close to tyrosin and valine in gelatin (Weiss, 1976). These amino acids occur in very small amounts in the pigskin and tendon samples (Laser Reuterswärd, Asp & Björck, 1984) as well as in gelatin (Eastoe, 1967). The higher TCA solubility values obtained for meat samples after pepsin digestion should be due to the larger amounts of these amino acids in meat proteins (Lawrie, 1979).

Low solubilities were obtained for both young and old pigskin samples after pancreatin treatment only (Table 2). After pre-swelling in HCl the solubilities increased significantly. The results of old unheated pigskin and insoluble collagen (Fig.

2) indicated that the same pH value was critical for the solubilization by pepsin as well as by pepsin/pancreatin. The data also show that the collagen solubilized in the pepsin/HCl step was further degraded by pancreatin to TCA soluble peptides. These results confirm the necessity of swelling for the susceptibility to enzyme action. Trypsin and chymotrypsin do not disrupt the collagen helix itself but attack only the non-helical telopeptides (Weiss, 1976). Table 2 also shows that the solubilities with and without TCA after HCl/pancreatin treatment were not significantly different. This indicates that the initial pancreatin solubilization was followed by denaturation of the monomer at 37°C and then by further degradation.

Figure 2 shows the TCA solubilities at varying pH for insoluble collagen and gelatin. Gelatin, which is randomized and water soluble, was almost completely degraded to TCA soluble peptides after pepsin/pancreatin treatment independent of the pH in the HCl/pepsin step. This was not true for insoluble collagen. In conclusion, the results showed that pancreatin degrades all the pepsin solubilized collagen to TCA soluble peptides.

Heated samples

The data in Table 1 show that unheated pigskin and tendon samples were highly solubilized and degraded by pepsin/pancreatin. A positive effect of heat treatment on solubilization was only obtained for some samples and the overall effect was not significant. Heat treatment performed at 74°C should have caused denaturation since insoluble fibres are known to denature at their shrinkage temperature. This occurs at 27°C higher than the denaturation temperature of soluble collagen, 36–41°C, but depends on the species (Bailey, 1968; Privalov, 1982).

Scalding of pigs is a mild heat treatment occurring in water at 60°C for 10 min. Some of the soluble pigskin collagen in immature pigs could be sufficiently labile to be denatured by scalding (Johns & Courts, 1977). The particles of the young scalded pigskin were bigger than the unscalded sample and looked more like the heated pigskin samples (Fig. 1). Since the particle size of the scalded and the heated samples were bigger than the corresponding unheated ones the possible positive effect of heating on solubilization could have been counteracted. Thus for the old pigskin sample the unexpectedly lower pepsin solubility after heating (Table 1) could probably also be due to the bigger particle size of the heated sample. A significant age effect was detected when comparing the solubilities of young and old heated pigskins. In old skin, as well as in young and old tendon, heat stable crosslinks are known to occur (Weiss, 1976; Sims & Bailey, 1981). In the present study, however, incubation with pepsin and pancreatin was more effective in solubilizing old tendon than old pigskin ($P < 0.01$). No significant age difference was obtained when solubilizing tendons at pH 1.5 with pepsin (Table 1). This is not in accordance with the findings of Etherington (1977) who obtained solubilities of 80% for 2-year-old and 65% for 10-year-old insoluble collagen from Achilles tendon. The conditions were different since he used pepsin/acetic acid (2 hr, 37°C) at pH 2.65.

Other studies

Neumann & Tytell (1950) obtained high solubility values, 95%, after pepsin treatment (pH 2, 37°C, 24 hr) of the Achilles tendon from cattle. Their results are in accordance with the value for insoluble collagen in the present study. Franke (1964) did not notice any differences in the pepsin digestibility of raw and heated pigskin. Both were about 97% digestible but no specification was given on scalding or incubation

conditions. The low digestibility, 38% and 48%, obtained by Loewit (1967) on raw pigskin and tendon after treatment in pepsin at pH 2.7 at 37°C for 3 hr, followed by pancreatin for 14 hr, could probably depend on the high pH value used. The large differences obtained by Harkness *et al.* (1978), in the solubility of rat tail and bovine tendon, 97% and 37%, after treatment with pepsin/HCl (pH 1.5, 37°C, 2 hr) were explained as a possible age effect, but this does not agree with the present results. Harkness *et al.* (1978) also compared human gastric juice to (unspecified) pepsin on the solubility of rat tail tendon, but did not find any differences.

Conclusions

Unscalded pigskin and unheated tendon were highly solubilized (75–94%) when incubated with pepsin and hydrochloric acid at pH 1.5 and pancreatin at pH 6.8 followed by TCA precipitation. A significantly lower solubility was obtained for pigskin from an old animal than from a young one. No age effect was recorded for tendons from calves and cows.

The pH during incubation with pepsin and hydrochloric acid was critical for the solubilization of the collagenous samples but not for the gelatin and meat samples. At a pH of about 2.5 the solubility started to decrease. Further treatment with pancreatin only slightly increased the solubility but had a prominent effect on the TCA solubility.

Solubilization of insoluble collagenous samples such as unheated pigskin and raw tendon after pepsin and pancreatin treatment indicate that digestibility could be high *in vivo*. Confirmation of these data should be performed *in vivo* however, since degradation of insoluble collagen is very sensitive to variations of pH during incubation with pepsin in hydrochloric acid.

Acknowledgments

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Effect of low-dose irradiation (radurization) on the shelf life of beefburgers stored at 3°C

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Summary

The bacteriological, chemical (FFA and peroxide value) and organoleptic quality of stored (3°C) radurized beefburgers were investigated. Treatment with gamma radiation at doses of 0.103 and 0.154 Mrad (1.03 and 1.54 kGy) resulted in an immediate reduction in total bacterial numbers of 82 and 92%, respectively. No 'pseudomonad-like' organism survived irradiation. Beefburger surface colour was influenced more than internal colour by irradiation; the larger dose (154 krad, 1.54 kGy) having the more pronounced effect. At the end of 15 days storage (3°C) 'spoilage' odours were not detected in the 1.54 kGy treated burgers but were detected in those irradiated at 103 krad (1.03 kGy). However 'irradiation' odours were detectable at both levels of treatment when burgers were exposed to the air. Irradiation had a larger effect on peroxide formation than on free fatty acid formation.

Introduction

Fresh beef has a limited shelf life at refrigeration temperatures. In efforts to extend this, attempts have been made to improve operational hygiene and to maintain the 'cold chain' (Patterson, 1967). Hygiene and temperature control in the processing of (fresh) meat therefore assume prime importance. Because of this, a continuous quest for other methods of preservation is made and one which shows promise is a low dose of less than 1 Mrad (< 10 kGy) ionizing radiation (Neimand, van der Linde & Holzapeel, 1981).

Irradiation processing of food has advantages, for example, radurization or radiation pasteurization significantly increases the storage life of meat dishes, eviscerated poultry and ground meat (Wierbicki *et al.*, 1975). Equally important advantages have been claimed for radicidation (reduction in the number of specific pathogens to a level where none are detectable) e.g. *Salmonella* in poultry and the elimination of *Trichinella spiralis* and *Taenia saginata* in pork and meat, respectively. However these benefits can be offset if the radiation dose is not controlled because high doses induce discolouration and flavour changes in meats (Batzer *et al.*, 1959); Hedin, Kurtz & Koch, 1960; Wick *et al.*, 1967). However, when proper conditions (dose and temperature) are employed, wholly acceptable products which are both safe and palatable can be produced. This study was made to investigate some of these conditions and to quantify their effects on the microbial status, organoleptic quality and chemical composition of beefburgers.

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Materials and methods

Beef

Freshly ground beef (24–25% fat) was purchased in a local supermarket in lots of *circa* 9 kg on each of four occasions. The beef was triple ground. Burgers were formed by weighing 90 g of mince into a sterile plastic Petri dish (15 × 100 mm). The formed burgers were placed in 'Cryovac' bags and a vacuum drawn on an AG 500 Busch multivac machine (Sepp. Haggenmuller KG, D8941 Wolfertschwenden, West Germany). The packs were randomly assigned to the following treatment categories:

(i) Frozen control (FC); burgers were immediately frozen at −45°C and then stored at −29°C. Before evaluation, burgers were thawed in a cold water bath for 15 min. These provided panellists with a reference similar to freshly ground beef which was not used since it would vary from day to day. Some were labelled reference (R).

(ii) Raw control (unirradiated) burgers; these were held at 3°C until required.

(iii) Burgers were cooled in an ice water bath at 0°C and irradiated. The source was a Gammacell (Atomic Energy of Canada Limited, Ottawa) containing 800 Ci ^{60}Co. The dose was *circa* 1.03 krad/min (10.3 Gy/min), as measured using the Fricke dosimetry method (Spinks & Wood, 1976) and the duration was 100 min. The dose absorbed in the meat sample was measured by the above method and was slightly less than the irradiation dose (approximately 90%) because of burger density and variation within the irradiation chamber. The temperature rise in the sample during irradiation varied between 0–4°C. This product was designated IR 103.

(iv) These were similarly treated as (iii) above except that burgers were irradiated for 150 min. They were designated IR 154.

Storage

Burgers were evaluated on the day of preparation (day 0) and subsequently at 4, 8, 11 and 15 days of storage at 3°C. The above procedure was replicated four times using four different lots of ground beef.

Microbial analyses

Two burgers from each treatment (except the FC) were analysed at each storage interval. A 10 g sub-sample was blended with 90 ml sterile peptone (0.1%) in saline (0.83%) diluent (ICMSF, 1978) for 1 min in a Colworth stomacher model 400 (Sharpe & Jackson, 1972). Dilutions were prepared with the same diluent and inoculated onto selected media. Total aerobic counts were determined by surface plating on Plate Count Agar (PCA, Oxoid CM 326). The plates were incubated aerobically for 2 days at 35°C and counted using a Biotran II automated colony counter (New Brunswick Scientific Company, Edison, New Jersey 08817). *Pseudomonas* spp. were enumerated by surface streaking plates of CFC medium (Mead & Adams, 1977). They were counted manually after 3 days at 25°C.

Chemical analyses

Peroxide value (PV) and percentage free fatty acid (FFA) were determined in duplicate on a 50 g beefburger from each of the treatments at each storage time. Procedures followed AOCS methods (1972). Peroxide value was expressed as eq oxygen per kg and FFA as percent oleic acid.

A pH measurement was made by blending 20 g of meat with 100 ml distilled water. The pH was read on a Fisher Accumet model 230 A meter (Fisher Scientific, Pittsburg, PA 15219). Four determinations were made and the mean calculated.

Colour measurements

At each storage interval, surface colour was measured on two burgers from each treatment using a Hunter colour difference meter (model No. D 25A-2, J.B. Atlas Company, Rexdale, Ontario). The instrument was standardized against the white standard tile (C2-8692) with values of L = 92.7; a = −1.0 and b = 0.3.

Organoleptic measurements

Sampling procedures and evaluation techniques were standardized. One burger per treatment, with a 4 cm centre portion removed, was evaluated under a McBeth skylight (model No. BBX-826, J.B. Atlas Company, Rexdale, Ontario). A thawed control sample labelled as a reference (R) was also evaluated. Six panellists individually scored the surface and interior colour (6 = excellent, 1 = poor, discoloured) and the intensity of spoilage and irradiation odours (6 = none, 1 = extreme) of each burger in different random orders. Prior to the study, panellists were trained over a 4 week period to identify and quantify irradiation and spoilage odours.

Statistical measurements

Chemical, microbiological, instrumental and organoleptic data were analysed using analyses of variance. Sources of variation consisted of treatments ($n = 4$), replications ($n = 4$) and storage times ($n = 5$). For all analyses with more than one observation per treatment per storage time, means were computed and used in the analyses. For the bacteriological data, linear and quadratic components for time were tested. For the other variables, Newman Keul's Multiple Range Test was used (Steele & Torrie, 1980).

Results and discussion

The total viable counts (log number/g) of the beefburgers are shown in Fig. 1. The corresponding counts for 'pseudomonas-like' organisms are presented in Table 1. One of the immediate effects of irradiation was a reduction in total numbers of 82 and 90% after exposure to 103 and 154 krad (1.03 and 1.54 kGy) respectively. However, results indicated that numbers increased with storage time and that there was a significant difference between irradiation treatments ($P < 0.001$). The interaction between treatment and time was also significant ($P < 0.05$), that is, the increase in counts with time varied between treatments. The 'pseudomonas-like' flora was inactivated after exposure to both levels of irradiation, but did not change significantly with time in the raw (unirradiated) samples.

Comminuted (ground) beef is a major potential vector for microorganisms of public health significance (Tiwari & Maxcy, 1971) as retail samples with total counts in excess of 10^6/g are very common. However, the present results suggest that the beef used in these experiments was of acceptable microbiological quality. Low dose irradiation of less than 1 Mrad (10 kGy) kills microorganisms of public health significance (Dempster & Lahola, 1983) and extends the shelf life of fresh meat. This has been already established (Wolin, Evans & Niven, 1957; Niven, 1958; Lea, MacFarlane & Parr, 1960; Niemand *et al.*, 1981; Tarkowski, Beumer & Kampelmacher, 1984) and is further confirmed here. Thus even after 15 days at 3°C irradiated samples (154 krad, 1.54 kGy) had mean total counts less than 10^6/g and numbers only increased by one log during that time. Such a quality product could have a bright future in the retail meat trade because point-of-sale ground (minced) beef with total viable counts in excess of 10^6/g are not uncommon (Dempster & Cody, 1978). If numbers can be controlled at this level, shelf life could be

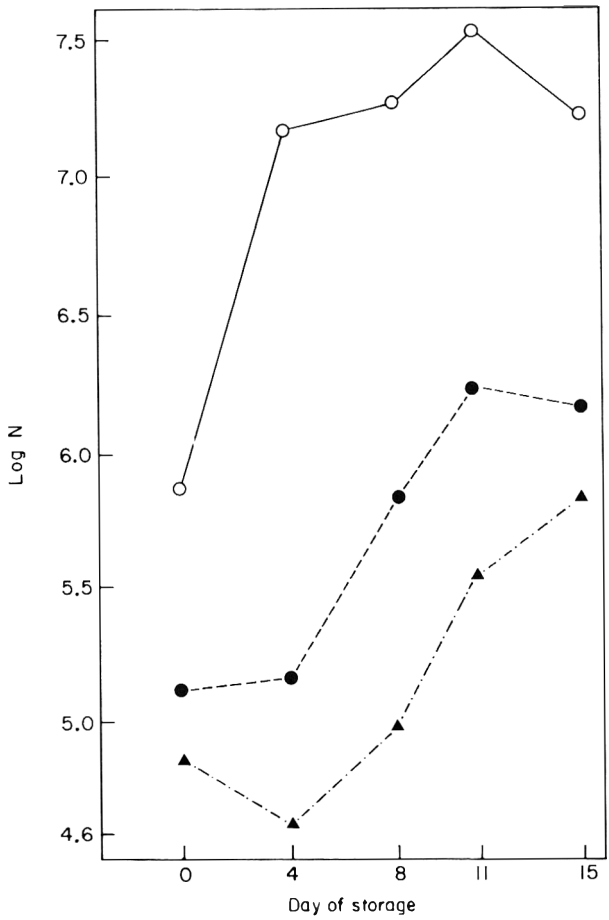

Figure 1. Means of total viable count (log count/g) or unirradiated (O), irradiated at 103 krad (1.03 kGy) (●) and 154 krad (1.54 kGy) (▲) beefburgers during storage at 3°C. The interaction between treatment and day of storage is significant ($P < 0.05$). SEM = 0.22.

Table 1. Means of 'pseudomonad-like' count on CFC medium* of vacuum packaged unirradiated beefburgers after storage at 3°C

Day of storage	0	4	8	11	15
Log count/g	5.08	4.8	5.56	5.4	5.1

*Medium of Mead & Adams (1977). S.E. of mean = 0.203.

considerably extended. That no 'pseudomonas-like' organism survived irradiation is additional proof of this improvement since *Pseudomonas* spp. is one of the main microbial types responsible for spoilage changes in meat stored at refrigeration temperatures (Gill & Newton, 1977; McMeekin, 1975).

The data for pH are shown in Fig. 2. Irradiation resulted in an immediate but small change in pH which persisted throughout storage; the larger irradiation dose resulting

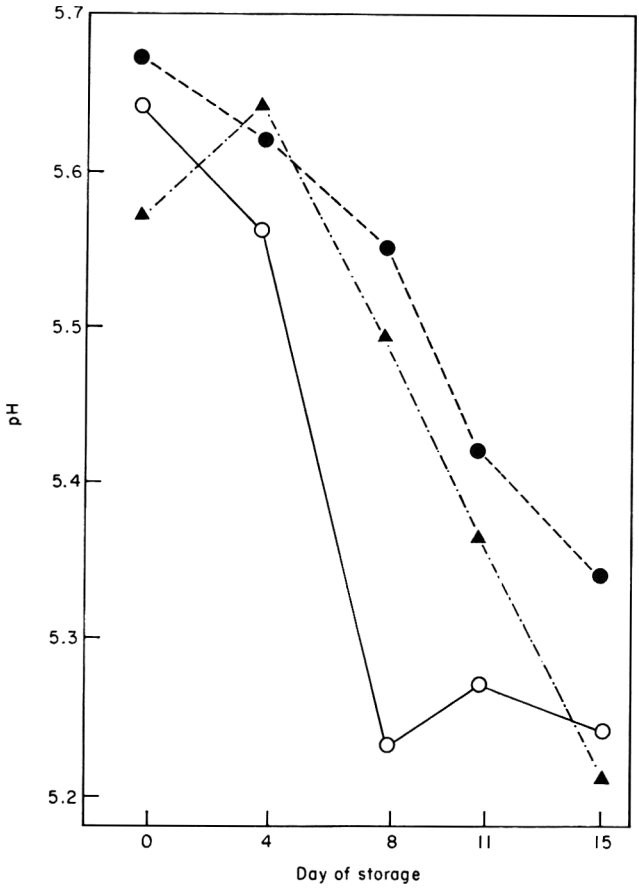

Figure 2. Means of pH values of unirradiated (○), irradiated at 103 krad (1.03 kGy) (●) and 154 krad (1.54 kGy) (▲) beefburgers during storage at 3°C. The interaction between treatment and day of storage is significant ($P < 0.01$). SEM = 0.039.

in a corresponding decrease in pH at all times except day 4. The decrease over 15 days was 0.4 pH unit for the raw (unirradiated) samples and 0.33 and 0.36 pH unit for the irradiated burgers. The interaction between time and treatment was significant ($P < 0.01$) and the difference between treatments was also significant ($P < 0.01$). This decrease in pH is at variance with other results. For example Batzer *et al.* (1959) noted that beef and pork irradiated at a sterilizing dose 5 Mrad (50 kGy) caused an increase in pH which persisted at 5 different temperatures (37– −20°C) throughout the storage period (12 months). However, their dose was much larger than the dose levels used here (32–48 times) and their storage period was also far in excess of 15 days. Coleby *et al.* (1961) studied the effect of temperature on pH in minced (ground) beef and pork after high dose irradiation (4.5 Mrad, 45 kGy). The changes brought about by irradiation were small and freezing diminished them (Coleby *et al.*, 1961). Lawrie *et al.* (1961) suggested that the pH increase noted by Batzer *et al.* (1959) was probably due to denaturation of the muscle proteins which is known to result in an increase in pH. Presumably the decreases in pH noted in the present study were due in part to the peroxides and hydroperoxides formed from lipid, see Table 5. The burgers had an average fat content of between 24–25%.

The effects of irradiation on beefburger colour scores are shown in Table 2 and the

Table 2. Mean values for the effects of irradiation and storage on the colour of beefburgers

Characteristic*	Storage time (days)	Treatment: Reference (R)	Frozen control	Raw (control)	103 krad (1.03 kGy) Irradiation	154 krad (1.54 kGy) Irradiation
Surface colour†	0	5.1^{ab}	4.8^{b}	$^{A}5.1^{ab}$	$^{A}5.5^{ab}$	$^{A}5.7^{a}$
	4	5.1	4.8	$^{A}5.2$	$^{AB}4.9$	$^{B}4.9$
	8	5.1^{a}	5.0^{ab}	$^{AB}4.6^{ab}$	$^{AB}4.9^{ab}$	$^{B}4.3^{b}$
	11	5.1	5.1	$^{B}4.4$	$^{B}4.5$	$^{B}4.4$
	15	5.2^{a}	4.9^{a}	$^{C}2.9^{b}$	$^{C}3.5^{b}$	$^{C}3.2^{b}$
Interior colour‡	0	5.1	5.0	$^{A}5.3$	$^{A}5.5$	$^{A}5.7$
	4	5.2	5.1	$^{A}5.3$	$^{AB}5.2$	$^{AB}5.2$
	8	5.3	5.0	$^{AB}4.8$	$^{AB}5.1$	$^{B}4.7$
	11	5.3	5.2	$^{AB}4.7$	$^{B}4.7$	$^{B}4.8$
	15	5.3^{a}	5.0^{a}	$^{C}3.4^{c}$	$^{C}4.1^{b}$	$^{C}4.0^{b}$

*Six point scale with 6 = excellent and 1 = poor or discoloured. Values are the means of 4 judgements by each of the 6 panellists.

†‡Standard errors for the storage by treatment interaction are 0.22 and 0.17, respectively.

^{a,b,c}Means within the same row with different superscripts are significantly different at $P < 0.05$.

^{A,B,C}Means within the same column with different superscripts are significantly different at $P < 0.05$.

Table 3. Mean values for the effects of irradiation and storage on the 'Hunter' colour data of beefburgers

Measurement*	Storage time (days)	Treatment: Frozen control	Raw (control)	103 krad (1.03 kGy) Irradiation	154 krad (1.54 kGy) Irradiation
Hunter 'L' value†	0	40.7	$^{C}39.7$	40.0	$^{B}39.4$
	4	40.0	$^{BC}41.1$	40.8	$^{A}41.2$
	8	41.5	$^{A}43.6$	41.9	$^{A}41.7$
	11	39.4^{b}	$^{AB}42.8^{a}$	41.4^{a}	$^{A}41.7^{a}$
	15	39.8^{b}	$^{AB}42.7^{a}$	42.1^{a}	$^{A}42.8^{a}$
Hunter 'a' value‡	0	11.1	$^{AB}12.4$	$^{A}12.9$	13.3
	4	11.5	$^{A}13.4$	$^{A}12.8$	12.6
	8	11.3	$^{AB}12.1$	$^{A}11.7$	11.8
	11	12.2	$^{B}10.2$	$^{A}12.6$	12.4
	15	11.4	$^{B}10.1$	$^{B}9.7$	10.7
Hunter 'b' value§	0	10.5	10.4	10.6	10.8
	4	10.0^{c}	11.3^{a}	10.6^{b}	10.8^{b}
	8	10.6	11.0	10.4	10.7
	11	10.1	10.5	10.6	10.7
	15	10.3	10.7	10.2	10.5

*Values are the means of 16 determinations, 4 per replication.

†‡§Standard errors for the storage by treatment interaction are 0.55, 0.67 and 0.27, respectively.

^{a,b,c}Means within the same row with different superscripts are significantly different at $P < 0.05$.

^{A,B,C}Means within the same column with different superscripts are significantly different at $P < 0.05$.

Table 4. Mean values for the effects of irradiation and storage on the odour profiles of beefburgers

Characteristic*	Storage time (days)	Treatment				
		Reference (R)	Frozen control	Raw (control)	103 krad (1.03 kGy) Irradiation	154 krad (1.54 kGy) Irradiation
Spoilage odour†	0	5.9	6.0	$^{A}5.9$	$^{A}5.8$	6.0
	4	6.0^{a}	5.9^{a}	$^{B}5.0^{b}$	$^{A}6.0^{a}$	5.9^{a}
	8	6.0^{a}	5.9^{a}	$^{C}3.1^{b}$	$^{A}6.0^{a}$	5.8^{a}
	11	5.9^{a}	5.9^{a}	$^{D}2.5^{a}$	$^{A}5.8^{a}$	5.9^{a}
	15	5.9^{a}	6.0^{a}	$^{D}2.1^{c}$	$^{B}5.2^{b}$	5.7^{a}
Irradiation odour‡	0	6.0^{a}	6.0^{a}	5.9^{a}	$^{A}4.2^{b}$	$^{A}4.0^{b}$
	4	6.0^{a}	6.0^{a}	6.0^{a}	$^{B}3.7^{b}$	$^{AB}3.8^{b}$
	8	6.0^{a}	6.0^{a}	6.0^{a}	$^{B}3.6^{b}$	$^{C}3.4^{b}$
	11	6.0^{a}	6.0^{a}	5.9^{a}	$^{AB}3.9^{b}$	$^{BC}3.5^{c}$
	15	6.0^{a}	6.0^{a}	6.0^{a}	$^{A}4.2^{b}$	$^{AB}3.8^{c}$

*Six point scale with 6 = no detectable spoilage or irradiation odour and 1 = extreme. Values are the means of 4 judgements by each of the 6 panellists.

†‡Standard errors for the storage by treatment interaction are 0.15 and 0.10, respectively.

^{a,b,c}Means within the same row with different superscripts are significantly different at $P < 0.05$.

^{a,b,c}Means within the same row with different superscripts are significantly different at $P < 0.05$.

'Hunter' colour data appear in Table 3. On day 0, the 154 krad (1.54 kGy) treated burgers were judged brighter (redder) in surface colour than the frozen control samples. This has already been observed in meat treated at much higher levels of irradiation than those used here. Thus in experiments with enzyme-inactivated meat Simic *et al.* (1979) showed that irradiated beef (39 kGy) had a persistent reddish colouration provided that air was excluded. Colour intensity is related to the levels of myoglobin in meat which is altered by irradiation.

With storage, the raw (unirradiated) and irradiated samples became progressively more discoloured (Table 2). Irradiation did not affect internal colour until the end of storage (day 15). At this time, the reference and frozen control had the best scores for internal colour with both irradiated samples intermediate and the raw (control) lowest in score. The Hunter colour difference data (Table 3) tend to confirm the findings of the panel. With storage, the 'raw' and 'irradiated' burgers became lighter (higher 'L' values) and they became less red or paler (lower 'a' values).

The mean scores for 'spoilage' and 'irradiation' odours are presented in Table 4. The reference (R), FC and 154 krad (1.54 kGy) treated samples developed no detectable spoilage odour during storage. However, some spoilage odour was detected in the burgers irradiated at the lower dose (103 krad, 1.03 kGy). As expected, the raw (control) samples spoiled with storage; the largest decrease in score was noted between days 4 and 8. Presumably this deterioration was at least partly due to the increase in 'pseudomonad' count which also showed the largest increase in numbers between days 4 and 8 (see Table 1). The mean score of the raw burgers (2.1) by day 15 represented 'very spoiled' on the 6 point scale. At this time, it is interesting to note that frozen

Table 5. Mean values for effects of irradiation and storage on Chemical data of beef patties

Measurement	Storage time (days)	Treatment: Frozen control	Raw (control)	103 krad (1.03 kGy) Irradiation	154 krad (1.54 kGy) Irradiation
Free fatty acids (%)†	0	0.8^{b}	$^{E}0.9^{a}$	$^{E}0.9^{a}$	$^{E}0.9^{a}$
	4	0.9^{b}	$^{D}1.1^{a}$	$^{D}1.1^{a}$	$^{D}1.1^{a}$
	8	0.9^{c}	$^{C}1.4^{a}$	$^{C}1.3^{b}$	$^{C}1.3^{b}$
	11	0.8^{c}	$^{B}1.6^{a}$	$^{B}1.5^{b}$	$^{B}1.5^{b}$
	15	0.8^{b}	$^{A}1.8^{a}$	$^{A}1.7^{a}$	$^{A}1.8^{a}$
Peroxide value (m eq O_2/kg)‡	0	1.2^{b}	$^{D}1.4^{b}$	3.3^{a}	3.7^{a}
	4	1.4^{b}	$^{CD}2.1^{ab}$	2.6^{a}	2.8^{a}
	8	1.7^{b}	$^{B}3.2^{a}$	3.2^{a}	3.5^{a}
	11	2.1^{b}	$^{A}4.1^{a}$	3.6^{a}	3.5^{a}
	15	2.2	$^{BC}2.8$	3.1	3.1

*Values are the means of 8 determinations, 2 per replication.

†‡Standard errors for the storage by treatment interaction are 0.02 and 0.30, respectively.

^{a,b,c}Means within the same row with different superscripts are significantly different at $P < 0.05$.

^{A,B,C,D,E}Means within the same column with different superscripts are significantly different at $P < 0.05$.

storage (−29°C) appeared to be superior to irradiation (103 krad, 1.03 kGy) in inhibiting spoilage.

No irradiation odour was discernible in the reference, FC and raw (control) initially or throughout storage as expected. However, irradiation immediately imparted a discernible odour to burgers (mean score 4.2 and 4.0 respectively) which dissipated slightly after exposure to air. Similar odours which developed in irradiated beef but which disappeared within minutes have been reported previously (Rhodes & Shepherd, 1966; Wick *et al.*, 1967). The former workers irradiated (0.35 Mrad, 3.5 kGy) beef in vacuum pack and it developed atypical odours described variously as 'liver like' or 'slight odour' indicating some indefinable difference from that expected of meat. However these disappeared within minutes and the samples were then indistinguishable in odour from fresh meat. Judges comments on the irradiated burgers in the present experiments ranged from 'wet dog', musty, 'burnt pop-corn' like to sulphury. Batzer & Doty (1955) reported that hydrogen sulphide was probably one of the components of the undesirable odours developed in meat during irradiation.

The chemical analyses (free fatty acid content and peroxide value) of the burgers are presented in Table 5. Irradiation did not immediately cause an increase in FFA as values were similar for the raw and treated samples. But FFA levels did increase after storage in all samples except the frozen control (FC). Autolytic changes resulting from the action of tissue enzymes may have contributed to this finding. The changes resulting from the continued action of tissue enzymes may then become the limiting factors influencing the useful shelf life of a product. However, these can be controlled by packaging *in vacuo* and by low temperature storage. Nevertheless, irradiation had a marked effect on peroxide value. Thus on day 0 both irradiated treatments had significantly higher peroxide values than the raw control and FC burgers, but these did

not change significantly with time. During storage (days 3 and 11), the raw and irradiated samples were similar in PV and all were significantly higher in PV than the control samples. At the end of storage (day 15) no significant differences between treatments were found. Most workers argue that the removal of oxygen from lipid material prevents the development of hydroperoxides and the peroxides and the production of off-flavours on irradiation (Coleby, 1959). This was partially effected here by vacuum packaging the burgers before irradiation.

Conclusions

Microbial spoilage of meat can be prevented by treatment with ionizing radiation. The present results suggest that low dose irradiation (150 krad, 1.5 kGy) but not less than 150 krad can improve the shelf life of beefburgers by at least 7 days at 3°C. This extension in shelf life is determined by the initial microbiological quality of the meat. However, irradiation at both levels did impart a distinct odour to the beefburgers.

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Cooking of pre-sliced meat

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Summary

Joints of bovine *M. semitendinosus* were sliced such that the slices remained upseparated. These joints and unsliced controls were stored at 0°C and later cooked at 175°C. The weight losses from pre-sliced joints during storage were on average 2.8% greater than those from the controls. Pre-slicing also increased the cooking losses by 6% but cooking time was reduced by 6%. The volume and diameter of pre-sliced joints during cooking were found to change more than the controls, but the change of joint length was reduced. The slices of *M. semitendinosus* could be separated easily after cooking but other tests showed that slices of rolled or fatty joints could not be separated to produce an acceptable product.

Introduction

The production of hot cooked meat from joints that have been pre-sliced in their raw state offers a number of potential advantages in domestic and catering situations. Primarily, carving problems would be removed, and portion control simplified since an exact number of slices of uniform thickness could be purchased. Tests carried out during the development of a multi-blade high speed slicer for unfrozen bacon showed that the machine would slice uncooked chilled meat such that the slices remained unseparated and the joint appeared intact.

Before the main experiment, pre-sliced joints of rolled beef sirloin were cooked to investigate handling problems and to provide data on cooking times, weight losses and dimensional changes. These trials showed that immediately after cooking it was difficult to separate complete slices of hot meat. The problem was worse with the top slices and when separating the thin layers of surface fat. The slices also had a wrinkled blotchy appearance and although the wrinkling was reduced by leaving the separated slices to stand for a few minutes, this led to drops of solid fat forming on the surface. Cooling the joints to a nominal 6°C made it even more difficult to separate the slices.

Although the small number of joints precluded a detailed comparison, the results of the trials indicated that pre-slicing reduced the cooking time and longitudinal shrinkage but increased the weight loss and radial shrinkage. The results of the trials were not promising because rolled joints have a tendency to fall apart, even when unsliced. It was therefore decided to carry out a detailed investigation using more suitable, lean, non-rolled joints of bovine *M. semitendinosus*. The results of these investigations are the primary concern of this paper.

Materials and methods

Pairs of *M. semitendinosus* were removed at 4 days post mortem from four beef

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carcasses that had been both chilled and held at −1°C. The muscles were conditioned for a further 4 days at 0°C before the membrane around each muscle was removed by a mechanical de-skinner. Two cylindrical joints (approximate diameter, length and weight of 90 mm, 85 mm and 650 g respectively) were taken from each muscle to obtain matched pairs of joints from opposite sides of the same carcass. The pH of 4 samples taken from each muscle was determined using the technique described by Bendall (1978). One joint of each pair was cut perpendicular to the fibre direction using a multi-blade slicer to produce 4 mm thick slices. All of the joints were weighed to ±0.1 g, placed on their curved surface in separate polystyrene trays, wrapped in polyethylene film and each pair held for a further 2, 3, 6 or 7 days at 0°C.

After storage each joint was removed from the tray and held in ambient air at approximately 23°C for 40 min. During this time, the volume of the joint was determined by wrapping the meat in aluminium foil and measuring the amount of water displaced from a containing vessel. The joint was also weighed and the diameter and height were both measured at four positions across the meat using a millimetre scale. Each joint was then placed, flat surface down, on a rack above a galvanized roasting pan before cooking in an oven pre-heated to 175°C. This domestic natural convection oven had a modified temperature control system comprising a proportional action controller and a platinum resistance sensor located 45 mm above the surface of the meat. Each joint of a pair was cooked separately, but on the same day, and the oven door was open during loading for 30 sec on each occasion. Air temperatures around the meat were monitored at three locations; 45 mm from the top and 55 mm from each side of the meat's surface. Meat temperatures were measured using a multi-point probe, consisting of 10 chromel-alumel thermocouples (0.076 mm diameter wire) at 5 mm or 10 mm intervals along a 'Tufnol' rod (3 mm diameter), inserted perpendicularly through the centre of a flat surface of each joint. All temperatures were recorded to ±0.1°C using a Solartron data logging system. The joints were removed from the oven when the lowest temperature sensed along the probe was 74±0.1°C. The joints were weighed and the linear dimensions and volume measured as described previously.

Results

After cooking the pre-sliced joints could readily be separated into slices of a uniform thickness and, as illustrated in Fig. 1, there was no obvious adverse effect on the appearance of the meat. The variability in appearance between slices in a treatment seemed to be no larger than that between treatments.

Figure 2 shows typical temperature–time histories at the centre of a pre-sliced and a whole joint and the oven air temperatures. After closing the oven door, the air temperatures required 9 (±1) min to recover to their control points; thereafter, the maximum variation of air temperature at any point was 14°C and the maximum deviation from 175°C (the mean temperature) was 17°C. The average cooking time to 74°C was significantly shorter ($P < 0.05$) for pre-sliced than whole joints (Table 1).

Analysis of variance showed that the mean weight of the pre-sliced joints did not differ significantly from that of whole joints either before or after storing in the chillroom at 0°C. However, the mean percentage weight losses from the pre-sliced joints were greater than the losses from the whole joints over the period of storage ($P < 0.05$) and cooking ($P < 0.01$). Figures 3 and 4 show the percentage weight loss during storage and cooking, respectively, against days in storage. All the pre-sliced joints, except those obtained from animal 2, lost substantially more weight during

storage (2.97 to 6.57% more) and cooking (6.05 to 8.74% more) than their unsliced controls. Considering all the samples the total loss during storage and cooking was 7.5% greater for pre-sliced than whole joints ($P < 0.01$).

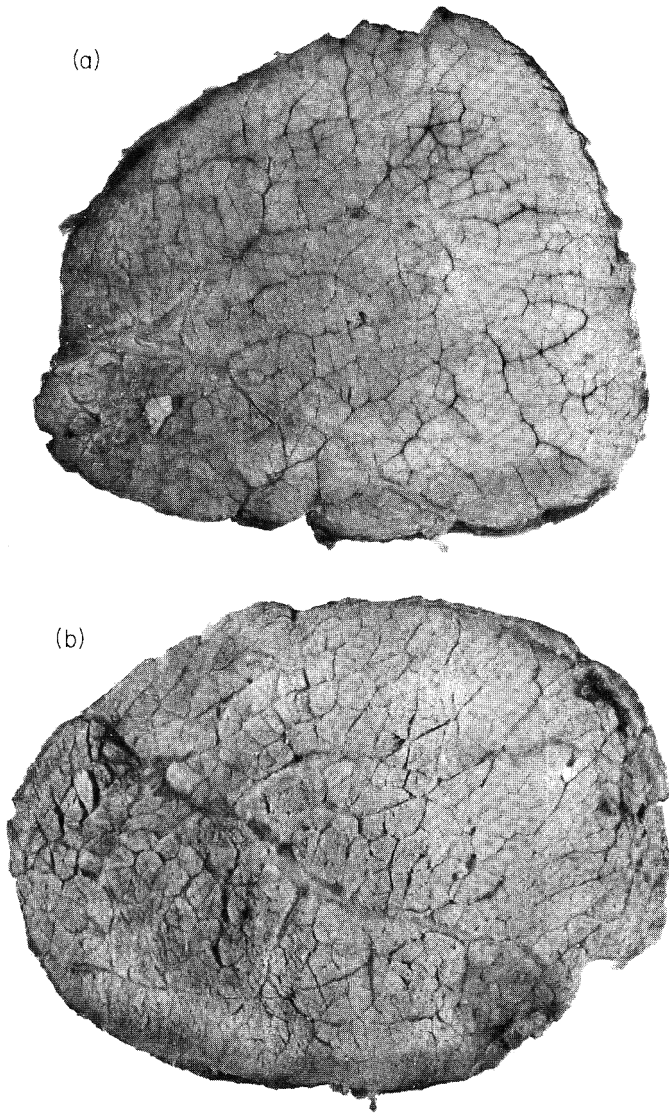

Figure 1. The appearance of meat prepared by cooking in a natural convection oven at 175°C: (a) sliced after cooking; (b) sliced before cooking.

The analysis of variance showed that all of the dimensional changes were affected by slicing (Table 1). The volume ($P < 0.05$) and diameter ($P < 0.01$) were reduced more in the pre-sliced joints than in the whole joints during cooking, but pre-slicing reduced the change in joint thickness ($P < 0.001$).

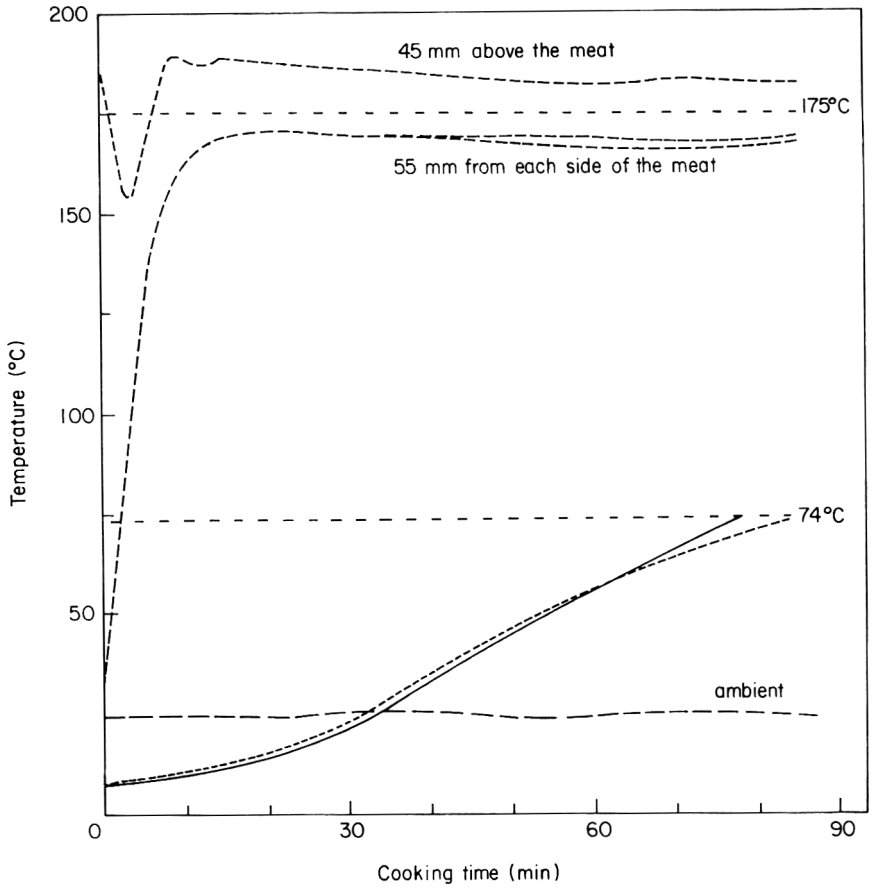

Figure 2. Typical temperature-time histories at the centre of a pre-sliced (————) and whole joint (– – – – –) and the air temperatures at three locations — — — — —).

Discussion and conclusions

Cooked pre-sliced joints of *M. semitendinosus* can readily be separated into individual slices and therefore the method may be used to eliminate the problems of carving small hot joints of lean meat. The only other advantage of pre-slicing was a statistically significant reduction in cooking time. Although this was interesting the actual magnitude, i.e. 5 min in a total cooking time of 80 min, is too small to have any practical implications. If the relative reduction of cooking time (6%) could be extrapolated to larger joints, commercial applications, especially those involving continuous cooking, might benefit since pre-slicing would allow higher throughput rates. However the disadvantages, even with the near ideal lean *M. semitendinosus* joints, tend to outweigh these two advantages. The average 3% increase in drip loss during storage would seriously reduce the sales appeal of the meat when presenting retail packs (Malton & James, 1983). A 6% reduction in cooking yield is also substantial, although in practice this might be offset by the elimination of cutting losses which result when conventional joints are sliced after cooking. Reduced cutting losses may be particularly significant in outlets such as restaurants but, its magnitude was not estimated in these experiments. Increased drip and cooking losses from the pre-sliced joints had been expected since

Table 1. The means, standard error of the differences and levels of significance of measurements on pre-sliced and whole joints of bovine *M. semitendinosus* cooked at 175°C, number of replicates=8

Measurement	Treatment		s.e.d.	Probability of F
	Pre-sliced	Whole		
pH	5.55	5.43	—	
Weight at 8 days post mortem (g)	651.6	647.0	—	
Weight loss during storage (%)	6.2	3.4	1.02	*
Weight before cooking (g)	611.0	625.2	—	
Cooking time to 74°C (min)	75.5	80.3	1.63	*
Weight loss during cooking (%)	39.3	33.3	0.97	†
Overall weight loss, storage and cooking (%)	43.1	35.6	1.43	*
Average diameter before cooking (mm)	88.8	86.6	—	
Change of diameter during cooking (%)	−17.0	−7.9	2.17	†
Average thickness before cooking (mm)	82.5	84.1	—	
Change of thickness during cooking (%)	−2.9	−13.0	1.38	‡
Volume before cooking (ml)	599	617	4.37	†
Change of volume during cooking (%)	−39.4	−34.6	1.36	*

Probability of F: $*P < 0.05$; $†P < 0.01$; $‡< 0.001$.

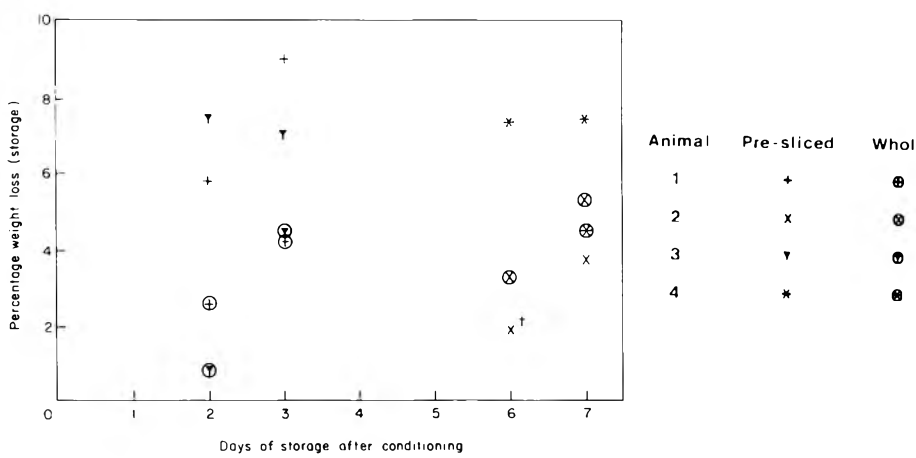

Figure 3. The effect of storage time in polystyrene trays at 0°C on the weight loss from pre-sliced and whole joints during storage.
†Coincident with whole (animal 4).

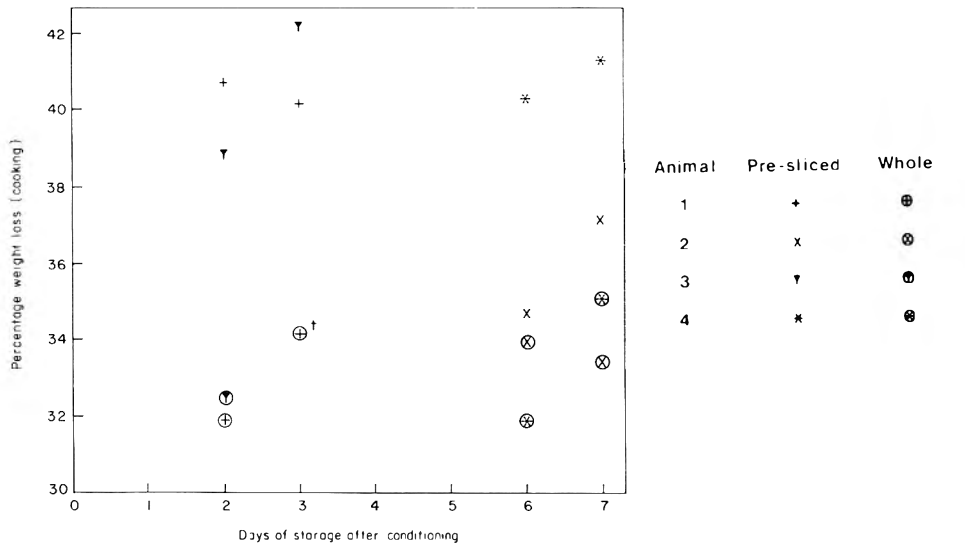

Figure 4. The effect of storage time in polystyrene trays at 0°C on the weight loss from pre-sliced and whole joints during cooking.
†Coincident with whole (animal 3).

drip loss is related to the area of cut surface (Penny, 1972), and cooking loss increases with decrease in cut fibre length (Bouton, Harris & Shorthose, 1976).

The drip and cooking losses from the pre-sliced joints obtained from animal 2 were noticeably lower than the mean values (Figs 3 and 4). There had been no obvious difference in the treatment of this animal and the ultimate pH of the meat (5.4), does not indicate a tendency towards dark cutting, which is known to affect drip loss. Excepting this single animal the overall trend in cooking and drip loss is clear and even including that exceptional animal the differences between sliced and pre-sliced data are statistically significant.

Despite difficulties in measuring dimensional and volumetric changes during the cooking of meat joints, such information is invaluable in the modelling of heat and mass transfer within meat (Burfoot & James, 1983). In work which used conditions similar to this investigation, Fenton *et al.* (1956) showed that the percentage change of thickness of whole joints was greater than the percentage change of diameter. While this agrees with the present work, the trend was reversed when pre-sliced joints were used probably because longitudinal shrinkage in the sliced joints was reduced due to the formation of cavities between adjacent slices.

Although the slices of *M. semitendinosus* could be separated easily, it was clear from the initial tests with rolled beef and subsequent unreported tests with pork, that major problems would be encountered in separating slices from many commercial joints. Most of the difficulties seemed to be caused by the effect of rolling and the adherence of fat layers, although this was less of a problem with the pork.

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Intermediate-moisture tropical fruit products for developing countries
II. Quality characteristics of papaya

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Summary

Intermediate-moisture papaya products (IMPP), were prepared after predrying treatments (pretreatments) of papaya slices. The pretreatments were: I blanching in boiling water or sugar syrup; II dipping in boiling; III cold (room temperature); sugar syrup, as well as a combination of pretreatments I and III. The influence of the pretreatments on quality characteristics of cabinet or sun-dried IMPP, such as: ascorbic acid (AA) and carotenoid retention, colour, pectin esterase (PE) activity, water activity (a_w) and microbial counts was studied and is presented in this paper.

The highest AA retention (66–91%) was observed in papaya slices directly dried in a cabinet, followed by those water blanched +cabinet dried (58–73%). The AA content of IMPP indicates that the product could be a rich source of vitamin C. Significantly lower retention of AA was observed in general in the sun dried samples. Most products maintained their typical orange colour, as well as their carotenoid content. Of the pretreatments tested only boiling caused significant discoloration and significant carotenoid losses. The total carotenoids content of the sun dried was lower than that of the cabinet dried samples. The relationship between the moisture content of IMPP and their a_w values suggests that IMPP with a moisture content of 28% or less will be relatively stable from the microbiological standpoint. IMPP are a promising means of utilizing surplus papayas in developing countries due to their attractive colour, flavour and other quality characteristics.

Introduction

In spite of the existence of large surpluses of fresh subtropical and tropical fruits, such as papaya, mango and banana, in many developing countries, only a limited variety and quantity of tropical fruit products (TFP) are produced for local or export markets. For example, 160 000 tons of bananas rejected for export, but otherwise sound, were wasted in Costa Rica in 1978 (Viquez, Lastreto & Cooke, 1981).

Intermediate-moisture tropical fruit products (IMTFP) appear to have potential markets. As shown by Levi, Gagel & Juven (1983) for papaya, IMTFP could be produced by simple production methods, while generally acceptable eating quality and reasonable storage stability under ambient conditions would be expected. The drying time of the IMPP was found to be considerably shortened and the drying temperature could be lowered following certain pretreatments such as blanching and/or osmotic dehydration. The direct sun drying of papaya slices was reduced from several days to several hours after the above treatments (Levi *et al.*, 1983).

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Except when harvested for the extraction of papain, papaya fruits are harvested mature but not ripe. Several methods of ripening by controlled atmosphere conditions, are mentioned in the literature (Akamine & Arisumi, 1952, 1953; Akamine, 1959), but in many developing countries the fruits are left to ripen under ambient humidity and temperature conditions.

The peel colour of the unripe mature papaya is green and changes gradually during ripening to yellowish-orange. The flesh (pulp) colour of the unripe papaya is yellowish-green and turns to yellowish-orange or deep orange during the ripening period (Rodriguez & de George, 1972). This coloration of the pulp is caused by various carotenoids, in particular β-carotene which is one of the main carotenoid components (Chan *et al.*, 1975). Devadas, Saroja & Murthy (1980) reported that the β-carotene content within a certain variety was 843 ± 13.2 μg/100 g edible portion. Pal *et al.* (1980), who studied the chemical composition of twelve papaya varieties, found wide variability in the vitamin A content between varieties as well as within the same variety, from *circa* 1600 to 6345 int. units/100 g.

The edible portion of the ripe papaya is reported to constitute *circa* 60–80% of the fruit (Khedkar, Patil & Dabhade, 1980; Levi *et al.*, 1983). The rest of the fruit (peel, seeds, central cavity liquid) must be removed before using the papaya for direct consumption or food production purposes because of its strong 'off-flavours', including the very bitter taste of the seeds (Khedkar *et al.*, 1980). He reported that the overall quality of hand peeled papaya was better than that of papaya peeled by other methods. Levi *et al.* (1983) obtained higher yields by lye peeling, with no significant difference between the quality characteristics (flavour, appearance, colour, texture) of hand peeled *versus* lye peeled papaya slices.

The dry matter content of the ripe papaya varies between *circa* 10 and 15%, on a fresh pulp basis. Wide variations were also observed in the contents of total soluble solids (TSS) e.g. sugars and acidity (Fock *et al.*, 1980; Levi *et al.*, 1983; Nath & Ranganna, 1981; Pal *et al.*, 1980). The pH of the papaya edible portion varies between 4.5 and 5.65 (Nath & Ranganna, 1981). Therefore, acidification of the papaya flesh is recommended so that a mild heat treatment can be employed for preservation.

Pal *et al.* (1980) found that the ascorbic acid (AA) content of fresh papaya pulp varies between *circa* 46 and 126 mg/100 g, while Baqar (1980) reported AA contents between 18 and 180 mg/100 g, in Papuan-New Guinean commercial varieties. Pectolytic enzymes, such as pectin-esterase (PE) and polygalacturonase, affect textural-structural properties of the papaya and its products (Brekke, Chan & Cavaletto, 1973; Chan *et al.*, 1975; Chan, Tam & Seo, 1981), while PE was found to be responsible for the gelation of liquiform papaya products.

Work on the development of papaya products includes studies of canned, frozen, dehydrated, liquiform and concentrated products (Brekke *et al.*, 1973; Chan *et al.*, 1975; Khedkar *et al.*, 1980; Levi *et al.*, 1983; Rodriguez & de George, 1972) but despite this, a very limited quantity and variety of papaya products are marketed to the best of our knowledge. Levi *et al.* (1983) reported on the technological data obtained during experiments to develop IMPP by a combination of several pretreatments of the papaya slices followed by cabinet or solar drying. Some important quality characteristics of the IMPP prepared in these ways are reported in this work.

Materials and methods

Batches of 5–50 kg of fresh, ripe (almost 100% yellow-orange peel coloration) papayas

were treated by washing, lye peeling (in boiling 10% NaOH) and halving, and the edible portion was sliced, as described by Levi *et al.* (1983). The slices were subjected (or not) to one or two of the following pretreatments: water or syrup (70% sucrose) blanching, boiling or cold syrup (70% sucrose) dipping and then dehydrated, by cabinet or sun drying to *circa* 70% dry matter (DM), as described by Levi *et al.* (1983). When potassium sorbate was added to the slices, this was achieved by dipping in a potassium sorbate solution of 5000–10000 ppm for 10–15 min, after blanching (if blanched).

Analytical methods

Representative samples of 0.5–1.0 kg of fresh, or *circa* 250–300 g of osmotically pretreated or dehydrated papaya slices, were thoroughly blended and analysed for total soluble solids (TSS), acidity (as citric acid) and pH as described by Ramirez-Martinez *et al.* (1977).

The ascorbic acid (AA) content was determined as described by Baqar (1980). The ascorbic acid retention values reported were calculated on a dry matter basis and are expressed as a percentage of the AA content of the fresh slices, from which the IMPP was prepared. The colour (reflectance) of the fresh or IMPP slices (L = lightness; +a = redness; +b = yellowness) was measured, and the PE activity was determined, as described by Levi, Ramirez-Martinez & Padua (1980). The total carotenoid content (expressed as β-carotene) was analysed and calculated by the method described by Chan *et al.* (1975), with a Varian Model DMS-9-UV, visible-spectrophotometer. The water activity (a_w) of the IMPP was measured at 20°C by placing *circa* 50 g of finely ground samples in 8-oz jars—with Hygrosensor elements attached to a Hygrometer indicator (model No. 15-3030E Hydrodynamics Inc., Silver Spring, MD., U.S.A.). Sensor calibrations were made against saturated $(NH_4)_2SO_4$ solution. An equilibrium time of not less than 24 hr was allowed before measurements were made.

Total aerobic counts were determined using the citric acid (CA) medium as described by Juven (1979). Viable counts of 'sugar-tolerant' microorganisms were determined on yeast extract agar with 50% D-glucose added (0.89 a_w). Microbial counts are expressed as log of colony-forming units/g. All plates were incubated for 5–7 days at 30°C. Counts were not made in product samples showing visible mould growth.

Results and discussion

Ascorbic acid content and retention

The range of the AA content of fresh papaya slices was between 70 and 113 mg/100 g. AA retention (apart from its nutritive value as vitamin C) is one of the indicators of overall quality retention of many fruit and vegetable products because of the relatively high temperature and oxidation sensitivity of AA. In Fig. 1 are given the average AA retention values of the IMPP prepared after certain pretreatments and dehydrated by cabinet or solar drying methods to about 70% dry matter (DM). The main reason for the lower retention after the heat pretreatments (water or syrup blanching; hot-syrup dipping) is probably the heat-enhanced oxidation of the AA. Sugar penetrates into the slices during osmotic treatment (Levi *et al.*, 1983), diluting the natural DM content of the papaya; therefore, the AA content (calculated on a DM basis) is lower and so is its apparent retention, in treatments II, IV, V, VI. The lowest AA retention was observed in the boiling syrup-dipped IMPP because of the DM dilution effect, as well as the destructive effect of prolonged heating (75 min).

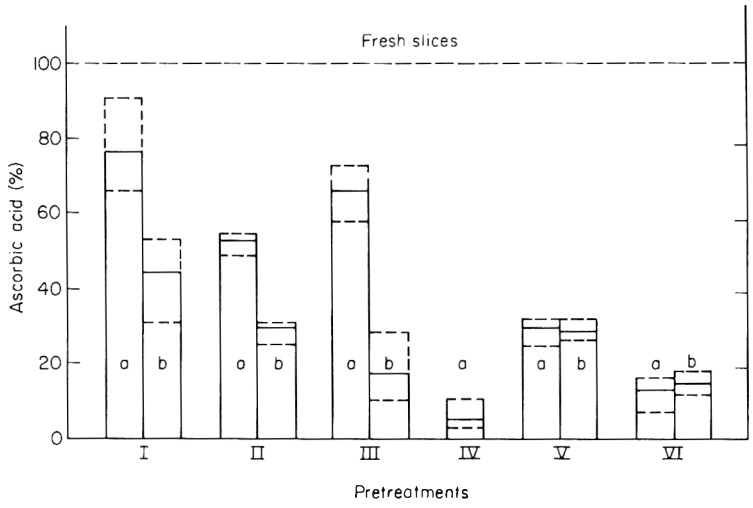

Figure 1. Ascorbic acid retention of IM papaya slices (on a dry matter basis). (a) Cabinet drying; (b) sun drying; I, direct drying; II, syrup blanching; III, water blanching; IV, boiling syrup dip; V, cold syrup dip; VI, water blanching + V.

Lower AA retention was observed after solar drying than after cabinet drying. This was probably due to the much longer time needed for sun drying after equal pretreatments than cabinet drying even if the latter is at a lower temperature (treatments I, II, III). The insignificant differences observed in AA retention between the cabinet and the solar dried IMPP after the cold syrup dipping, were probably due to the high DM solution effect (up to 250–300%) of this pretreatment, as well as the relatively short drying period (Levi *et al.*, 1983).

In order to evaluate the nutritional value of the AA (as vitamin C) of the different IMPP (after 3 months' storage), the actual AA contents of the sun or cabinet dried IMPP after the different pretreatments, compared with that of the fresh papaya slices, are given in Table 1. From this point of view, the best source of vitamin C is the directly cabinet dried slices, while even mild heat treatments lowered the AA content significantly. The lowest AA content observed was in the slices heat treated for the longest time (treatment IV). Thus, the IMPP could be a reasonable source of vitamin C, as could directly dried products, particularly if cabinet drying is used.

Colour characteristics

It is difficult to quantify colour characteristics, even with colour prints. Therefore, the colour of the slices was estimated also by objective measurements with a 'Colour Difference Meter' (reflected light). The influence of the degree of ripeness of the papaya fruits—as determined by their peel colouration—on the colour characteristics of the fresh slices is given in Table 2. The slices were prepared from a particular batch of mature green papayas, which were ripened as described by Levi *et al.* (1983). The colour intensity increased with increasing peel colouration (and ripeness), resulting at full ripeness in a darker orange colour with lower L and higher a and b values. A slight difference in the measured colour (not detected visually) was observed between 80 and 100% ripe papayas. Similar trends in the colour changes were observed during ripening of different varieties of papaya and papayas of different harvest dates.

Table 1. Effect of pretreatments and drying method on the ascorbic acid content (in mg/100 g) of intermediate moisture papaya slices*

Treatment No.	Fresh slices	Pretreatment	Following pretreatment†	Drying method	
				Cabinet‡	Sun‡
I	99	None	—	441	193
II	78	Syrup blanch⁺	65	263	156
III	82	Boiling water blanch (5 min)	64	269	134
IV	113	Boiling syrup⁺⁺ (90 min)	48	68	—
V	70	Cold syrup⁺⁺ (24 hr)	71	161	144
VI	100	Combined (III) and (V)	44	74	73

*Dried to 70±3% DM content.
†Before drying.
‡Similar data were obtained in other experiments.
⁺5 min boiling (70° Bx).
⁺⁺70% sugar.

The effect of the pretreatments (syrup dips or blanching) on the colour characteristics of the IMPP is shown in Table 3. The colour characteristics of the fresh papayas from which the IMPP were prepared, were very slightly but not significantly different. The IMPP heated for a long time (treatment II) showed significant darkening (low L value) and destruction (and dilution) of the yellow pigments (b). Shorter heat treatments (blanching) also caused some destruction of the colour components, resulting in lower b values. On the other hand, the red components were of higher visual value (+a) but this could have been caused by the effect of removal of the entrapped air bubbles during blanching.

It seems that there is no direct correlation between the DM dilution (cold syrup treatment) and the reflected colour of the IMPP. Higher colour values were observed in

Table 2. Effect of the degree of ripeness of var. 'Solo' papaya on the colour characteristics of fresh slices

Peel colour* (%)	Colour characteristics			
	L	+a	+b	Visual
0–10	60.9	9.4	33.9	Greenish
50±5	57.0	21.1	35.6	Yellow-Orange
80±5	57.4	27.1	38.0	Orange
100	59.2	30.0	38.6	Orange
r†	−0.49	0.99	0.99	
P‡	>0.2⁺	0.01⁺⁺	0.01⁺⁺	

*As defined by the percent of the area of yellow-orange peel coloration (Levi *et al.*, 1983).
†*r* = Correlation coefficient between percent of peel colouration and the colour components of the slices: L = lightness; +a = red; +b = yellow.
‡*P* = Significance of correlation of the above.
⁺No correlation.
⁺⁺99% correlation.

Table 3. Colour characteristics of intermediate-moisture papaya products (IMPP)

Treatment No.	Pretreatment	L		+a		+b		Visible colour‡	
		Cabinet	Sun	Cabinet	Sun	Cabinet	Sun	Cabinet	Sun
I	Direct drying	58.7	52.1	14.2	14.3	34.7	33.7	Opaque	Opaque
II	Boiling syrup (75 min)	31.2	—	13.1	—	13.3	—	Dark brown	—
IIIa*	Syrup blanch† (5 min)	48.3	44.1	13.6	14.5	27.2	24.5	Opaque	Translucent
IIIb	Syrup blanch† (5 min)	51.0	48.0	18.5	15.8	31.4	27.2	Opaque	Opaque
IV	Water blanch (5 min)	46.3	37.7	21.0	11.9	27.2	17.2	Opaque	Translucent
Va	Cold syrup† (22 hr)	61.5	58.3	18.5	16.3	31.4	30.0	Translucent	Translucent
Vb	Cold syrup† (22 hr)	48.4	55.0	17.3	19.8	29.9	34.5	Translucent	Translucent
Vc	Cold syrup† (24 hr)	44.2	42.8	16.5	13.7	27.6	29.0	Translucent	Translucent

*a, b, c, separate experiments with different batches of papaya.

†In 70% sugar solution.

‡Orange, except in treatment II.

the cold syrup pretreated slices, both after the cabinet and the sun drying (compare treatment V a and b with I). The above effect could also result from the removal of the entrapped air bubbles, which disperse the light. Air removal could also explain the translucent appearance of the slices after certain pretreatments.

The effect of the natural colour of the papaya on the colour changes during the pretreatments and the drying is recorded in Table 4. In this case the intensity of the colour is given as a×b—because of the interrelationship between these two components (yellow and red) of the orange colour.

The direct cabinet drying (treatments I and III) did not cause great changes in the colour characteristics of the IMPP, as compared with the same in the fresh papaya slices. Blanching (Ib, IIb) diluted the colour, probably by causing some destruction of the components of the orange colour (a×b). The effect of the blanching on L is not clear because in one case (Ib) L was brighter (higher value), while in the other (IIb) a darker colour (lower L) was observed after blanching. The blanched + cabinet dried slices were darker in colour (lower L) and apparently lost some orange (a×b) colouration as compared with the fresh slices. The lighter colour and the lower orange value (a×b) of the cold syrup dipped slices were most probably due to the penetrating syrup (colour dilution) effect. Greater darkening and orange decoloration were observed following sun drying. The blanching effect on syrup dipped slices was obvious because the blanched dipped slices, as well as those also dried, were darker and had a lower a×b value than the syrup-treated or the fresh slices. Lower L and a×b values were observed after sun drying than after cabinet drying which can be explained as due to the photosensitivity of the carotenoid components of the colour. The storage stability of the colour can possibly be ensured by SO_2 addition, but this requires investigation.

Significant correlation was found between the AA content and the colour values (L, a×b) of the IMP slices. When comparing the above of 11 untreated, or pretreated, sun or cabinet dried samples the following linear correlation was found: AA *versus* L $-r = 0.683$; $0.01 < P < 0.05$; AA *versus* a×b $-r = 0.745$; $0.01 > P > 0.001$. It seems that heat and oxidation are the main factors affecting both the degradation of the AA, and the discolouration, of the IMP slices.

Carotenoids

The total carotenoid content of the fresh papaya slices, expressed as β-carotene, was between 1800 and 2300 μg/100 g, on a DM basis. After the pretreatments, a certain discolouration of the IMPP was observed which was found to depend also on the drying method employed. One of the main reasons for such discoloration was the photosensitive nature of the carotenoid compounds. Therefore, the colour may also change because of their configuration and their degradation due to exposure to heat and light.

Figure 2 shows the retention of the total carotenoid content (TCC, expressed as β-carotene) by the IMPP, dried after different pretreatments. The TCC was calculated as the percentage of the TCC of the fresh fruit, on a DM basis. After direct cabinet drying the retention was higher than after direct sun drying, probably because of the intensive 'sun-light' degradation effect on the carotenoids. The cold syrup dip resulted in still lower retention (dilution effect), and again the TCC was higher after cabinet drying. Blanching probably had a stabilizing effect on the TCC: therefore, the retention value was considerably higher for both the cabinet and sun dried IMPP. Comparing this high retention value of the carotenoids (above 90%) with the reflected colour components (a×b) in Table 4, it is seen that a direct correlation between carotene content and

Table 4. Reflected colour of fresh slices and the resulting colour changes during pretreatments and drying

Treatment No.	Pretreatment	Drying method						Remarks
				Cabinet‡		Sun‡		
		L	a×b	L	a×b	L	a×b	
Ia*	None (fresh slices)	52.1	548	49.5	533	45.0	385	Direct drying
b*	Water blanched	58.8†	430†	54.7	397	34.4	178	Blanching + drying
IIa*	Fresh slices	63.3	884	—	—	—	—	
b*	Water blanched	51.6†	444†	46.3	573	35.7	205	Blanching + drying
IIIa*	Fresh slices	59.2	623	56.3	540	34.7	321	Direct drying
b*	Cold syrup dip (24 hr)	62.4†	569†	58.3	581	39.5	535	Dip + drying
c*	Syrup blanch + IIIb	60.7†	480†	51.0	581	48.0	432	Blanching + dip + drying
IVa*	Fresh slices	63.3	504	—	—	—	—	
b*	Water blanch + IIIb	56.5†	367†	50.5	504	47.7	450	Blanching + dip + drying

*Treatments b and c were prepared from the fresh slices used in treatment a.
†Before drying.
‡Dried (with or without pretreatment) to about 70 ± 3% DM content.

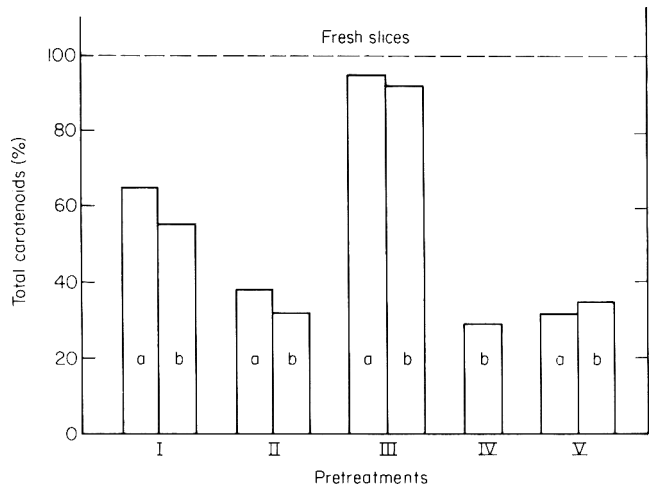

Figure 2. Retention of total carotenoids (as β-carotene) in IM papaya slices (on a dry matter basis). (a) Cabinet drying; (b) sun drying; I, direct drying; II, cold syrup dip; III, water blanching; IV, water blanching + II; V, syrup blanching + II.

colour does not exist specifically after sun drying. After the syrup blanching + cold syrup dip, the observed higher carotenoid retention of the sun dried slices is not significant. Similar trends were observed in the TCC retention in three similar experiments, differing only in the different TCC of the fresh slices. No correlation was found between the retention values of the ascorbic acid and the carotenoids.

Pectinesterase activity

One of the most important quality characteristics of the IMPP is their texture. Pectolytic enzymes are responsible for pectin degradation, which affects the texture of dried fruits. The PE activity of the IMPP after different pretreatments and cabinet or

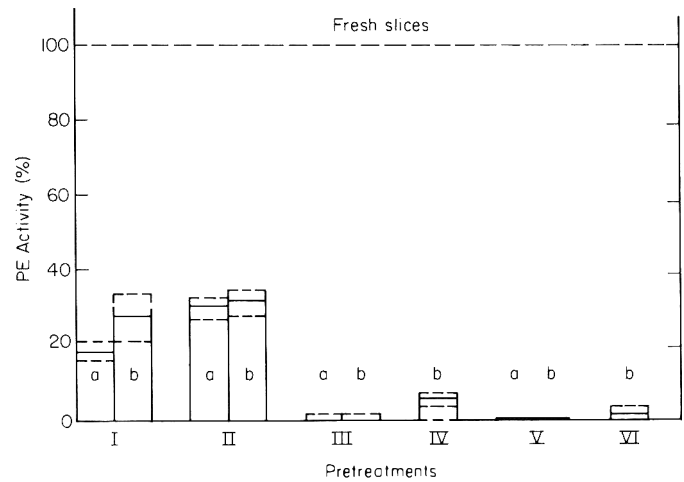

Figure 3. Residual pectin esterase (PE) activity in IM papaya slices (on a dry matter basis). (a) Cabinet drying; (b) sun drying; I, direct drying; II, cold syrup dip; III, water blanching; IV, syrup blanching; V, water blanching + II; VI, syrup blanching + II.

Table 5. Effect of predrying treatments and of methods of drying, on microbial counts of papaya slices (log of CFU/g)

Treatment No.	Treatments	Before drying		After drying*			
		Total aerobic	Sugar tolerant	Total aerobic		Sugar tolerant	
				Cabinet	Sun	Cabinet	Sun
I	None (fresh slices)	2.3–3.4	< 1.0–2.2	3.7–4.1	5.9–7.0	< 1.0–1.0	3.6–3.9
II	Water blanching	2.8–3.7	< 1.0–1.0	2.0–4.0	Mouldy†	< 1.0–1.0	Mouldy†
III	As treatment II + K sorbate‡	2.7–3.7	< 1.0–1.0	—	2.8–4.0	—	< 1.0
IV	Syrup blanching	1.3–2.6	< 1.0	2.7–3.1	Mouldy†	< 1.3–4.1	Mouldy†
V	As treatment IV + K sorbate‡	—	—	—	2.3	—	1.5
VI	Cold syrup dip	2.9–4.0	< 1.0–1.5	2.4–2.9	3.5–3.7	1.0–1.5	1.0–2.1
VII	Boiling syrup dip	< 1.0–1.3	< 1.0	1.5–4.5	—	1.0–1.1	—

*To about 70% DM, followed by 2–3 weeks' storage.

†Mouldy—more than 50% of the dried samples were covered with visible mould, and microbial counts were not performed.

‡Between 400 and 800 ppm in the IMPP.

CFU—Colony Forming Units.

solar drying, is shown in Fig. 3. The initial PE activity of the fresh papaya slices was between 12.3 and 25.6 PE units per 100 g DM. In order to be able to compare the PE inactivation, the different PE activities in the fresh slices are given as 100%, while the residual PE activity after each pretreatment, or drying, is given as the corresponding percentage of the initial one, all calculated on a DM basis.

From the values given in Fig. 3, one can see that about 80% of the initial PE was inactivated after direct cabinet drying, and about 70% by direct solar drying (lower temperature, longer period). As expected, water blanching almost completely inactivated PE and very low PE activity was observed in both the cabinet and the sun dried papaya. Higher PE activity was observed after syrup blanching, showing a possible sugar protecting effect which will be investigated further. The same phenomenon was observed in the sun dried IMPP after a combined syrup blanching + cold syrup dip pretreatment. No PE activity was detected after the combined water blanching cold syrup dip pretreatment in the dried papaya. As expected, the heat inactivation effect of the combined heating time-temperature factor during drying of syrup dipped IMP slices (treatment II) was less, probably due to the lower temperature for a shorter time after the syrup dip (Levi *et al.*, 1983).

Moisture content, water activity and microorganisms' survival

The storage stability of dried foods depends mainly on their water activity (a_w). Concerning microbiological stability, it is widely documented that the chances of certain microbial spoilage occurring, depend on the a_w of the product. Most bacteria do not grow below 0.91 a_w, while in IMF products (a_w below 0.8) fungal growth is usually the main microbiological spoilage problem. Environmental factors such as pH, redox potential and temperature are known to influence the level of a_w required for microbial growth, while the main factor determining the a_w is the product's moisture content.

After analysing eighteen solar or cabinet dried samples at various degrees of dryness (between 14.5 and 60.4% moisture content), a significant correlation was found between the moisture content (x) and the water activity (y). The equation representing the linear correlation is:

$$y = 0.0069\,x + 0.506.$$

The linear correlation coefficient, r, was +0.949, which means that the correlation is very highly significant ($P < 0.001$). The standard deviation (G) was G = 0.09, while the standard error of the estimate, was SY = 0.0287.

Assuming that growth of microorganisms (with the exception of certain osmotolerant moulds) will not occur below an a_w of 0.7, reasonable microbiological storage stability of IMPP at room temperature can be expected at a moisture content of < 28%, as calculated from the above equation. In order to prevent the growth of osmotolerant microorganisms in IMF Products, the storage stability could be assured by the addition of certain preservatives.

The degree of initial contamination of the IMPP (at the end of the process) will affect their storage stability: therefore, microbiological counts of the pretreated slices were performed immediately after the pretreatment and in the IMPP, within 2–3 weeks' storage at ambient conditions (Table 5).

Total aerobic counts were done to assess the destructive effect of the various treatments on the microbial contaminants. The level of sugar tolerant microorganisms is taken as an index of potential microbial spoilage of the product. Yeasts constituted the majority of sugar tolerant microorganisms recovered from the various samples. The

effects of the predrying treatments on the microbial counts of the IMPP are presented in Table 5. The lower counts (in the pretreated but undried samples) observed were in the boiling syrup dipped samples. No significant differences were observed between the untreated and the osmotically or short blanch treated slices. The same situation was observed also after cabinet drying, in both the total and sugar tolerant microbial counts. On the other hand, after sun drying, the total and sugar tolerant counts of the untreated as well as of the blanched slices were higher, as was anticipated. Addition of potassium sorbate reduced the counts significantly. The lower counts observed in the IMPP after the cold syrup dip can be explained by the unfavourable (high osmotic pressure) conditions for recontamination during the drying process. The above results indicate only the potential storage stability of the IMPP and extended storage tests are needed before practical conclusions can be drawn on storage stability.

Conclusions

IMPP which were shown to be promising from the technological point of view for developing countries (Levi *et al.*, 1983) possess also promising quality characteristics. They are a rich source of vitamin C, possess a pleasing colour and flavour, and give promise of being reasonably stable under ambient storage conditions.

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Effect of crop maturity on agronomic and sensory quality characteristics of continental-style canned peas

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Summary

The effects of crop maturity on yield, seed size distribution, tenderometer values and canning quality of two continental pea cultivars, Mini and Kriter are described. The relationships between raw material characteristics, financial value of the crop (based on the system of payment used in France), and the quality of the processed product are discussed.

Introduction

Continental-style canned vining peas produced in France differ from the canned garden peas produced in the U.K. in several respects. French canners use a different range of vining pea cultivars, most of which have smaller and paler coloured seeds than their U.K. counterparts. Traditionally the French have used round or smooth seeded peas for canning (Thung & Gersons, 1966) although now both round and wrinkle seeded types are grown (Unilec, 1981).

In France peas for canning are always size-graded, with the two smallest grades (those less than 8.2 mm diameter, collectively termed 'sortes fines') fetching a premium price at both grower and retail levels. French peas are canned without the artificial green colouring used in the U.K., but often with the addition of herb and vegetable ingredients for added flavour (Vandommele, 1981). The French term for this type of product is 'a l'étuvée'.

In view of the developing interest in continental-style canned peas in the U.K., as shown by the increased availability of these products, an investigation of the relationship between maturity, yield, seed size distribution and sensory quality of the canned produce of two or the most popular French pea cultivars, Mini (wrinkle seeded) and Kriter (round seeded) was undertaken.

Methods

Six plots (1.8×23.0 m) of each cultivar were sown on 19 May 1983 at the Campden Food Preservation Research Association, in Gloucestershire. Single, whole plot harvests were taken daily for 6 days (23–28 July for Kriter and 26–31 July for Mini) as soon as sampling indicated that the crop had attained a tenderometer (TR) value of 90 or above. The tenderometer (Martin, 1937) is the standard commercial instrument for measuring maturity of peas in many countries. The peas were vined using a stationary plot viner. The yields of vined peas and the TR values of the ungraded samples were recorded. The peas were then sieved into four standard EEC size grades: less than

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7.5 mm diameter (extra fins); 7.5–8.2 mm diameter (très fins); 8.2–8.75 mm diameter (fins); and greater than 8.75 mm diameter (moyens). The seed yield and TR value of each of these grades was recorded. All samples of peas in the 'très fins' and 'extra fins' grades (i.e. the 'sortes fines') were blanched for 15 min at 93°C in three parts de-ionized water to one part tap water. The samples were cooled and filled into UT (73 mm internal diameter, 446 ml capacity) cans and covered with a brine containing 38 g sugar and 19 g salt/l. The cans were sealed and processed at 120°C for 15.5 min.

Sensory appraisal of the quality of the canned samples was carried out after 1 month's storage. Samples of Kriter were assessed by the QAV method (Adams, Bedford & Geering, 1981). Three replicates of each sample were assessed by each of three tasters who scored for attributes of colour, flavour and texture on a scale of 1–5, where a score of 1 represents a low level of the attribute and a score of 5 represents a high level of the attribute. Samples of Mini were assessed for texture attributes only by the Sensory Profile Method (Lyon, 1981) using three replicates of each sample and five tasters. Profile analysis provides a more sensitive technique than the QAV method for detecting differences in attributable intensity. Attribute scoring was carried out by placing a mark on a line 6.25 cm in length, at a point which corresponded to the degree of intensity which the assessor detected for the attribute. The marks on the line were then converted to a score of 0–50. The scores obtained by both methods of sensory appraisal were analysed by computer using the Mann-Whitney *U* Test for non-parametric comparisons. The significance level of the difference in attribute mean scores between any two samples of the same variety was determined by Fishers Modified LSD procedure.

Results

Tenderometer values

The TR readings of each size grade at each harvest are presented in Table 1. Whenever possible each value represents the mean of three readings, except where

Table 1. Effect of time of harvest on tenderometer readings of ungraded and size-graded raw peas

		Tenderometer readings				
Cultivar	Harvest date (July)	Ungraded sample	Moyens grade	Fins grade	Très fins grade	Extra fins grade
Kriter	23	113	—	134	124	106
	24	132	145	139	128	104
	25	142	147	152	146	118
	26	153	164	165	156	133
	27	> 180	> 180	> 180	> 180	168
	28	> 180	> 180	> 180	> 180	> 180
Mini	26	95	—	123	109	85
	27	108	—	132	116	95
	28	131	—	147	139	108
	29	149	—	169	158	127
	30	170	—	> 180	177	146
	31	> 180	—	> 180	> 180	> 180

there was insufficient seed or the first reading was in excess of the maximum on the tenderometer scale (TR 180). The results demonstrate that substantial variation occurs in the TR values of different seed size grades at any one harvest.

Seed size distribution

The proportion of seeds in each size grade at each harvest date are presented in Table 2. With increasing crop maturity the proportion of seeds in the 'extra fins' grade decreased and there was a corresponding increase in the proportion in the 'fins' and the 'moyens' grades. The percentage of seeds in the 'sortes fines' category decreased by 30% for Kriter and 21% for Mini, from the first to the last harvest.

Table 2. Effect of time of harvest on pea seed size distribution

		% in each grade				
Cultivar	Harvest date (July)	Moyens	Fins	Très fins	Extra fins	Sortes fines
Kriter	23	1	6	38	55	93
	24	2	15	55	28	83
	25	4	16	49	31	80
	26	7	26	48	19	67
	27	9	24	49	18	67
	28	8	29	46	17	63
Mini	26	0	3	34	63	97
	27	0	10	43	47	90
	28	0	19	43	38	81
	29	0	20	55	25	80
	30	0	28	52	20	72
	31	0	24	56	20	76

Yield and crop value

The comparative yield and crop value for each of the six harvests is presented in Table 3. The value of the crop was calculated from the total yield and from seed size distribution using the French system of two-tier payments. Under this system the price of the 'sortes fines' grade depends on the TR value of the sample (with a maximum limit

Table 3. Effect of time of harvest on relative yield and crop value

	Cultivar: Kriter		Cultivar: Mini	
Harvest number	Yield (% of max)	Value (% of max)	Yield (% of max)	Value (% of max)
1	97	100	83	89
2	91	90	86	90
3	100	97	100	100
4	95	86	90	90
5	97	18	81	13
6	95	20	87	12

of TR 150). Since specific TR values for the 'sortes fines' grade were not available, an average price for this grade was used in the calculations. The value of the 'moyens' and 'fins' grades is approximately half that of the 'sortes fines' grade and is not related to the TR values. For Kriter the maximum yield was obtained at the third harvest, although maximum crop value occurred at the first harvest. For Mini both the maximum yield and maximum value occurred at the third harvest. The decrease in value of both cultivars at harvests 6 and 7 was because the 'sortes fins' grade had by this time exceeded the TR 150 limit.

Table 4. Effect of tenderometer (TR) value on sensory appraisal of texture in canned pea samples

		Mean scores for texture attributes		
Cultivar	TR value of sample	Firmness of skins	Firmness of flesh	Mealiness of flesh
Kriter[1]	106 (Control)	1.89	2.67	2.67
	118	2.00	2.78	3.00
	124	2.11	2.89	3.44
	128	2.11	3.11	3.56
	133	2.56	3.78	4.00*
	146	2.67	3.89*	4.11*
	156	3.11*	4.11*	4.22*
Mini[2]	95 (Control)	15.00	11.20	16.53
	109	13.40	10.93	15.47
	116	20.87	15.53	22.07
	127	26.00†	21.47†	25.47‡
	139	30.27‡	27.73‡	32.22‡
	146	32.00†	28.40‡	32.80‡
	158	32.33‡	30.80‡	34.40‡
	177	34.93‡	37.13‡	32.87‡

Confidences of differences from control based on Fishers Modified LSD procedure: *95%, †99%, ‡99.9%.

[1]Scoring range 0–5;

[2]Scoring range 0–50.

Sensory appraisal of canned samples

Significant differences in the scores for texture occurred in both cultivars and these are presented in Table 4. The samples with the lowest TR values (TR 106 for Kriter and TR 95 for Mini) were deemed to be of optimum quality and were used as the controls for statistical comparisons. With Kriter increased mealiness of the flesh was detected at TR 133, TR 146 and TR 156; increased firmness of the flesh was evident at TR 146 and TR 156 and increased firmness of the skins was apparent at TR 156. With Mini, highly significant increases in the scores for mealiness, firmness of flesh and firmness of skins occurred at TR 127, TR 139, TR 146, TR 158 and TR 177.

Discussion

The results indicate that maximum yield, maximum value of the crop and optimum quality of the canned produce may not always be achieved at the same stage of crop

maturity. Thus, although the highest yield and the highest crop value occurred on the same date (harvest 3) for Mini, for Kriter a period of 2 days separated the peaks in yield (harvest 3) and crop value (harvest 1).

Regarding the quality of the canned produce, significant differences in the scores for texture attributes occurred between the samples of Kriter from the first harvest (TR 106 control) and those from the third harvest (TR 146). The samples at TR 146 were significantly firmer and more mealy in flesh texture, and hence were of inferior quality.

The profile analysis method of quality appraisal which was used to assess texture differences between the canned samples of Mini, clearly revealed the effect of increasing TR values. No significant differences occurred between the control sample at TR 95 and the samples at TR 109 and TR 116. At TR values of 127 and above there was a highly significant increase in the scores for all three texture attributes, which represented a pronounced deterioration in the overall quality of these samples. In terms of canned quality therefore, the optimum stage to harvest Mini was the first or second harvests when both the 'très fins' and 'extra fins' grades were below TR 127. At the third harvest, the TR value of the 'extra fins' grade was only TR 108, whilst that of the 'très fins' grade (which constituted 43% of the total harvested) was TR 139. The latter was therefore of inferior quality. Thus, although Mini produced the highest yield and gained the highest value at the third harvest, the quality of the 'très fins' grade at this harvest was less than optimum.

In conclusion, the continental system of size-grading vining peas for canning and of premium payments for the smallest size grades, means that the producer must have an understanding both of how a cultivar performs in relation to seed size development, and of the relationship between the TR of bulk and size-graded samples. These factors, together with the yield, determine the financial returns that will be received for the crop.

The work reported here has demonstrated the nature of these relationships for two popular continental cultivars. In addition, the results of sensory quality appraisal have revealed that at TR values of around 130, a significant increase in the firmness and mealiness of the canned samples can be detected by the taste panel. Commercially, however, peas of the 'sortes fines' grade are accepted for canning up to a limit of TR 150, as this value represents a compromise between poor quality in the canned produce if more mature peas were used, and loss of crop yield if less mature peas were stipulated. Although the trained and experienced panel of tasters could detect texture differences between the samples above and below TR 130, it is probable that these changes would not be obvious to the majority of consumers, and therefore a limit of TR 50 is acceptable in practice.

Acknowledgment

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Influence of packaging on the shelf life of frozen foods I. Carrot cubes*

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Summary

The effect of packaging and storage conditions on the shelf life of frozen carrot cubes were studied. The packaging had the greatest significance on shelf life when the storage temperature was warm, or when carrot cubes were subjected to storage conditions in an open retail display cabinet. Packages of low oxygen and water vapour permeability and transparency proved to be most advantageous.

Introduction

The dependence of the quality of frozen foods on storage temperatures and storage time has been studied quite exhaustively. However, the product, processing before freezing, and packaging also have an effect on the quality. The significance of packaging on the quality of frozen foods had received little attention up to a few years ago. In the selection of packages and packaging methods more attention has been paid to economical and technical aspects than to the protective effects of packages e.g. against freezer burn, oxidation of fat and formation of snow.

The influence of packaging on the shelf life of different frozen foods, especially on vegetables, has not been studied very widely. Steinbuch (1979, 1980) has studied the significance of packaging in the frozen storage of unblanched vegetables and noticed that vacuum packing improves the quality of such products as mushrooms, asparagus, parsley and celery. The shelf life of mushrooms is 3 months in a vacuum package instead of 2 weeks in a normal package when the storage temperature is −20°C. For a long time it has been known that the water vapour permeability of a package should be low enough for vegetables to be protected against drying and the resulting quality changes. The objective of this study was to find the optimal type of package from the point of view of quality for frozen carrot cubes, and to investigate quantitatively the effect of storage conditions on the shelf life.

Materials and methods

Pre-processing and packaging

To get representative samples, both the freezing and packaging of carrot cubes were carried out on an industrial scale. Before freezing, the carrot cubes were blanched, freezing being performed in a fluidized bed.

Test conditions

The significance of packaging on the shelf life of carrot cubes was studied in three closed frozen food storage cabinets, the dimensions of which were 1200×1800×

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2000 mm. The set temperatures of the cabinets were −12°C, −15°C and −18°C. There were four shelves in the cabinets. Packages of carrot cubes were stored on the two top shelves, where the temperature was lowest and fluctuated most. To balance the temperature differences between different positions of the shelves, the packages were circulated once a month on and between the two shelves on which they were stored. The actual centre temperatures of carrot cube packages varied during storage from −12.1 to −15.7°C at a nominal −12°C, from −15.4 to −19.3°C at −15°C and from −18.3 to −21.9° at −18°C. The cabinets were defrosted manually twice weekly. This procedure caused the temperatue of the carrot cubes to increase by 1.0 to 1.5°C. There was no illumination in the cabinets when the door was closed. The packaging material had no effect on the product temperature. In addition, the shelf life of carrot cubes was tested in an open display cabinet simulating retailing conditions. The set temperature of the display cabinet was −15°C. Because the cabinet was symmetrical the carrot cubes packed in the metallized PETP/LDPE bags were stored in the left half of the cabinet and the cubes packed in the LDPE bags in the right half.

The differences between the centre temperatures of the packages, as influenced by their differing infra-red reflectance and absorption properties, are presented in the results. The open display cabinet was defrosted automatically twice a day. After the defrost, the centre temperature reached the temperature before the defrost after 5 hr. The test packages were stored in the top layer of the cabinet (below the load line). The packages were circulated in the top layer twice a month, to balance the temperature differences in different positions of the top layer. The cabinet was equipped with its own light (OSRAM 40 W/32 warm de Luxe), which was switched on every morning and switched off every night from Monday to Friday. On Saturdays and Sundays there was no illumination. Reference samples were stored at −25°C to −27°C in an industrial storage.

Packages tested

In the closed frozen food cabinets, the storage life of carrot cubes was tested in three different cardboard and in six different plastic packages. Cardboard packages were made from: lightly low density polyethylene (LDPE)-coated folding boxboard (inside coating), water-repellent (WR) folding boxboard (inside coating) or aluminium foil laminated cardboard (outside coating). All the cardboard packages were so-called expresso-boxes, closed by heat sealing. The plastic packages were: low density polyethylene (95 μm) bag (LDPE bag), low density–high density polyethylene multilayer (60 μm) bag (LD-HD polyethylene bag), linear low density polyethylene (95 μm) bag (LLDPE bag), polyamide/low density polyethylene (20/65 μm) bag (PA/LDPE bag), polyester/low density polyethylene (12/50 μm) bag (PETP/LDPE bag) or metallized polyester/low density polyethylene (12/50 μm) bag (Met. PETP/LDPE bag). The polyethylene bags were white-pigmented. The package of the reference sample was an LDPE (95 μm) bag. The effect of nitrogen on the shelf life was studied using the three last-mentioned plastic bags. In the open display cabinet, the storage life was tested in a white-pigmented LDPE (95 μm) bag and in a metallized PETP/LDPE (12/50 μm) bag. The weight of all the packages was approximately 300 g.

Evaluation of samples

Quality changes of carrot cubes during storage were followed principally by sensory evaluation, which was made at intervals of 1–2 months for 14 months. The quality was evaluated both before and after cooking. The quality before cooking was evaluated by

two–three trained persons using for appearance and odour a nine-point scale (9 = extremely desirable; 1 = extremely undesirable) and for the amount of snow using a three-point scale (0 = no snow; 3 = a great deal of snow).

The quality of the samples after cooking was evaluated by a trained ten member taste panel for the acceptability of odour, texture and taste using a nine-point scale (9 = extremely desirable; 1 = extremely undesirable). For this evaluation carrot cubes were steamed for 10 min after which they were rationed into dishes and covered by styrox and glass. On the basis of hedonic scores, the quality of samples was graded into first class or second class. If the quality was in the third class, the sample was regarded as unacceptable. The limit of 1st class for odour, texture and taste was ≥ 6.5 points, and for overall points ≥ 21. The limit of second class for odour, texture and taste was ≥ 3.5 points and for overall points ≥ 12. The losses of weight were also determined by weighing five samples of each package before storage, in the middle of storage and at the end of storage.

Permeability of packaging materials

Oxygen and water vapour permeabilities and transparency of the packaging materials were determined in order to find out the effect of material characteristics on the shelf life of carrot cubes. Oxygen permeability for plastic materials was determined at room temperature, −2°C and −14°C by a Gas-Perm-Unit (constructed by the Central Institute for Industrial Research, Oslo, Norway) connected with a gas chromatograph. The water vapour permeability of plastic materials was determined at room temperature and +4°C by a Korput-apparat, in which the determination is based on the loss of weight. The water vapour permeability of cardboard materials was determined at +23°C and −20°C by the SCAN-P 22:68 method of the Finnish Pulp and Paper Research Institute. The transparency of plastic materials with diffused light was determined by equipment developed by the Electrical Engineering Laboratory of this Research Centre, and with u.v.-light using a u.v.-spectrofotometer.

Statistical analyses

On the basis of statistical calculations it was possible to plot the mean overall taste panel scores for the different packages versus time. Straight lines (correlation > 0.9 in most cases) appear with different slopes for loss of quality per time unit. By calculating confidence intervals of slopes (95% probability), statistically significant differences between packages could be found.

Results

Permeability of packaging materials

Of the packaging materials studied, PE films had the highest oxygen permeability both at room temperature and at frozen temperatures (Table 1). The least permeable was metallized PETP/LDPE laminate. PA/LDPE laminate and PETP/LDPE laminate were also relatively impermeable, especially at −2°C, at which temperature oxygen permeability was the same as that of metallized PETP/LDPE at room temperature. In contrast, the oxygen permeability of PE films at −14°C was many times greater than that of PA/LDPE and PEPP/LDPE laminates at +23°C. Water vapour permeability of all the plastic films was low even at room temperature, and at +4°C permeability was only 10–25% of that at +23°C (Table 1).

Table 1. Oxygen permeability, water vapour permeability and transparency of plastic films

Plastic film	Oxygen permeability (ml/m² 24 hr atm)			Water vapour permeability (mg/dm² 24 hr)		Transparency (%)	
	+25°C (RH 50–70%)	−2°C	−14°C	+23°C (RH 100%/10%)	+4°C	u.v.	diffuse light
LDPE (95 μm, pig)	1660	437	204	9.9	< 1.0	0.0	33.4
LD-HD-multil. film (60 μm, pig)	2380	672	328	13.1	3.5	1.2	57.6
LLDPE (95 μm, pig)	2670	547	281	23.7	6.5	0.3	52.0
PETP/LDPE (12/50 μm)	25	3	—	14.7	2.8	52.1	85.3
Met. PETP/LDPE (12/50 μm)	< 5	< 2	—	3.2	1.8	1.0	0.9
PA/LDPE (20/70 μm)	70	12.4	—	13.6	1.6	58.2	89.0

Table 2. Water vapour permeability of cardboard materials

Cardboard	Water vapour permeability (mg/dm² 24 hr)	
	+23°C	−20°C
Al-foil laminated	1	—
Lightly LDPE-coated	31	30
WR-coated	4820	820

Water vapour permeability of cardboard materials at +23°C was higher than that of plastic films (Table 2). WR cardboard had a very high permeability. At −20°C the permeability of cardboards, not taking into account WR cardboard, was nearly the same as for plastic films at room temperature. However, the methods of determination were not the same for the two types of materials. The fall in temperature does not seem to affect the permeability of cardboards as much as that of plastic films. Transparent films had the highest transparency both for diffused light and for u.v.-light (Table 1). The best light reflection was found with metallized PETP/LDPE laminate. White pigment reduces u.v., as well as the diffused light-transparency very significantly (PE films). Transparency and reflectance for infra-red radiation were not measured directly. Their effects on the temperature of the packages are presented in Fig. 1.

Sensory quality

The sensory quality before cooking was not significantly influenced by temperature and the type of package, with the exception of the WR cardboard box, when samples were stored in closed frozen food cabinets. On the other hand the quality of the reference sample before cooking deteriorated markedly more slowly than that of other samples, and even after 14 months' storage the quality before cooking was perfect. The appearance and odour both received scores of 7–8 (max 9), whereas the appearance and odour of the best sample after 14 months in the closed frozen food cabinet received only 4–5 points. The quality of the reference sample decreased after cooking nearly as

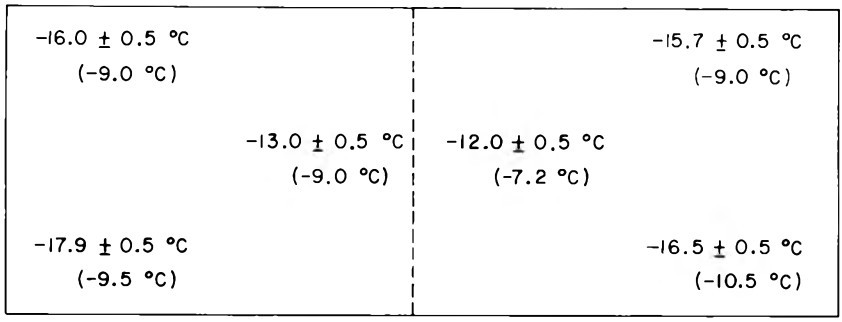

Figure 1. Temperatures in the centre of packages of carrot cubes stored in the top layer of an open display cabinet where the set temperature was −15°C; left side: metallized PETP/LDPE bag and right side: LDPE bag. The temperatures in the figure were determined while the cabinet was illuminated. Without illumination the temperatures were 0.5°C colder. Numbers in brackets refer to the highest temperatures during the defrosting cycles.

quickly as the quality of carrot cubes packed in plastic packages and stored at −15°C and −18°C (Fig. 2). Thus the lowering of the storage temperature from −15°C to −25°C does not seem to increase the shelf life of carrot cubes packed in plastic packages. For the consumer, the quality after cooking is evidently more important than that before cooking. For this reason, only the quality after cooking is treated in the following.

In closed frozen food cabinets significant differences between the packages were found only at −12°C (Table 3, Figs 2 and 3), where the best packages for quality were tight plastic bags. The main differences were in the odour and taste. At −12°C the

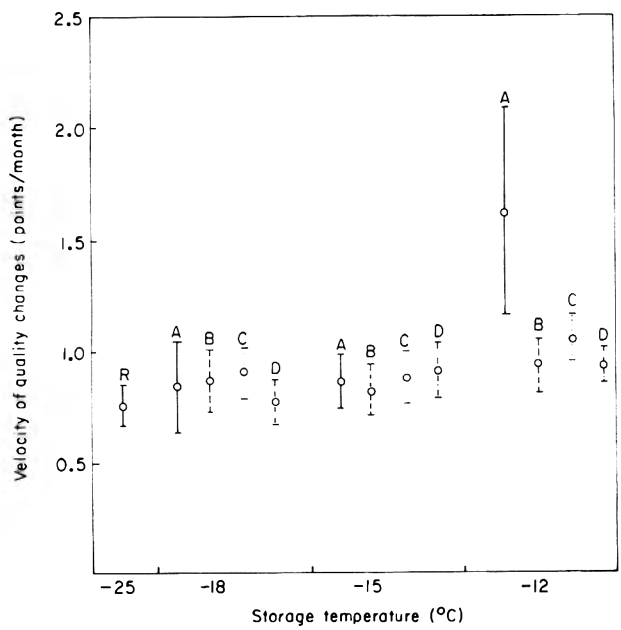

Figure 2. The effect of different kinds of plastic packages and storage temperature on the rate of quality changes in carrot cubes stored in closed frozen food cabinets; vertical lines: confidence interval of the rate (95% probability); R = reference sample, A = LDPE bags (LDPE, LD-HD and LLDPE bags), B = PA/LDPE bag, C = PETP/LDPE bag and D = metallized PETP/LDPE bag.

Table 3. The shelf life of frozen carrot cubes packed in different packages and stored in closed frozen food cabinets. I = first quality class (see text). II = second quality class (acceptable quality). The shelf lives were determined on the basis of hedonic scores given after cooking

	Shelf life (months)					
	−12°C		−15°C		−18°C	
Package	I	II	I	II	I	II
Al-foil laminated cardboard box	2–3	10–12	3–5	> 12.5	3–5	> 11.5
Lightly LDPE-coated cardboard box	2–4	10–11.5	3–4	> 12.5	4.5–6	11.5–13.5
WR cardboard box	1.5–2	8.5–10	3–4	10.5–12.5	4.5–6	11.5–13.5
LDPE bag	2–4	8.5–10	3–4	> 12.5	3–4.5	> 13.5
LD-HD polyethylene bag	2–4	7–8.5	3–4	12.5	3–4.5	> 13.5
LLDPE bag	1.5–2	7–8.5	3–4	> 12.5	4.5–6	> 13.5
PA/LDPE bag	2–4	> 13.5	3–4	> 12.5	4.5–6	11.5–13.5
PA/LDPE bag+N_2	2–4	> 13.5	4–5.5	> 12.5	3–4.5	> 13.5
PETP/LDPE bag	2–4	11.5–13.5	3–4	> 12.5	4.5–6	> 13.5
PETP/LDPE bag +N_2	4–5	7–8.5	3–4	> 12.5	4.5–6	> 13.5
Met. PETP/LDPE bag	4–5	> 13.5	3–4	> 12.5	3–4.5	> 13.5
Met. PETP/LDPE bag+N_2	4–5	> 13.5	3–4	> 12.5	4.5–6	> 13.5
Reference (−25°C)	4.5	> 13.5				

deterioration of odour and taste was faster than that of the texture. On the other hand, the texture of carrot cubes, including that of the reference, was regarded as watery throughout the storage time at all storage temperatures and nearly in all packages. The texture of carrot cubes packed in the WR cardboard box was clearly poorer than in other packages.

The water vapour permeability of the WR cardboard box was very high. For this reason, the carrot cubes in this package were very dry and tough and they resembled raisins at the end of the storage (note also the weight losses). The taste was sweeter than that of carrot cubes in other packages. Obviously for this reason the carrot cubes in this package were considered acceptable a surprisingly long time. On the other hand, at −12°C in the carrot cubes stored in cardboard boxes, especially in the WR cardboard box, 'off-odour' and 'off-flavour' caused by cardboard were observed. At −12°C the carrot cubes packed in PE bags kept their quality significantly less well than those packed in tight plastic packages (Fig. 2, Table 3). Defects in quality were various 'off-odours' and 'off-flavours', which were often described as odour and flavour of plastic and chemicals. However, at −15°C and −18°C the carrot cubes packed in PE bags kept their quality as well as carrot cubes packed in tight plastic packages. The best cardboard package at all temperatures tested was an aluminium foil laminated cardboard box, at −12°C it being even better for keeping quality than PE bags (Table 3). Nitrogen did not improve the preservation of the quality of carrot cubes as compared with normal packagings (Table 3). The after-cooking quality of carrot cubes remained in the first quality grade for 1.5–6 months, depending on packaging and temperature (Table 3). The shelf life was highest in the most impermeable packages (PA/LDPE, PETP/LDPE, and metallized PETP/LDPE bags) and increased temperature only slightly diminished the shelf life in the first quality grade. The shortest shelf life (1.5–2

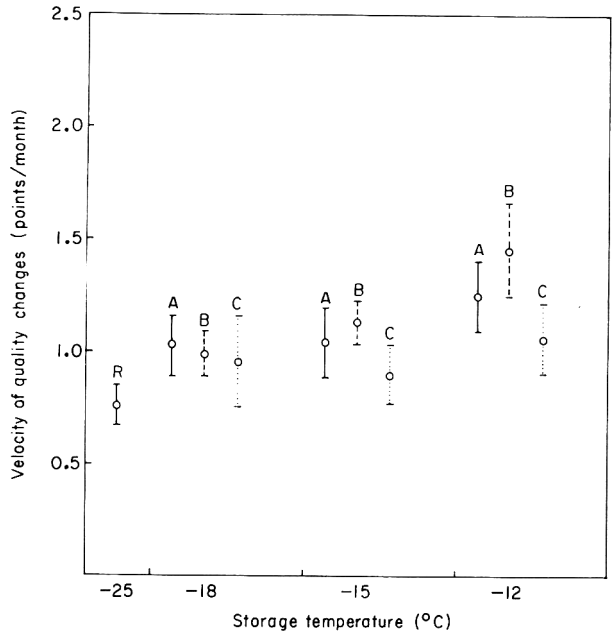

Figure 3. The effect of different kinds of cardboard packages and storage temperature on the rate of quality changes in carrot cubes stored in closed frozen food cabinets; vertical lines as in Fig. 1; R = reference sample, A = a box of lightly LDPE-coated folding boxboard, B = a box of WR-coated folding boxboard and C = aluminium foil laminated cardboard box.

months) in the first quality grade was observed in the WR cardboard box and in the LLDPE bag, when the storage temperature was −12°C. In the case of these two packages the effect of temperature was very significant; at −18°C the quality kept in the first grade for 4.5–6 months, and the shelf life was comparable to that of the reference sample at −25°C.

The package had the greatest effect on the quality of carrot cubes stored in the top layer of an open display cabinet (Table 4 and Fig. 4). In the metallized PETP/LDPE laminate bag, the quality after cooking was maintained nearly as well as that of the reference sample. In the white-pigmented LDPE bag (the same package as the reference) the quality, texture and taste deteriorated significantly faster than in the metallized package, the acceptability time of the latter being about 2.5 times that of the former. However, the shelf life in the first quality class was less than 1.5 months regardless of package (Table 4). In the metallized bag the quality was acceptable throughout the storage time (11 months), but in the LDPE bag only for 3–5 months.

Table 4. The shelf life of frozen carrot cubes stored in the top layer of an open display cabinet at −15°C and packed in two different packages. I = first quality class, II = second quality class

	Shelf life (months)	
Package	I	II
LDPE bag	< 1.5	3.5
Met. PETP/LDPE bag	< 1.5	> 11

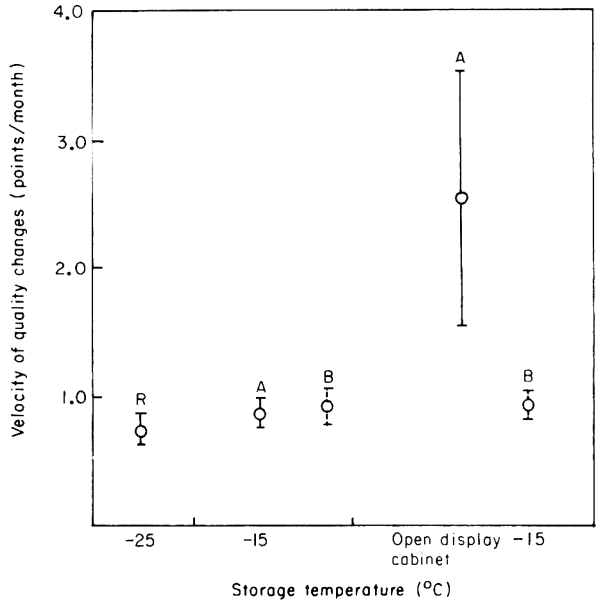

Figure 4. The effect of package on the rate of quality changes in carrot cubes stored in closed frozen food cabinet or in the top layer of an open display cabinet at −15°C; vertical lines as in Fig. 1; R = reference sample, A = LDPE bag and B = metallized PETP/LDPE bag.

This was evidently a result of the very low oxygen permeability of metallized laminate at −15°C, whereas low density polyethylene (95 μm) has an oxygen permeability of about 200 ml/m² 24 hr atm at −15°C (Table 1). Thus oxidation reactions occur, catalysed by u.v.-light, although the u.v.-transparency of pigmented polyethylene is low. Metallized polyester/low density polyethylene also provided better protection against the tendency of light and infrared radiation to increase the temperature (Fig. 1). The temperature in the metallized bag was 1–2°C colder than in the LDPE bag. Hawkins, Pearson & Raynor (1975) discovered that 95% of the energy to which the frozen foods in an open display cabinet are exposed is caused by infrared radiation.

Weight losses and amount of snow

Carrot cubes suffered losses of weight principally only in cardboard packages, the losses being significant only in the WR cardboard box (Table 5), where the weight losses after 9 months varied from 19.0 to 31.5% depending on the storage temperature. This was the result of the very high water vapour permeability of WR cardboard. Weight losses were not produced in packages stored in an open display cabinet. However, the amount of snow caused by fluctuating temperature was markedly higher than in the packages stored in the closed frozen food cabinets (Table 6). In the LDPE bag the amount of snow was evaluated even after 3 months' storage with maximum points; 3. For this reason, carrot cubes in the LDPE bag stored in an open display cabinet were dry and also hard. In the metallized bag the amount of snow did not increase to maximum points during the 11 months. Snow also accumulated in the packages stored in closed frozen food cabinets (Table 6). The amount of snow was rather smaller at −18 than at −12°C, especially after 12 months' storage. In addition, in cardboard packages where weight losses were produced, the amount of snow was markedly smaller than in plastic packages. The water which evaporated from carrot cubes also evaporated out of the

Table 5. The weight losses of carrot cubes packed in different packages and stored in closed frozen food cabinets and in the top layer of an open display cabinet

	Weight losses (%)						
	Closed frozen food cabinet						Open food cabinet
	−12°C		−15°C		−18°C		−15°C
Package	9*	14	9	14	9	14	9
Cardboard packages							
Al-foil laminated cardboard box	0.24	0.77	0.29	0.81	0.22	0.79	—
Lightly LDPE-coated cardboard box	0.30	0.62	0.21	0.56	0.14	0.37	—
WR cardboard box	31.5	39.1†	26.2	44.2	19.0	34.7	—
Plastic packages	‡	‡	‡	‡	‡	‡	‡

*Months.
†After 10 months' storage.
‡No weight losses.

cardboard packages, because the water vapour permeability of cardboards, especially that of WR cardboard was sufficiently high even at frozen temperatures.

Table 6. The amount of snow in different packages after 12 months' storage, averages of evaluations on three-point scale

	Amounts of snow			
	Closed cabinets			Open cabinet
Package	−12°C	−15°C	−18°C	−15°C
Al-foil laminated cardboard box	1.25	1.50	1.00	—
Lightly LDPE-coated cardboard box	1.50	1.50	1.00	—
WR cardboard box	—	0.75	0.25	—
LDPE bag	—	1.75	1.50	3.00*
LD-HD polyethylene bag	—	2.25	1.50	—
LLDPE bag	—	1.50	2.25	—
PA/LDPE bag	2.00	2.25	1.75	—
PA/LDPE bag+N_2	1.50	1.50	1.25	—
PETP/LDPE bag	2.00	1.50	1.50	—
PETP/LDPE bag+N_2	—	0.75	1.25	—
Met. PETP/LDPE bag	1.75	2.00	1.25	2.75
Met. PETP/LDPE bag +N_2	1.50	1.75	0.50	—
Reference (−25°C)	0.50			

*After 3 months.

Discussion and conclusions

On the basis of the results obtained, the type of package has the greatest effect on the shelf life of frozen carrot cubes stored at warm storage temperatures and in conditions where carrot cubes are subjected to light, infra-red radiation and fluctuating temperatures. This is of particular importance for storage under the storage conditions in an open retail display cabinet. The shelf life of carrot cubes stored in the top layer of an open display cabinet was in a tight metallized package 2–3 times that in a LDPE bag. This was without doubt caused by the low oxygen and water vapour permeabilities and by the low transparency to light and infra-red radiation of the metallized laminate. The temperature of the product was in a metallized bag 1–2°C colder than in a LDPE bag. The price of the metallized laminate is about three times that of LDPE, but is comparable to that of cardboard boxes. Particularly in open display cabinets, the more permeable packages do not adequately prevent quality changes such as the forming of snow, drying, 'off-odour' and 'off-flavour'.

The results are partly in contrast with earlier observations and with the general Time-Temperature-Tolerance (TTT) concept. Thus in our studies, the shelf life of carrot cubes in plastic packages was in the temperature range from −15 to −25°C virtually independent of temperature. This emphasizes the role of packages in determining the shelf life of frozen vegetables.

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Influence of packaging on the shelf life of frozen foods II. Baltic herring fillets*

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Summary

The influence of packaging and storage conditions on the shelf life of fillets of Baltic herring was studied. Packaging affected the quality under all the storage conditions studied particularly under retailing conditions. Under conditions simulating frozen storage, the shelf life in vacuum packages of low oxygen and water vapour permeability was twice that in cardboard packages. Under storage conditions in an open retail display cabinet, protection against the chemical and temperature-elevating effects of light and infra-red radiation was found to be essential for good shelf stability. In a vacuum package covered with metallized cardboard the shelf life was at least twice that in a vacuum package without covering.

Introduction

The significance of packaging on the shelf life of various frozen foods has not been studied very extensively. Primarily the dependence of quality of frozen fish and meat products on packaging materials and packaging methods has been investigated to some extent (Bramsnaes & Sörensen, 1960; Bøgh-Sørensen & Jensen, 1981; Lindsay, 1977; Löndahl & Danielson, 1972; Yu, Sinnhuber & Crawford, 1973). These investigations indicate that particularly fat fish like trout, and also peeled shrimps, individually quick-frozen, retain their quality in vacuum packagings of low oxygen and water vapour permeability as much as twice as long in a polyethylene bag or cardboard package. The high quality life (HQL) of many meat products is also in vacuum packages two times that in a PE bag. Further, it has been shown for example for sliced smoked bacon that it is not sufficient just to vacuum pack, it is also necessary to use a packaging material with a very low oxygen permeability (Bøgh-Sørensen & Jensen, 1981). On the other hand investigations where packaging material has been only wrapped around a product indicate that packaging material and packaging method have only little significance on the storage life of frozen meat. According to Jeremiah (1980) there are no consistently significant differences among protective wraps (for example aluminium foil and PE wrap) in maintaining the quality of various fresh and cured pork cuts. The results of Gokalp, Ockerman & Plimpton (1979) indicated that frozen beef retains its quality better in a plain polyethylene wrapping than in a PE wrapping, vacuum sealed with a metal clip, or in a PE wrapping filled with CO_2, not sealed but gently folded.

The protective effect of correctly chosen packaging methods and materials seems to improve the shelf life especially for frozen fish and meat products. The purpose of this study was to investigate quantitatively the effects of various alternative packagings on the shelf life of frozen Baltic herring fillets stored under different conditions.

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Materials and methods

Preparation and packaging

In order to obtain results which would be relevant to industrial products, all the pre-processing, packaging and freezing stages were carried out in an industrial scale. Fillets were frozen to blocks of 300 g in a plate freezer on the day they were caught. All fillets packed in test packages were from the same catch having similar size and fat levels.

Test conditions

Storage tests were performed at −12, −15 and −18°C in the same closed frozen food cabinets as those used for carrot cubes (Ahvenainen & Mälkki, 1985). The actual centre temperatures of Baltic herring fillet packages during the storage varied from −11.5 to −13.3°C at a nominal −12°C; from −13.6 to −14.8°C at −15°C; and from −17.3 to −19.1°C at −18°C. The defrost, performed twice a week, raised the temperatures by about 0.5–1.0°C. In order to balance the uneven temperature distribution in the cabinet, the packages were circulated once a month on the shelf on which they were stored.

In addition, the shelf life was tested in an open display cabinet simulating retailing conditions. The set temperature of this cabinet was −15°C. The centre temperatures measured are presented in the results. The open display cabinet was defrosted automatically four times a day, and the return to the temperature before defrost took ½ hr. The test packages were stored in the top layer of the cabinet. Otherwise the experimental conditions were the same as those stated for carrot cubes (Ahvenainen & Mälkki, 1985). Reference samples were stored at −25 to −27°C in industrial storage.

Packages tested

The following packagings were used:

(a) in the closed frozen food storage cabinets:

(i) box of latex-coated (inside and outside coating) folding boxboard (experimental), hot-melt glue sealed;

(ii) as No. 1, but the herring fillets were wrapped in thin low density polyethylene film (LDPE) 15 μm;

(iii) box of low density polyethylene (LDPE)-coated (inside and outside coating) cardboard, hot-melt glue sealed;

(iv) deep-drawn package, bottom polyamide/low density polyethylene (PA/LDPE) multilayer of 175 μm, lid polyamide/low density polyethylene (PA/LDPE) laminate 20/70 μm;

(v) as No. 4, bottom laminate 122 μm;

(vi) as No. 4, bottom laminate 110 μm, lid 20/50 μm; and

(vii) low density polyethylene (LDPE) bag, 50 μm, polyvinylidene chloride (PVDC) laquered (experimental).

Vacuum was applied to packages Nos 4–6 and nitrogen filling after vacuum to Nos 4–7. Storage was in closed frozen food cabinets.

(b) in the top layer of an open display cabinet: (no. 4 as above with vacuum and)

(viii) No. 4 as above with vacuum, covered with a metallized (aluglass) cardboard box (metallized layer on the outside of a box).

(c) in the industrial reference storage:

No. 4 as above with vacuum, covered with a box of low density polyethylene (LDPE)-coated (inside and outside coating) folding boxboard.

Evaluation of samples

Quality changes of frozen herring fillets during storage were followed principally by sensory evaluation, which was made at intervals of 1–2 months during about 1 year. The quality was evaluated both before and after cooking. For the evaluation, the fillets were thawed in a refrigerator overnight. The evaluation before cooking was performed by a group of two–three trained persons using for appearance a two-point scale (2 = extremely desirable; 0 = extremely undesirable) and for odour a 4-point scale (4 = extremely desirable; 0 = extremely undesirable). For the evaluation after cooking, the thawed fillets were wrapped in aluminium foil and steamed for 15 min. The quality after cooking was evaluated by a trained ten member taste panel. For the acceptability of appearance a two-point scale, for odour a four-point scale, and for taste a ten-point scale were used. If a half of the taste panel members gave points below 5 to the taste, the sample was regarded as unacceptable. Losses of weight, thiobarbituric acid (TBA) number, peroxide number and free fatty acids were also determined.

Permeability of packaging materials

Oxygen and water vapour permeability of the packaging materials of fillets were determined as described previously (1985) by us.

Statistical analyses

Overall taste panel scores for the different packages were plotted *versus* time. Straight lines (correlation ≥ 0.9) appeared with different slopes indicating a loss of quality per time unit. By calculating confidence intervals of slopes, statistically significant differences between packages (95% probability) were found.

Results

Permeability of packaging materials

The oxygen permeability of all plastic materials used in packages for fillets of Baltic herring was quite low, especially at −2°C (Table 1). The most impermeable was the PA/LDPE multilayer laminate 175μm. However, the permeability after deep drawing was not determined. Also the water vapour permeability of plastic materials was low (Table 1).

Table 1. Oxygen permeability and water vapour permeability of plastic films

	Oxygen permeability (ml/m^2 24 hr atm)		Water vapour permeability (mg/dm^2 24 hr)	
Plastic films	+25°C RH 50–70%	−2°C	+23°C RH 100%/10%	+4°C
PA/LDPE (20/50 μm)	80	12.6	28.4	5.1
PA/LDPE (20/70 μm)	70	12.4	13.6	1.6
PA/LDPE-multil. (175 μm)	25	5.0	17.1	2.9
PA/LDPE-multil. (122 μm)	40	—	27.1	5.7
PA/LDPE-multil. (110 μm)	40	10.6	14.0	2.0
PVDC-laq. LDPE (50 μm)	60	—	27.0	—

By contrast, the water vapour permeability of cardboard materials was quite high even at −20°C (Table 2). The permeability of latex-coated cardboard decreased most with lowering of temperature.

Table 2. Water vapour permeability of cardboard materials

Cardboard	Water vapour permeability (mg/dm² 24 hr) +23°C RH 50%	−20°C
Latex-coated	210	38
LDPE-coated	29	22
Metallized	66	26

Sensory quality of herring fillets

Packaging and storage temperature had a markedly greater effect on the shelf life of herring fillets than that of carrot cubes (Ahvenainen & Mälkki, 1985). At −18°C, herring fillets kept their after-cooking quality in all packages about 1.5–2 times as long as at −12°C (Table 3). The reference sample retained both its before- and after-cooking quality very significantly better than the samples in the closed frozen food cabinets at −12–18°C (Fig. 1). The only exception was a vacuum deep drawn package (bottom from PA/LDPE multilayer laminate, 175 μm), in which herring fillets kept their quality at −18°C only fairly significantly more poorly than the reference (Fig. 1). This package was the most advantageous for the quality of fillets stored in closed frozen food cabinets (Table 3, Fig. 1), the shelf life being 1.5–2.2 times that in cardboard packages, depending on the storage temperature. The differences in thickness of the deep drawn laminates tested had a significant effect on the shelf life (Table 3). The thinnest laminate was also from a practical point of view weaker, since deep drawing it was quite difficult to handle and the corners of a package broke very easily. The storage life of herring

Table 3. The effect of packaging and storage conditions on the shelf life (acceptability time) of fillets of Baltic herring stored in closed frozen food cabinets and in the top layer of an open display cabinet

Package	Shelf life (months)* In closed cabinets −12°C	−15°C	−18°C	In open display cabinet −15°C
1	4	5.5	6	—
2	5	4	6	—
3	5	5.5	6	—
4 with vacuum	8	11	13	6
4 with N_2	8	8	10	—
5 with vacuum	6.5	11	12	—
5 with N_2	6.5	8	8	—
6 with vacuum	6.5	8	10	—
6 with N_2	5	5.5	8	—
7 with N_2	4	5.5	8	—
8	—	—	—	> 11
Reference (−25°C)	> 13			

*Shelf life has been determined according to the quality after cooking.

fillets in the two different cardboard packages tested was similar. Thin LDPE wrapping around herring fillets improved the quality a little but not significantly. In packagings with nitrogen, herring fillets retained their quality less than in corresponding vacuum packages, but in general not significantly less (Fig. 1). In a PVDC-lacquered bag, in which herring fillets had been packed with nitrogen, herring fillets kept their quality better than in cardboard packages, especially at −18°C. However, the difference was not significant, although the permeabilities of the material were low (Table 1). It is

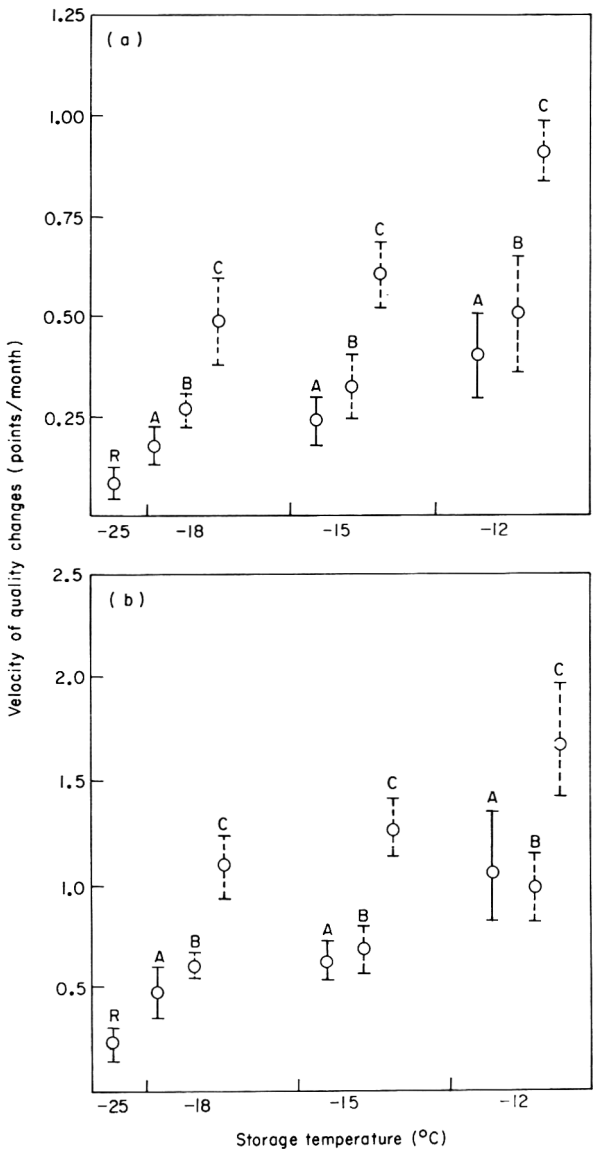

Figure 1. The effect of packaging and storage temperature on the rate of quality changes in Baltic herring fillets stored in closed frozen food cabinets. (a) Quality changes before cooking and (b) Quality changes after cooking; vertical lines: confidence interval of the rate (95% probability); R = reference sample. A = deep-drawn package with vacuum (bottom from 175 μm PA/LDPE multilayer laminate). B = deep-drawn package with nitrogen. C = LDPE-coated cardboard box.

obvious that if a PVDC-lacquered bag had been thicker, the keeping of quality would have been better, but because the thickness was only 50 μm, the corners of the bag were broken in most cases. Also a decrease in the shelf life when nitrogen was used was observed.

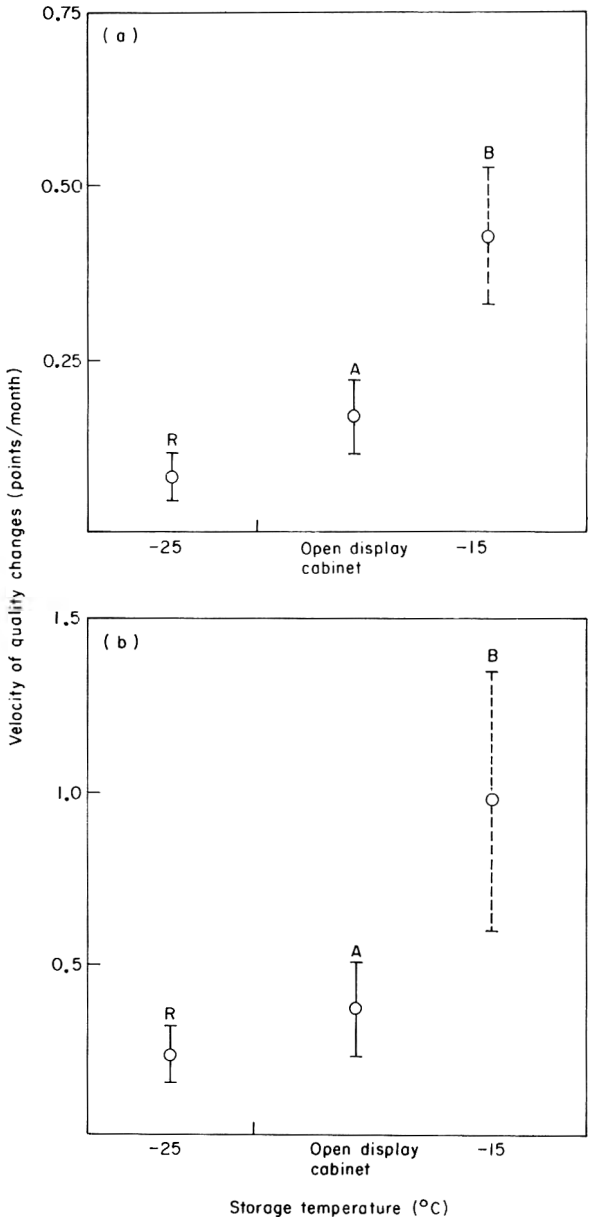

Figure 2. The effect of packaging on the rate of quality changes in Baltic herring fillets stored in the top layer of an open display cabinet. (a) Quality changes before cooking and (b) quality changes after cooking; vertical lines as in Fig. 1; R = reference sample, A = deep-drawn package with vacuum covered with a metallized cardboard box, B = deep-drawn package with vacuum without covering.

The quality changes produced during the storage were typical of frozen fish. Taste and odour became old, rancid and bitter, and light and brown spots made the appearance poor. Especially in non-heat-sealed cardboard packages, the fillets were dry at the end of the storage. The velocity of quality changes depended on the package and storage conditions. In cardboard packages which were not quite tight and in which the wrapping or packaging material was not tight around the product, fish fillets deteriorated faster than in vacuum packages with low oxygen and water vapour permeability. Even at −18°C fish fillets packed in cardboard packages or in a PVDC-lacquered bag had a slightly rancid odour and taste after 7.5 months' storage. In addition, in cardboard packages and in the PVDC-lacquered bag, the appearance of fish fillets suffered more than in vacuum packages. Also in nitrogen packages fish fillets had more defects in appearance, mainly light spots, than in vacuum packages. In vacuum packages the quality changes were relatively slow. Packaging had the most important effect on the keeping of the quality of herring fillets stored in the top layer of an open display cabinet. In the vacuum deep drawn package covered by a metallized cardboard box the quality was retained very significantly better than in the vacuum package without covering (Table 3). During a storage period of 11 months the velocity of quality changes in herring fillets packed in a metallized box was not significantly higher than that of the reference sample (Fig. 2). The metallized cardboard box protected the vacuum package very well from the effects of light and infra-red radiation. The temperature of fish fillets inside this package was about 4°C lower than in the vacuum package without covering (Fig. 3). The temperature differences between these two types of packages were obviously due partly to the reflectance of the metallized layer to light and infra-red radiation and partly to the small isolating air space remaining between the cardboard and the vacuum package. Without the metallized cardboard box, the acceptable time of herring fillets stored in the open display cabinet at −15°C was only 6 months in the vacuum deep drawn package, whereas in the closed frozen food cabinet at −15°C it was 11 months.

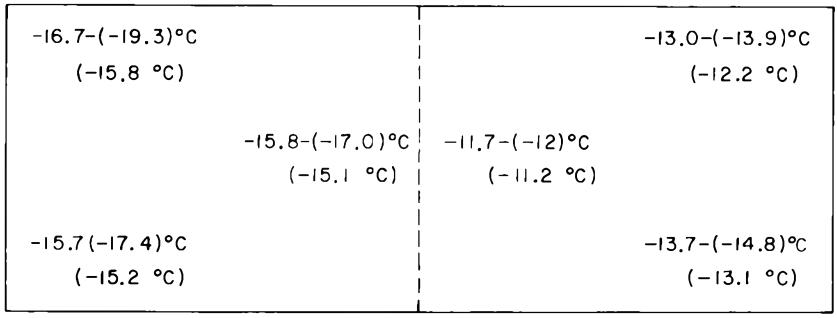

Figure 3. Temperatures in the centre of packages of Baltic herring fillets stored in the top layer of an open display cabinet adjusted to a temperature of −15°C. Left side: vacuum deep-drawn package covered with a metallized cardboard box and right side: vacuum package without covering; numbers in brackets refer to the highest temperatures during the defrosting cycles.

Weight losses and chemical changes

Fillets of Baltic herring had weight losses generally only in cardboard packages. The highest losses of weight were observed in the latex-coated cardboard box, being 3.8–5.3% at the end of the storage, depending on temperature. Thin LDPE wrapping also decreased weight losses in this package to one tenth. The small weight losses

(0.19–0.44% at the end of storage) in the thinnest plastic packages were a result of broken corners. TBA numbers, which correlate with oxidation of fats, varied with storage time. In vacuum and nitrogen packages, the TBA number of fish fillets was markedly lower than in cardboard packages during the whole time of storage. Peroxide numbers were also higher in cardboard packages than in vacuum and in nitrogen packages. After 4 months' storage at −12°C the peroxide number of the herring fillets was in the latex-coated cardboard package 19.9 mval/kg, in the vacuum package (No. 4.) 2.5 mval/kg and in the nitrogen package (No. 4.) 4.6 mval/kg. At −18°C the corresponding numbers were 9.2, 3.0 and 2.7, respectively. A thin LDPE wrapping did not decrease TBA and peroxide numbers. This was obviously due to the high oxygen permeability of LDPE and the looseness of the wrapping.

Discussion

The greatest differences between the packages tested were in good correlation with the oxygen and water permeabilities of the materials, as was expected. The protective effect of the vacuum packages with low oxygen and water vapour permeabilities allows a shelf life in retail storage cabinet temperatures of up to two times that in permeable packages. On the other hand, in open display cabinets vacuum packing is not sufficient if its transparency is high. For this reason, for retailing conditions, the fish should be protected by a vacuum package with low oxygen and water vapour permeability and low transparency and absorptivity at the long wave lengths. The mechanical strength of the package should also be good. The most advantageous package is a vacuum package covered with another packaging material (e.g. cardboard) with low transparency and good mechanical strength, as this investigation indicated.

However, even in good package fillets of Baltic herring could obviously not be stored at temperatures above −25°C for a long time without producing defects in quality. In retail stores where storage times of frozen foods are relatively short, the present realistic storage temperatures could be maintained, if fish fillets were packed in a vacuum package covered by an aluminium foil laminated or metallized package.

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Heat stability of fish muscle proteins

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Summary

The heat stability of the proteins present in cod, siganus and tilapia fish muscle were compared using differential scanning calorimetry and by measuring the solubility of the proteins in weak and strong salt solutions following heat treatment at selected temperatures from 25° to 100°C for 1 hr. It was found that the stability of both the collagens of the connective tissue and the myosins of the myofibrillar proteins varied between species, the more stable proteins being those from the fish found in the waters of higher ambient temperatures. The stability of the sarcoplasmic proteins also exhibited some species dependence but the actins present in the three species were all of similar thermal stability. The thermograms obtained by scanning calorimetry indicated that during frozen storage some changes in the nature of the myosin molecule occurred. It is suggested that scanning calorimetry may be a rapid and simple means of following the so-called cold store denaturation of frozen fish but more work is required to confirm this.

Introduction

The stability of fish proteins is of importance in relation to the properties of processed fish products. Thus freezing, heat and infusion of salt solutions can all affect the functional properties of the proteins resulting in increased or decreased organoleptic acceptability. Proteins of mammalian origin, particularly myosin and collagen are known to be more stable than those derived from fish. For example, Connell (1961) studied the myosins present in several different cold water fish species and compared them with those found in ox, rabbit, chicken and frog. Although only small differences were found in stability between the myosins of the fish species, these proteins were all much less stable than those derived from ox, rabbit or chicken muscle; frog myosin appeared to be intermediate in stability between that for mammals and cold water fish. Connell concluded that myosins of different species had adapted to the different body temperatures and that there was a connection between this phenomenon and the resistance of a muscle towards changes caused by processes such as freezing and frozen storage or dehydration. It is also well established that the connective tissue proteins (collagens), of various species increase in stability with increase in their *in vivo* body temperatures (Menashi *et al.*, 1976).

Fish may be caught in waters with average temperatures ranging from close to freezing point up to 30 or 35°C. From Connell's results and others (Hamoir, McKenzie & Smith, 1960; Cheng, Hamann & Webb, 1979) it would be expected that the stability of the proteins of fish found in waters of such widely differing temperatures would also

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vary. This in turn may well affect the optimum processing conditions for the ultimate product whether it be frozen storage temperature, temperature of smoking or drying, or the salt concentration necessary to obtain a satisfactory gel. Indeed, Jones & Disney (1977) suggested that in some respects the technology of warm water species could have closer similarities to meat technology than to cold water fish technology.

In this study two techniques were used to gain a further insight into the heat stability of temperate and tropical water fish muscle. The first involved solubility studies in which changes in the extractability of the proteins in 0.05 M and 1.0 M salt solutions were measured after heating at different temperatures. This technique is widely used to follow the so-called cold store denaturation which occurs during frozen storage of fish (Mills, 1975).

The second technique was a differential scanning calorimetry study (DSC) in which the weak heat effects associated with the denaturation of proteins is monitored. To date a considerable amount of work has been carried out using this technique on a range of protein foods. One of the most intensively studied systems has been the striated muscle of meat producing animals (Ledward & Lawrie, 1975; Stabusvik & Martens, 1980; Wright, Leach & Wilding, 1977). These studies have all shown that there are at least three distinct transitions in the thermograms of these meats. A transition with a peak temperature at about 60°C has been ascribed primarily to myosin denaturation whilst one centred at about 81°C has been ascribed to actin denaturation (Wright *et al.*, 1977; Stabusvik & Martens, 1980). A further transition with a peak temperature at about 67°C has been ascribed to the denaturation of sacroplasmic and/or collagenous proteins (Wright *et al.*, 1977; Stabusvik & Martens, 1980). Peak maximum temperature T_{max} from thermograms obtained by DSC are usually a few degrees above the actual denaturation temperature at which the transition begins. However, these onset temperatures (T_0) are often difficult to discern in the complex thermograms given by many foods and the use of peak maximum temperatures to describe such transitions is becoming accepted practice and is used in this paper.

Scanning calorimetry studies on the isolated, purified myofibrillar proteins of meat have also been undertaken (Wright, 1978) as have studies on the acid soluble and acid insoluble collagens of a range of cold water fish and animal species (Menashi *et al.*, 1976). These studies have yielded fundamental insights into the structure of these proteins and it is against this background that the current study of the heat stability of tropical fish muscle proteins was carried out.

The fish species studied in the work were cod (*Gadus morhua*; a temperate marine fish), siganus (*Siganus oramin*; a tropical marine fish) and tilapia (*Sarotherodon aureus/niloticus* Cross; a tropical freshwater fish). For cod and tilapia the bulk of the work carried out was with fresh (non-frozen) samples. However, additional experiments were carried out to determine the effect of extended chill storage and freezing and frozen storage. Only frozen samples of siganus were available.

Materials and methods

Fish samples

Fresh (non-frozen) cod fillets were purchased from local retail suppliers and held chilled until required. For the samples of frozen cod, fresh fillets were wrapped in polythene and frozen in still air at −20°C and kept at this temperature until required. Samples of siganus were caught in reef traps in the Seychelles, killed by a blow on the

head and sent to the U.K. packed in ice. On arrival at the Tropical Development and Research Institute in London the fish were filleted, freed from skin and as far as possible from connective tissue, vacuum packed in polythene bags and frozen in liquid nitrogen before storage at −30°C until required. Whole tilapia reared in the laboratory were sacrificed by a blow on the head, and packed in ice. Most of the experimental work was carried out on this freshly iced material but in addition some samples were analysed after up to 9 days storage on ice or after freezing and storage in still air at −20°C. Intra-muscular collagen was prepared from fresh cod and tilapia tissue by removing all of the water and salt soluble proteins as outlined by Herring *et al.* (1969) and teasing out the collagenous fibres from the residue. It should be noted that different fish were used in the solubility and calorimetric studies.

pH

The pH of all muscle samples was determined by homogenizing triplicate 1 g samples of tissue in 100 ml of water and recording the pH of the suspension with a combined glass electrode.

Total nitrogen and non-protein nitrogen content

These were determined on duplicate 2 g samples of fish muscle using the micro-Kjeldahl method (AOAC, 1980). For non-protein nitrogen determinations, protein nitrogen was precipitated using 10% trichloroacetic acid and the nitrogen content of the solution determined.

Separation of protein fractions by salt extraction

A method based on that by Howgate & Ahmed (1972) was employed involving a two stage extraction using 1.0 M potassium chloride and 0.05 ionic strength phosphate buffer. Corrections were made for non-protein nitrogen and each determination was made in duplicate. The exact nature of all the different proteins extracted by these salt solutions can be open to question but the 1.0 M potassium chloride extract will contain the bulk of the myofibrillar and sarcoplasmic proteins, and the 0.05 ionic strength phosphate buffer will contain the bulk of the sarcoplasmic proteins alone. There is the possibility that on heating the relative distribution of the proteins within the solutions may change slightly, for example due to the solubilization of collagen or changes in the tropomyosin faction. It is not considered however, that this will have a major effect on the overall pattern of results. For the purposes of this paper the protein extracted by 1.0 M potassium chloride will be called the total extractable protein and be assumed to consist mainly of myofibrillar and sarcoplasmic proteins. The proteins soluble in 0.05 ionic strength phosphate buffer will be assumed to consist mainly of sarcoplasmic proteins and the difference between the two values to be mainly due to soluble myofibrillar proteins. This is in line with the common use of these terms in the literature.

Preparation and heat treatment for the solubility studies

Using a scalpel and forceps the muscle tissue from several fish fillets was scraped free of skin and most of the associated connective tissue. The tissue was then chopped into small pieces and mixed to give a relatively homogenous starting material (Love, 1970). Samples of this fish muscle (5 g) were placed in glass Petri dishes, vacuum sealed in heat resistant plastic bags, placed in an oven preheated to the required temperature and left for 1 hr. The samples were then removed and placed in a refrigerator until required.

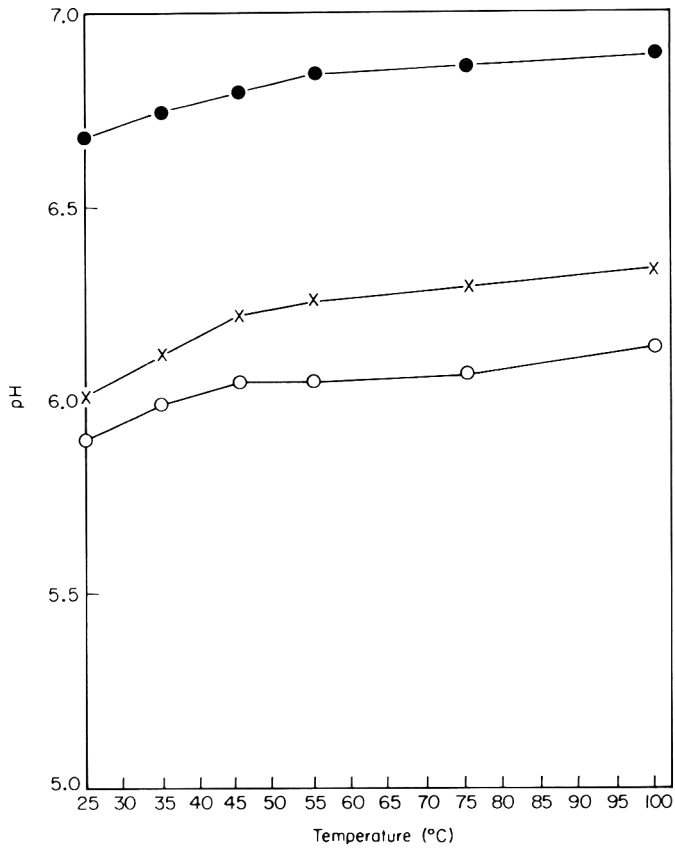

Figure 1. Effect of heating for 1 hr at selected temperatures on the pH of cod (●). siganus (○) and tilapie (×) muscle.

Scanning calorimetry

Calorimetric measurements were made using a Perkin-Elmer DSC-II differential scanning calorimeter at a scan speed of 5°C/min. Samples of 10–20 mg were weighed and sealed into aluminium sample pans for the analysis. The collagen samples were soaked in excess distilled water prior to and during analysis. Peak areas were measured by planimetry.

Results

Figure 1 shows the effect of heating at various temperatures for 1 hr on the pH of freshly chilled samples of cod and tilapia, and of frozen siganus. Although the initial pH values varied quite considerably there was in all cases a gradual increase in pH with increased temperature of cooking, irrespective of species. Figures 2, 4 and 3 respectively give the effect of heating for 1 hr at temperatures between 25 and 100°C on the total protein nitrogen soluble in 1.0 M potassium chloride, the protein nitrogen soluble in 0.05 ionic strength buffer and the difference between the two, each expressed as a percentage of the total protein nitrogen. Again, freshly chilled samples of cod and tilapia and frozen samples of siganus were used. The results show that the total extractable protein remained at its initial level until temperatures of 35–40°C were reached for the tilapia and siganus muscles whereas the cod muscle protein began to lose its extractability in

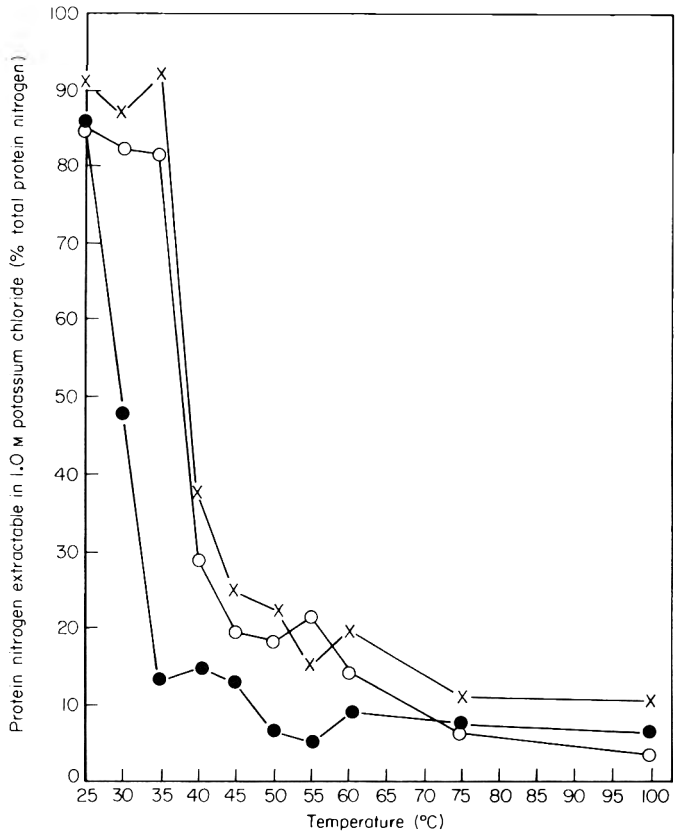

Figure 2. Effect of heating for 1 hr at selected temperatures on the protein nitrogen extractable in 1.0 M potassium chloride in cod (●), siganus (○) and tilapia (×) muscle.

salt solutions at temperatures between 25 and 30°C, i.e., 10°C lower. By reference to Figs 3 and 4 it is apparent that the initial large change in protein extractability is due to changes in the myofibrillar fraction. Changes in the protein fraction soluble in 0.05 ionic phosphate (mainly sarcoplasmic protein) are more gradual starting at 30°C for cod and 35–40°C for tilapia and siganus and extending to a temperature of 50°C for cod and 75°C for tilapia and siganus (Fig. 4). These results were obtained using pooled muscle tissue from one batch of fish of each species and it is known (Connell, 1964) that protein solubility can vary in fish from batch to batch. Nevertheless, an additional study with a different batch of fish gave results very similar to those shown in Figs 2, 3 and 4 and to avoid duplication the results have not been included.

Typical thermograms for intact, freshly chilled cod and tilapia muscle are shown in Fig. 5 where it is seen that distinct differences do exist. The first (low temperature) transition observed for cod, which by analogy with meat should relate primarily to myosin denaturation, appears as a doublet and occurs at a temperature over 10°C less than that seen from the tilapia muscle, which itself is singlet in nature. The second peak, which is quite pronounced in cod muscle and has a T_{max} of about 53°C, was not visible in the thermograms of several tilapia muscle samples although in some of these thermograms there was some indication of a small peak at a T_{max} of about 60°C. There was no significant difference between the positions of the third peak, which by analogy with meat is presumably due mainly to actin denaturation. These transitions were centred at about 73°C in both cases.

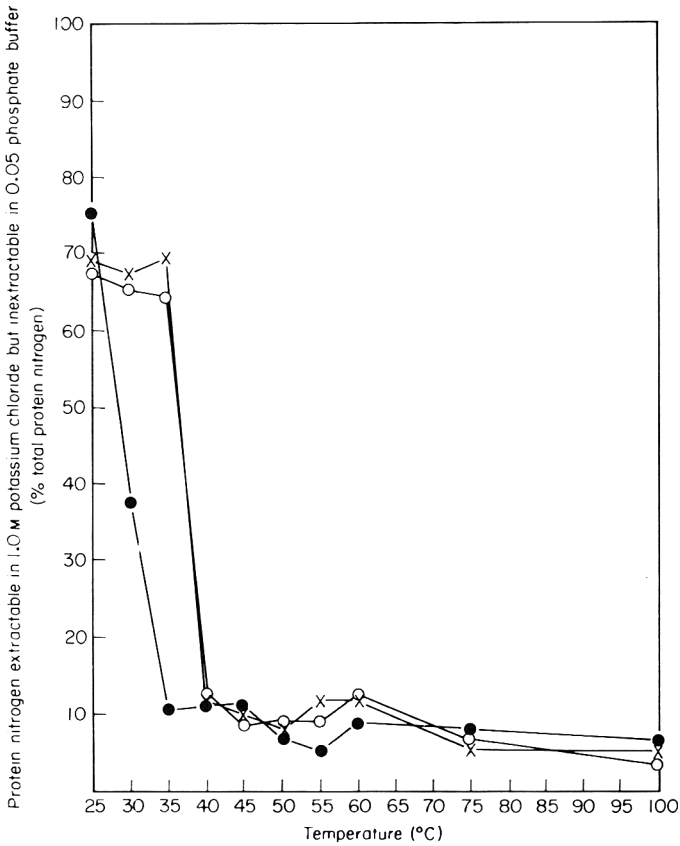

Figure 3. Effect of heating for 1 hr at selected temperatures on the protein nitrogen extractable in 1.0 M potassium chloride but inextractable in 0.05% phosphate buffer in cod (●), siganus (○) and tilapia (×) muscle.

In order to determine whether chill storage affected the results, samples of intact cod and tilapia muscle were subject to DSC analysis after periods between 19 and 216 hr on ice. It was found that in every case the profile of the thermograms obtained for both fish species was similar to that shown in Fig. 5. Table 1 gives the mean T_{max} values and the standard deviations for the three thermogram transition peaks for these samples. The samples from each batch are assessed together regardless of storage time and it is apparent that the standard deviations are quite low. Similarly, the between batch variation is small.

Freezing though, did appear to cause some changes in the thermograms. Thus, for both the cod and tilapia T_{max} for the first peak (or doublet peak in the case of cod) decreased and the T_{max} values for the higher temperature transition in the cod thermograms also decreased to some extent (Table 1). However in both cod and tilapia the position of the peak at about 73°C was not affected by freezing. It must be remembered though that T_{max} does not relate to any unique physical characteristic of the system but rather will depend on several parameters such as heating rate and sample size and shape as well as the denaturation temperature, and thus the small differences recorded in Table 1 must be viewed with some caution. More convincing evidence that changes in the nature of the proteins of cod muscle and tilapia muscle have occurred on freezing and subsequent storage is afforded by the fact that the relative size of the low temperature (myosin) peak, or doublet, decreased after freez-

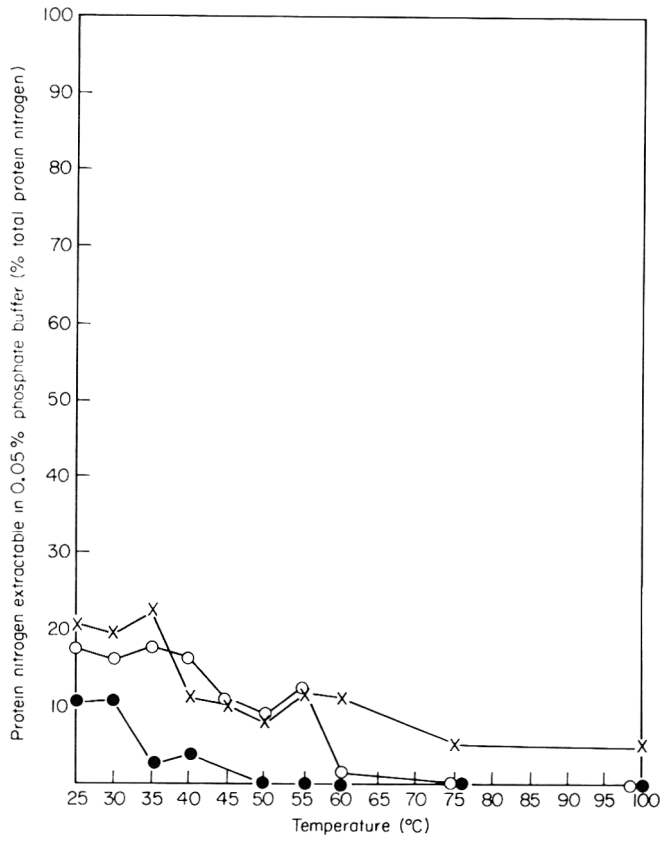

Figure 4. Effect of heating for 1 hr on the amount of protein nitrogen extractable in 0.05% phosphate buffer in cod (●), siganus (○) and tilapia (×) muscle.

ing. On a unit weight basis the relative area of these transitions decreased on freezing by about 30% in the case of both tilapia and cod muscle samples (Table 2). Thus the enthalpies associated with these transitions must be significantly less following freezing and subsequent frozen storage. There was little evidence for any freezing induced changes in the peak with a T_{max} of about 73°C, i.e. the actin peak.

Only frozen samples of siganus muscle were available. However, the thermograms had the characteristic three peaks as found in chilled and frozen samples of cod and tilapia. The first peak was singlet in nature with a T_{max} of 46.4±0.5°C and the second peak was clearly discernible with a T_{max} of 57.1±0.5°C (Table 1).

Table 3 summarizes the peak and onset temperatures for the isolated collagens of freshly chilled tilapia and cod muscles. These samples exhibited sharp transitions (Fig. 6) such that the onset temperatures could be determined relatively precisely and as previous workers (Finch & Ledward, 1972, 1973; Menashi *et al.*, 1976) have used this value to characterize the denaturation of this protein, it was recorded. These earlier studies gave an onset temperture of 31°C for insoluble cod skin collagen. In this study, however, the two samples of cod had mean values of 34.0 and 40.3°C respectively which presumably reflects differences in the water temperatures in which they wer caught. The two tilapia samples, reared in the laboratory under similar conditions gave almost identical T_0 values.

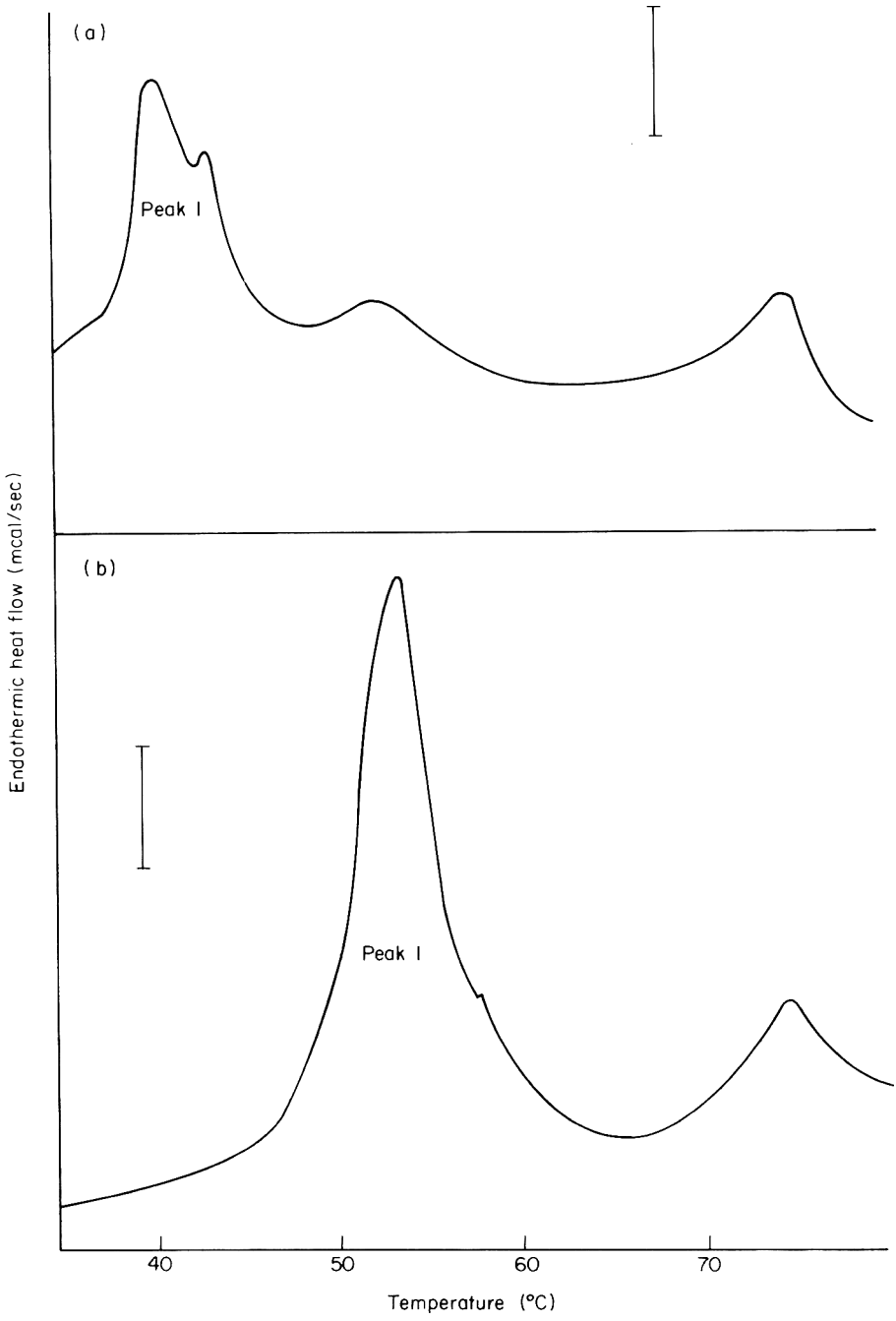

Figure 5. Thermograms obtained on heating 11.27 mg of unfrozen cod muscle (a) and 10.07 mg of unfrozen tilapia muscle (b). The vertical bar represents an endothermic heat flow of 0.02 mcal/sec.

Discussion

The initial pH of the samples used in this study varied quite considerably and when heated the pH of all samples increased by about the same amount for equivalent heat

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Table 1. Peak transition temperatures (T_{max}) for the proteins present in different fish muscles.

Sample	T_{max} (°C)		
	Peak 1*	Peak 2	Peak 3
Cod A (fresh) pH 6.4 (3)	41.7±2; 44.6±0.3	53.2±0.2	73.8±0.2
Cod B (fresh) pH 6.8 (16)	42.9±0.9; 45.8±0.7	54.4±0.8	73.8±0.5
Tilapia A (fresh) pH 6.2 (10)	52.0±0.8	small shoulder sometimes seen at 60°C but very small	73.2±0.4
Tilapia B (fresh) pH 6.4 (8)	52.7±0.3		74.4±0.4
Cod A (frozen) pH 6.4 (3)	40.0±0.1; 42.2±0.3	51.3±0.3	73.3±0.3
Cod B (frozen) pH 6.8 (9)	41.1±0.9; 43.8±0.5	52.8±0.5	73.8±0.3
Tilapia A (frozen) pH 6.2 (6)	50.6±0.5	As fresh	75.2±1.1
Tilapia B (frozen) pH 6.4 (16)	51.0±0.6	As fresh	73.6±0.3
Siganus (frozen) pH 6.3 (4)	46.4±0.5	57.1±0.5	72.6±0.5

All values are the means ± standard deviation of the number of determinations shown in brackets.

*Peak 1 is the singlet or doublet peak assumed to correspond to myosin denaturation.

Peak 2 is the small peak usually seen at a temperature intermediate between the myosin and actin denaturation peaks.

Peak 3 is the most stable transition observed in the thermograms and is assumed to correspond to actin denaturation.

treatment. It should be noted however, that these results were obtained on pooled muscle tissue from one batch of fish of each species. It is well known that the pH of fish flesh can vary according to fishing ground, season etc. (Love, 1970). The increase in pH occurred in a regular manner throughout the temperature range 25–100°C and there appears to be no particular zone where the changes are more pronounced as might be expected if such changes were associated with the denaturation of the different protein fractions. This is perhaps unexpected since it may be reasonable to assume that the changes in pH are due to an overall decrease in available acidic groups in the muscle and that such groups would also play a part in the inter- and intra-muscular links which are involved in the insolubilization of the protein in the heated muscle. Thermal denaturation of myofibrillar proteins of pork and beef (Bendall, 1962) has been claimed to be accompanied by a shift in isoelectric point to a more alkaline region. However Connell

Table 2. Relative peak areas/mg of sample of the low temperature transitions (Peak 1 of Fig. 1) seen in the thermograms of fresh and frozen cod and tilapia samples

Sample	Relative peak area	Level of significance of the decrease seen on freezing
Fresh cod, A pH 6.4 (3)	1.17±0.07	
Frozen cod, A pH 6.4 (3)	0.75±0.22	$P < 0.05$
Fresh cod, B pH 6.8 (10)	1.01±0.26	
Frozen cod, B pH 6.8 (8)	0.69±0.18	$P < 0.01$
Fresh tilapia A pH 6.2 (8)	2.32±0.64	
Frozen tilapia A pH 6.2 (3)	1.67±0.26	not significant $P > 0.05$
Fresh tilapia B pH 6.4 (3)	3.42±1.05	
Frozen tilapia B pH 6.4 (12)	2.12±0.4	$P < 0.01$

All values are the mean ± standard deviation of the number of samples shown.

& Howgate (1964) undertook a study from which they concluded that there was no evidence with either cod or beef that the numbers of ionizing groups change significantly on heat coagulation.

The extraction of protein from the fish and meat products by salt solutions has been employed widely as a means of measuring changes, often termed denaturation, in their

Table 3. Onset, T_0 and peak, T_{max} temperatures for the denaturation of tilapia and cod intramuscular connective tissue collagen

Sample	T_0 °C	T_{max} °C
Cod A (4)	34.0±0.5	40.1±0.1
Cod B (4)	40.3±1.0	44.4±0.9
Tilapia A (4)	57.0±0.9	62.0±0.8
Tilapia B (4)	57.2±0.3	61.3±0.5

All values are the mean ± standard deviation of the number of determinations shown.

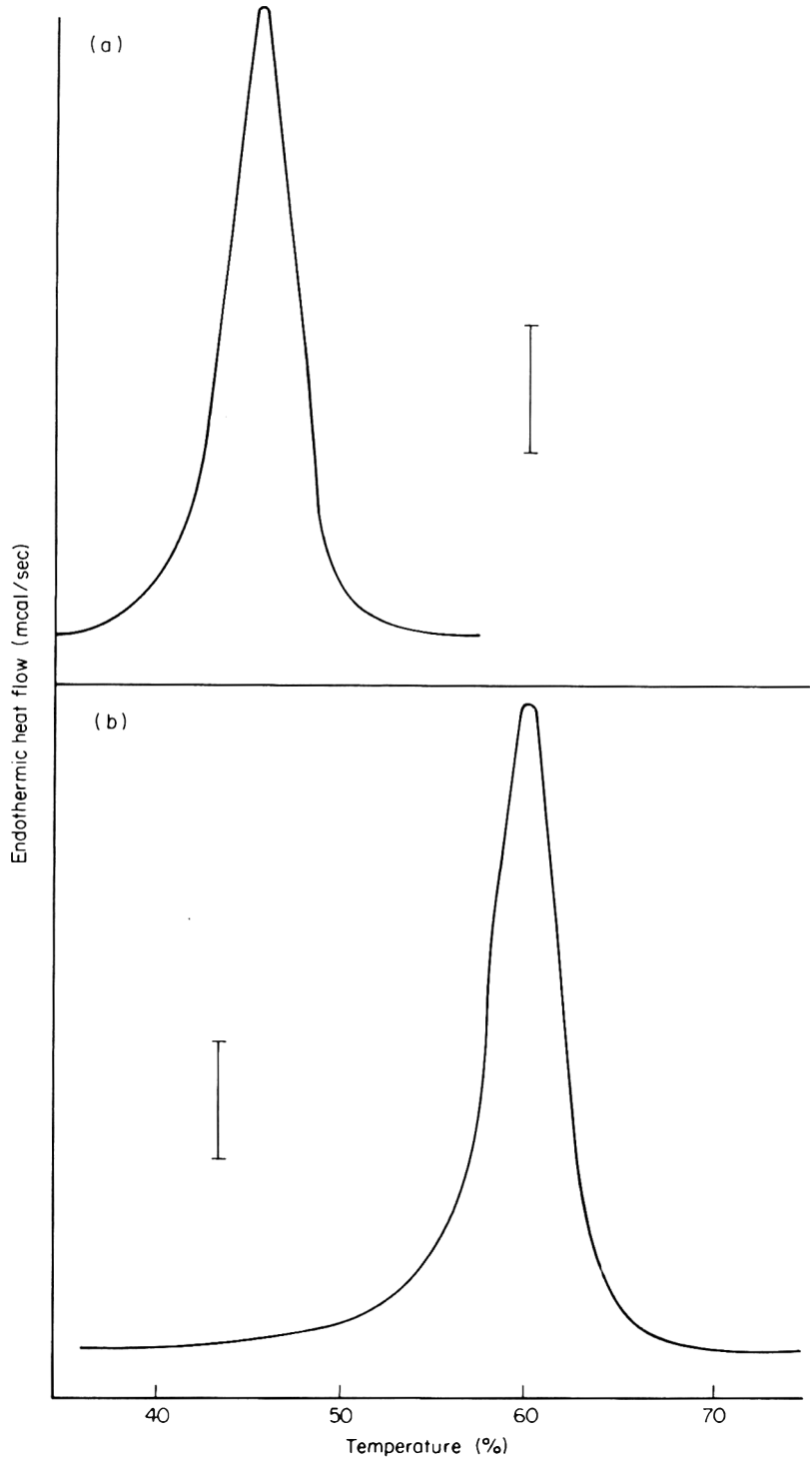

Figure 6. Thermograms obtained on heating 11.00 mg of intramuscular cod collagen (a) and 12.52 mg of intramuscular tilapia collagen. The vertical bar represents an endothermic heat flow of 0.05 mcal/sec and in both cases the samples were heated in the presence of excess distilled water.

proteins. Frozen fish is perhaps the best example of this where it has been established that there is a direct relationship between taste panel texture scores and protein nitrogen extractability.

Protein extractability, however, only gives an indirect measure of protein changes and the results must be interpreted with caution. For example Connell (1964) pointed out that two main factors govern the removal of protein from a muscle into a solvent; the intrinsic solubility of the protein in the solvent and the ability of the solubilized protein to diffuse out of the muscle into the extract. Thus, the sharp change which occurred at 25–30°C for cod and 35–40°C for tilapia and siganus in Fig. 3 may not be due to all the myofibrillar proteins becoming insoluble. The myofibrillar fraction of muscle proteins consists mainly of myosin, actin and tropomyosin. Of these three, myosin is by far the least stable and Howgate & Ahmed (1972) postulated that changes in the salt solubility of myofibrillar proteins of cod heated at 30°C were in fact only due to changes in the myosin component; the actin remaining unchanged and soluble but unable to be extracted without the mysoin.

It is reasonable to make the same assumption here since some 85–90% of the protein becomes inextractable at temperatures below 45°C where it may be expected that actin would not be affected. There would appear, therefore, to be approximately 10°C difference between the temperature at which cod muscle myosin becomes inextractable and the temperature at which tilapia or siganus myosin becomes inextractable. It should be noted that the cod and tilapia samples were chilled whereas the siganus had been rapidly frozen and stored for a few weeks at −30°C. Freezing and frozen storage under these conditions, however would not be expected to have a major effect on protein extractability. Since cod is found in waters at about 6°C whereas tilapia and siganus would be in water of between 25 and 30°C, the results presented here would seem to support Connell's (1961) suggested relationship between the stability of myosin and the normal body temperature of the animal.

The same 10°C temperature difference would appear to apply to the stability of the proteins soluble in 0.05% phosphate buffer, mainly sarcoplasmic proteins, although in this case there was no sharp decline in the extractability but rather a gradual change with temperature. This fraction of the muscle proteins is made up of a large group of different proteins, mainly enzymes, and it is not unexpected that they would have a range of heat stabilities.

Although no attempt was made to study the heat stability of the intra-muscular collagens using solubility techniques, limited studies were made using DSC. In line with the previous findings of Menashi *et al.* (1976) an increase in denaturation temperature with *in vivo* body temperature was found. It is well established that the thermal stability of collagen is directly related to the total imino acid content (proline and hydroxyproline) the stability increasing with increasing concentrations of these residues. This suggests that the number of imino acid residues per collagen molecule in cod is less than that in tilapia. In fact the denaturation temperature of the tilapia collagen (T_0 57°C) is very similar to the values found for the intramuscular connective tissue collagen of both pork (T_0 59°C) and rabbit (T_0 58°C) (Chizzolini, Ledward & Lawrie, 1975) suggesting a similar imino acid content in all three species, i.e. about 200 residues per 1000 compared to the value of 155 residues per 1000 found in cod (Menashi *et al.*, 1976). These studies on the fish collagens show that at least in the case of cod, the second peak in the whole muscle thermogram (T_{max} 53–54°C) cannot correspond to collagen denaturation which has T_{max} of about 40 to 44°C (Table 3). Thus, the peak at a T_{max}

of about 53°C presumably represents the denaturation of some of the sarcoplasmic proteins or a further myosin transition and further studies are necessary to determine unequivocally the nature of the peak. By analogy, the peak at about 67°C seen in the thermographs of beef and rabbit muscle does not correspond to the denaturation of collagen as has been suggested by Stabusvik & Martens (1980). Further evidence that collagen denaturation is not observed in the thermograms of intact muscle is afforded by the results obtained with the tilapia muscle where no significant peak was observed at the T_{max} of this collagen (62°C) when 10–20 mg of muscle were studied (Fig. 5).

It is not very surprising that no peak due to collagen denaturation is observed in intact muscle as collagen only constitutes about 5% of the total protein present and thus in a typical DSC analysis, when about 10 mg of muscle is studied, the collagen concentration is about 0.1 mg, which on denaturation absorbs so little energy that it would not be easily detected in the complex thermograms generated. Thus all the peaks seen on a typical muscle thermogram are due primarily to transitions involving myosin, actin and possibly sarcoplasmic proteins. The first peak (or doublet in the case of cod) is undoubtedly due primarily to myosin denaturation although it seems probable that some small part of the transition may also be related to the denaturation of some of the less stable sarcoplasmic proteins. It is readily apparent from Table 1 that the stability of the myosins from different fish species do vary quite markedly; e.g., cod myosin being denatured at a temperature about 10°C lower than that of tilapia. This is in agreement with the findings of the solubility studies. The temperature at which these transitions start to occur, however, are slightly higher than those found in the solubility experiments. This is not unexpected as time and temperature would be important in relation to the observed effect and the two studies were carried out under very different heating regimes and on fish of different initial pH.

It is interesting to note that the myosin of tilapia muscle is significantly less stable ($\sim$ 10°C) than that from beef or rabbit (Table 1, Wright, 1978), although its collagen is of similar stability (Table 3, Chizzolini *et al.*, 1975). Just as the differences in collagen stability can be related to their amino acid composition andsequence (Menashi *et al.*, 1976) so the differences observed in the myosins are presumably related to the composition and sequence of amino acid residues within the helical chains enabling a greater or lesser number of stabilizing interactions to take place. However, non-protein components of the muscles may also be important in leading to different levels of stability. For example, it has been found that some of the lipids present in fish muscle bind to, and consequently stabilize, actomyosin (Hamada *et al.*, 1982). Analysis of the fish muscle used in this work showed that the lipid (plus phospholipid) content of the tilapia muscle was 1.03% compared to 0.75% for the cod suggesting that additional lipid protein interactions may help stabilize the tilapia myosin. However, it does not seem likely that this small difference in lipid content is a significant factor in modifying the stability of the myosin studied. The DSC results obtained with siganus showed that the T_{max} for the first peak (myosin denaturation) occurred a little above the temperature of the transition in cod but some way below that for tilapia. This result is at variance with the solubility studies where it was found that the myosin of both muscles became inextractable within the same temperature range. However, the siganus muscle had been frozen and the possible effects of this on the DSC results are discussed later. The solubility studies showed that it was not only the myosin fraction of the muscles that increased in stability with *in vivo* body temperature but also the sarcoplasmic proteins. Such differences in stability were not unexpected as it is known, for example, that the myoglobins

from different species differ in stability (Ledward, 1983). For the reasons outlined above the second peak in the DSC thermograph is probably due, at least in part, to the denaturation of some of the sarcoplasmic proteins.

Although the stabilities of the myosins and sarcoplasmic proteins in the different species varied, it is seen from Table 1 that the protein(s) giving rise to the high temperature transition at about 73°C in the thermograms do not differ significantly in stability. By analogy with meat this peak is due to actin denaturation and suggests that in all the muscles studied the actin components are of similar stability. It is interesting to note, however, that rabbit and beef actins are known to denature at about 80°C (Ledward & Lawrie, 1975; Wright *et al.*, 1977) and this work therefore clearly demonstrates that actins from meat animals differ in stability, and presumably composition, to those derived from fish.

Although the effect of freezing was initially studied as a check on the different methods of sample preparation used in the investigation, other interesting results were obtained. For cod and tilapia for which both fresh and frozen samples were available, it was found that freezing appeared to reduce T_{max} values slightly. Insufficient data was available to indicate whether this effect was real or whether it was due to other changes such as the heating rate within the samples. It is not possible at this time therefore, to say whether the differences in T_{max} found for myosin from fresh tilapia and frozen siganus was due to differences in intrinsic stability or an effect of freezing. Changes in the area of the myosin denaturation peak per unit weight of sample between fresh and frozen cod were statistically significant as were the differences observed for one of the tilapia samples Table 3). In the other tilapia samples although there were apparent differences, these were not statistically significant due to large variations in peak area and hence large standard deviations. Nonetheless, the results of this study suggest that quantification of the magnitude of the decrease in peak area may well be a simple means of estimating the amount of change undergone by the proteins of fish muscle during freezing and frozen storage. Although cod and tilapia myosins have been shown to have different thermal stabilities, in this study the results seem to indicate that freezing and frozen storage under similar conditions results in a similar amount of denaturation, since for both species the peak size was reduced by 30–40%. However, the results of this study are limited and more work is required to confirm them and particularly to study the decrease in area with time in the frozen state.

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Rheological property of myosin B fibre and the fibre of the mixture of myosin B and soya protein; and the interaction between myosin B and soya protein

LIANG-CHUAN LIN AND TATSUMI ITO

Summary

Protein fibre was prepared in native state from concentrated myosin B solution, soya protein solution and the mixture of them, and the extensibility of the resulting fibre was examined under various conditions with a tension meter devised for the analysis of strain of fibre. Gel electrophoretic analysis in the presence of SDS and scanning electron microscopic observations of protein fibre were also done in order to elucidate the nature of the interaction between myosin B and soya protein. A fragile fibre was obtained simply by extruding the concentrated protein solution into buffer with weak acidity, but it was changed to one having strong extensibility after thermal treatment, i.e., extensibility of the fibre increased with increasing temperature. The pH value of bathing solution was critical to produce protein fibre having good rheological property: pH 5.5 was the best for the development of protein-protein interaction. Extensibility of protein fibre made from the mixture of myosin B and soya protein decreased with increasing the mixing amount of soya protein. However, the change of rheological property of myosin B during storage was protected by soya protein. It was found that soya protein binds to the actin moiety in the myosin B complex simply by mixing them even without heating.

Introduction

Myosin and actin are the major structural proteins which make up 10% of the muscle mass. They form the complex named myosin B during the extraction of myofibrillar protein with high ionic strength solution. The fibre of myosin B prepared from saline extract of skeletal muscle consists of a random network of filaments (Beck *et al.*, 1969). In comminuted meat products, myosin B is regarded as one of the most important factors in determining the quality of meat products, such as binding quality and water holding capacity (Fukazawa, Hashimoto & Yasui, 1961; Siegel & Schmidt, 1979a,b). It has been reported that the quality of meat products is influenced by the extent of denaturation of myosin B, i.e., the physical properties of meat products are dependent upon denaturation pattern of myosin B (Hashimoto *et al.*, 1959). Nakayama & Sato (1971) demonstrated that the binding quality of reconstituted actomyosin as well as myosin B (natural actomyosin) is superior to that of myosin alone. Haga & Ohashi (1982) have reported that heat induced gelation of washed actomyosin, in which Ca^{2+} sensitivity is lost during washing, is superior to that of myosin B in the range of 50–100°C and the difference in rheological properties between the gels of non-treated and washed myosin B is due to the difference in microstructure of the gels.

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Recently, soya protein has been used as an additive in meat products not only to improve their nutritional values and lower the cost in manufacturing meat products (Skurry & Osborne, 1976; Nada, Sofos & Allen, 1977) but also to stabilize the quality of meat products by introducing desirable functional properties of soya proteins, such as moderate emulsifying, stabilizing and gelling abilities, into meat products (Wolf, 1970). Publications regarding the fabrication of edible soya products, including soya flours, soya concentrates and soya isolates, into meat products have been accumulated in a recent few years. It has been found that the soya proteins are a useful aid in the manufacture of many kinds of meat products, such as sausages and beef patties, in which soya proteins are used to bind meat particles together (Seideman, Smith & Carpenter, 1977). However, Siegel *et al.* (1979c) have shown a negative effect of soya protein (soya isolate) on the texture, flavour and colour of meat products, although the soya proteins used in emulsified meat products and in restructured meat products are able to improve the yield of cooked product, water binding ability and fat absorption of the products.

The storage proteins 7s (conglycinin) and 11s (glycinin) components are the major constituents of soya protein. The 7s and 11s components make up 70% of the total soya protein (soya globulin) (Wolf, Babcock & Smith, 1961). They are clearly different in their functional properties: heat induced gel of 11s component having a higher water holding capacity, tensile values, hardness and extensibility than 7s gel (Saio & Watanabe, 1978). According to Yamamoto, Fukazawa & Yasui (1973), the mixture of myosin and soya protein aggregated to a lesser extent than the individual proteins did upon heating. However, myosin and the 7s component were found to be associated after exposure to temperatures in the range of 75–100°C, although no evidence for the formation of aggregate was obtained when the mixture was incubated at lower temperatures (2 and 25°C) (King, 1977). Peng *et al.* (1982a) have reported that the soya 11s component also interacts with myosin at temperatures between 85–100°C. In addition, they have found that the basic subunit of the 11s component interacts with the myosin heavy chain, while the acidic subunit shows no interaction. Because of the difference in functional properties between muscle proteins and soya proteins, between myosin and myosin B, and between the soya protein 7s and 11s components, the nature of the interaction between these proteins has not been elucidated precisely so far. Therefore, it is important to know the functional properties of muscle proteins, soya proteins and the mixture of these proteins in order to elucidate the mechanism of the interaction between muscle proteins and soya proteins, and also to make the best use of soya proteins in the meat products.

This paper deals with the study of the protein-protein interaction in the fibres made from myosin B (natural actomyosin), soya proteins and the mixture of them. In general, soya protein fibre is usually prepared by extruding an alkaline solution of soya isolate through spinnerettes into an acid bath and then stretching the resulting fibres in order to give the toughness and chewiness to the products (Thulin & Kuramoto, 1967). In the present study we have prepared the fibre by extruding concentrated protein solutions through a syringe into buffers having varying pH values in order to avoid the possibility of the denaturation of protein, which usually occurs during spinning from alkaline environment into acidic media. The examination of the extensibility of the resulting fibre certainly provides real rheological features of protein-protein interaction during processing of meat products containing soya proteins.

Materials and methods

Materials and reagents

Japanese White rabbits (male, 2.5–3 kg) were purchased from Kyudo Company (Saga, Japan), an animal breeding company. Defatted soya flakes were kindly donated by Fuji-Oil Company Limited (Osaka). Sephadex g-100 and DEAE Sephadex A-50 were the products of Pharmacia. Aquacide II-A (content of CMC is 92%) was purchased from Calbiochem-Behring Corp. (California, U.S.A.). Other reagents were of analytical grade.

Preparation of myosin B

Myosin B was extracted from rabbit longissimus muscle with 5 vol. of Weber-Edsall solution for 24 hr at 0°C and purified by repeating the usual precipitation-dissolution cycle three times according to the procedure of Watanabe (1975).

Preparation of soya 7s and 11s component

Soya 7s component was substantially prepared according to the procedures of Roberts & Briggs (1965) and King (1977). Soya storage proteins were extracted from defatted soya flakes with 5 vol. of distilled water with stirring for 2.5 hr at room temperature. The extract was filtered through two layers of cheese cloth and allowed to stand overnight at 0°C. The precipitated fraction (CIF: cold insoluble fraction) was removed by centrifugation. The supernatant liquid was fractionated by adding ammonium sulphate (50–90% saturation) and the resulting precipitate was dissolved in standard buffer (0.035 M K_2HPO_4, 0.026 M KH_2PO_4, 0.4 M NaCl, 0.01 M β-mercaptoethanol, pH 7.6). Then, the pH of the solution was adjusted to 4.5 with 2 M acetic acid, followed by dialysis against distilled water at 0°C for 48 hr to precipitate crude 7s component. After collecting the precipitate by centrifugation, the resulting crude 7s component was purified by gel filtration on Sephadex G-100 column (5.0×80.0 cm) with the standard buffer (Koshiyama, 1972). The eluate was monitored at 280 nm and the first peak containing 7s component was concentrated with Aquacide II-A.

Soya 11s component with 90–95% purity was obtained from CIF essentially by the method of Wolf, Babcock & Smith (1962). CIF dissolved in the standard buffer was fractionated with two steps of ammonium sulphate fractionation; 51–66% saturation at pH 7.6, followed by 26–40% saturation at pH 4.0. The resulting crude 11s component was purified by DEAE-Sephadex A-50 column chromatography using a linear NaCl concentration gradient system (0.1–1.0 M) (Catsimpoolas *et al.*, 1967). Eluate was monitored at 280 nm and the main peak containing 11s component was concentrated with Aquacide II-A.

SDS polyacrylamide gel electrophoresis

Soya 7s and 11s components, myosin B and mixture of myosin B and soya proteins were electrophoresed on SDS polyacrylamide gels according to the procedure of Weber & Osborn (1969). Electrophoresis of soya proteins dissolved in 0.1% SDS, 0.1% β-mercaptoethanol and 10 mM sodium phosphate (pH 7.0) was carried out on 5% gel (for 7s component) and 10% gel (for 11s component) containing 1% SDS and 0.1% β-mercaptoethanol. An aliquot (20 μg) of the sample was applied onto the gel and was electrophoresed at 8 mA/tube for 3 hr (for 7s component) and 5 hr (for 11s component).

When protein fibres were electrophoresed, protein fibres were at first collected by filtration through two layers of cheese cloth. In the case of the mixture of myosin B and

soya protein, the supernatant liquid was obtained by centrifugation at 0°C for 15 min at 3000 rpm following the filtration of protein fibre. Then, protein fibre was directly dissolved in 0.1% SDS, 0.1% β-mercaptoethanol and 10 mM sodium phosphate (pH 7.0), 25% glycerol and then heated in a boiled water bath for 3 min.

The suspension of soya protein and the supernatant liquid of the fibre made from the mixture of myosin B and soya protein were dissolved in the above solution by dialysing against 100 vol. of the above solution. Electrophoresis was done on 10% gel containing 1% SDS and 0.1% β-mercaptoethanol at constant current of 8 mA/tube for 5 hr. Gels were stained with an aqueous solution of 0.25% Commassie Brilliant Blue R-250, 45.5% methanol and 0.2% acetic acid for 2 hr at room temperature. Then, the gels were destained in an aqueous solution containing 7.5% acetic acid and 5% methanol. Densitometric scan of the gels were performed with a Hitachi 557 Double Wavelength-Double Beam Spectrophotometer using the absorbance range of 0–4. The wavelengths for scan and for reference were 700 and 550 nm, respectively.

Preparation of protein fibre

Using a syringe with a needle of 0.5 mm in diameter, concentrated protein solutions (20 mg/ml unless otherwise mentioned) were extruded into buffers having varying pH values (pH 1.08–4.0, 0.1 M sodium citrate, dibasic-HCl; pH 4.3–6.0, 0.1 M acetic acid-sodium acetate; pH 7.0–8.0, 0.1 M Tris-maleate; pH 9.0, 0.1 M Tris-HCl; pH 10.0–12.0, 0.1 M sodium carbonate-sodium bicarbonate) in a test tube. The needles

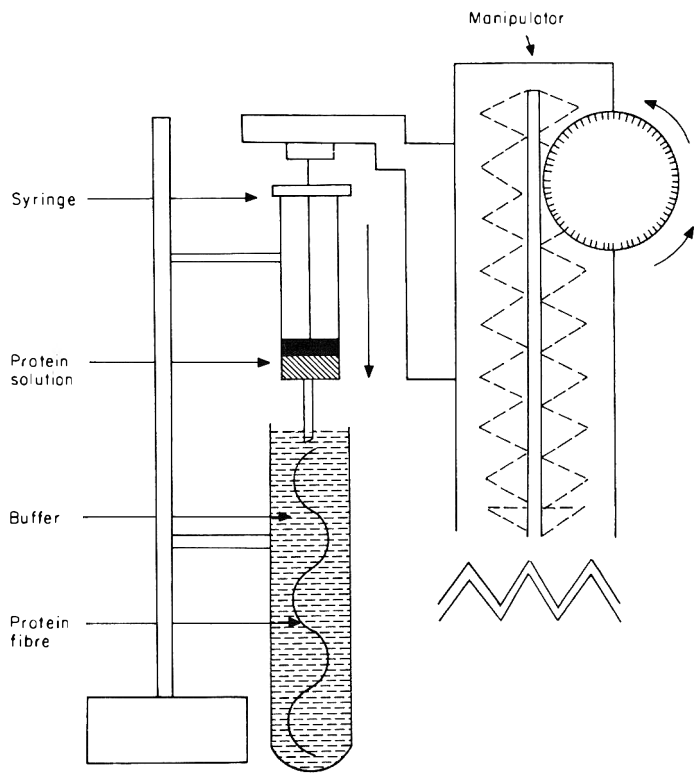

Figure 1. Diagram of the apparatus for the preparation of protein fibre. A protein sample in a syringe was pressed out into a test tube by the downward movement of the hand of manipulator. The speed for the extrusion of protein sample was about 8.3×10^{-1} μl/sec.

were dipped into the buffers and protein solutions were mechanically pressed out using a manipulator to produce protein fibres (Fig. 1). The temperature of bathing buffer was 20±2°C. The resulting myosin B fibre and the fibre of the mixture of myosin B and soya protein in each buffer were heated in a water bath at 70°C for 15 min (unless otherwise specified), and then immediately cooled to room temperature by diluting with 9 vol. of cold water. The heat treated protein fibres were very stable, so that it was easy to handle them, while non-treated fibres with heat were very fragile to handle.

Extensibility of protein fibre

The extensibility of the fibres, prepared under various conditions, was examined with a tension meter before and after heat treatment: the fibres (6.0 mm in length) were fixed to two thin vertical glass rods of the tension meter with a minute amount of Aron Alpha A (an adhesive agent for surgery) and then the fibres were extended mechanically at the speed of about 7.5 mm/sec until the fibres were broken (Fig. 2). The curve for breaking force *versus* time (or extended length of the fibres) was recorded on a graph paper. This experiment was repeated nine times for each kind of fibre preparation. The diameters of the fibres were measured with an ocular micrometer attached to a stereoscopic microscope at the magnification of 10×. The breaking force (BF) and apparent elasticity modulus (AEM) of the fibres were calculated from the recorded diagrams according to the following equation: (Muller, 1973):

$$\mathrm{AEM} = \frac{f \times l_0}{r^2 \times \triangle l},$$

$$\mathrm{BF} = \frac{f' \times l_0}{r^2 \times \triangle l'},$$

where f is the stress of the fibre at the applied force, f' is the stress of the fibre (breaking force) at the breaking point (BP), l_0 is the initial length of the fibre (mm), $\triangle l$ is the extent of strain of the fibre at the applied force, $\triangle l°$ is the extent of strain of the fibre at

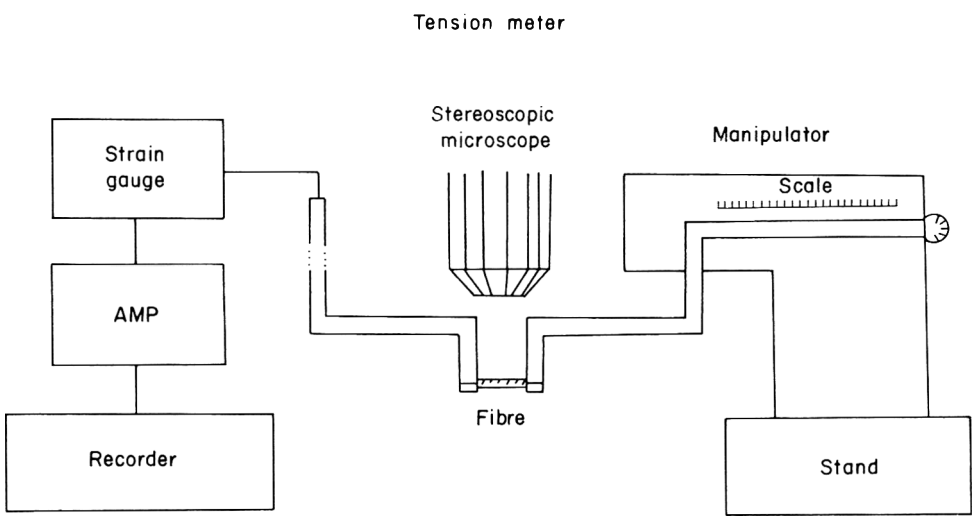

Figure 2. Schematic diagram of tension meter. The left rod is attached to a strain gauge (UL-53807, Shinkoh Tsushin Kogyo Company) which is connected to an amplifier-recorder system (DS-6003, Shinkoh Tsushin Kogyo Company and F-42 CP Riken Denshi Company) and the right one is attached to the arm of micrometer which is used to measure the extended length.

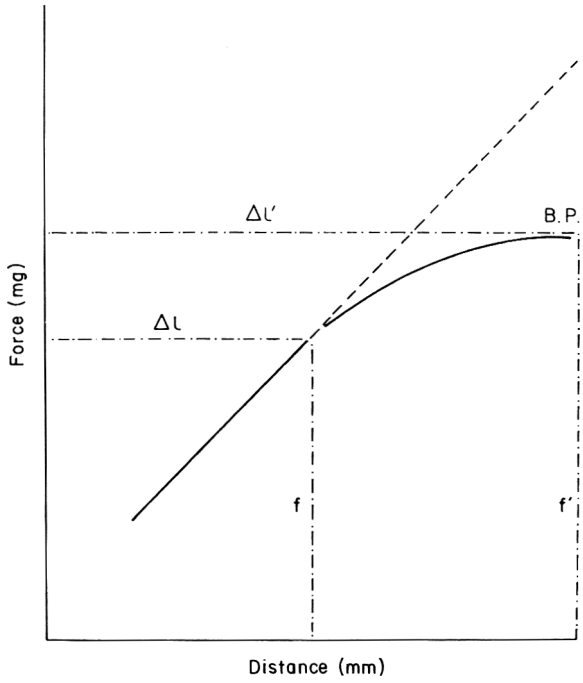

Figure 3. Diagram showing the relation between tension (force) and distance (the change of the length of fibre) during the measurement of the extensibility of the fibre. Solid line represents values of actual measurement, while dotted line represents the values which obey Hook's law. The overlapping of solid line and dotted line indicates that the relation between tension and distance is in the range which obeys Hook's law. The relation between tension and distance at breaking point (BP) does not obey Hook's law.

the breaking point, and r is the diameter of the fibre at the initial length. Both f and $\triangle l$ are within the agreeable limit of Hook's law, while f' and $\triangle l'$ are beyond the limit of Hook's law (Fig. 3).

In order to investigate the change of AEM and BF of protein fibre during storage, unheated fibres were stored in acetate buffer at 25°C for 3 days. Then, the fibres were heated at 70°C for 15 min, followed by cooling to room temperature as described above. Sodium azide (10 mM) was added to the buffer in order to inhibit the growth of bacteria during storage.

Protein concentration

Protein concentration was determined by the Biuret method of Gornall, Bardawill & David (1949), which had been standardized by bovine serum albumin.

Scanning electron microscopy

Scanning electron microscopic observations were made according to the procedure of Sjöstrand (1967). A drop of sample (protein fibre) on a aluminium film coated slide glass was freezed with isopentane containing dry ice. After freeze drying, the sample was coated with gold using a vacuum evaporator (Model HUS-4GB, Hitachi Limited). Samples were examined with Hitachi SSM-2A using an accelerating voltage of 10 kV.

Results

Electrophoresis of soya 7s and 11s component

SDS polyacrylamide gel electrophoretograms and its densitometric scan of 11s component are shown in Fig. 4a. As shown in this Figure, the 11s component is composed of three subunits with apparent molecular weights of 44200 (acidic subunit), 37200 (acidic subunit) and 22300 (basic subunit). No other band was detected in the preparation of 11s component in the present study. Also, the electrophoretogram and its densitometric scan of Fig. 4b show that 7s component consists of three subunits, α', α and $\beta+\gamma$ subunits, the molecular weights being 58000, 57000 and 46000 respectively. In addition to these subunits, a contaminant, supposedly derived from 11s component, appeared on the gel (Roberts & Briggs, 1965), so that the purity of 7s component in the present study, calculated from the intensity of densitometric scan, was about 90%. The subunit composition of 7s and 11s components used in the present study is consistent with those of Vu Huu Thanh & Shibasaki (1977) and Catsimpoolas *et al.* (1971), respectively.

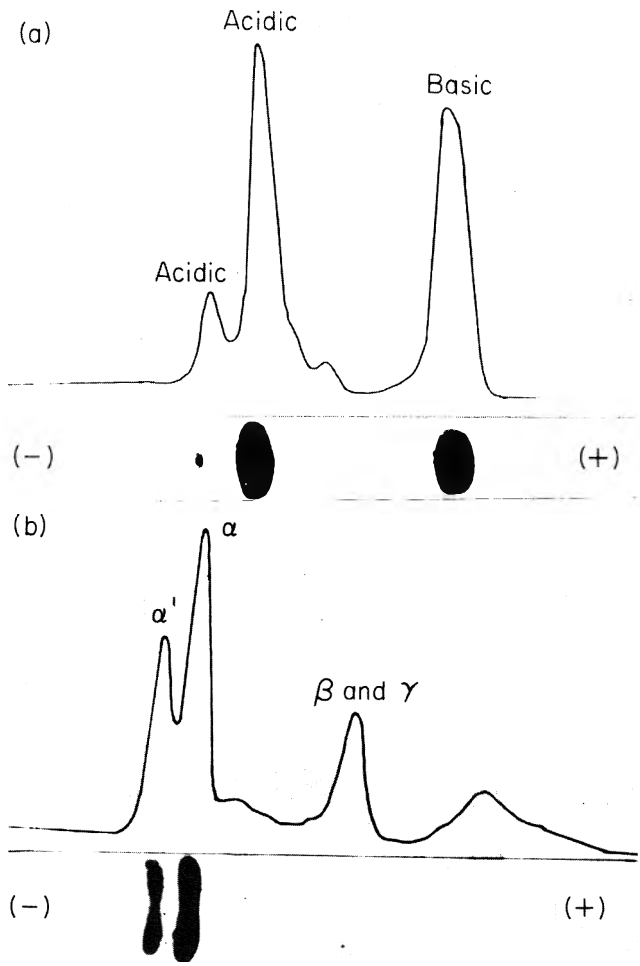

Figure 4. Sodium Dodecyl Sulphate (SDS) polyacrylamide gel electrophoretograms and their densitometric scans of soya protein 7s and 11s components. The sample was electrophoresed from left to right in the figures. (a) 11s component; (b) 7s component.

Fibre formation of myosin B, soya protein and mixture of them

Figure 5 shows the appearance of protein fibres made from concentrated myosin B, soya protein (a mixture of 7s and 11s components unless otherwise specified; the mixing ratio was 1:1) and the mixture of myosin B and soya protein in a buffer with low pH value (pH 5.5) at room temperature. The mixing ratios (weight ratio) of myosin B to soya protein were 4:1, 3:2, 1:1, 2:3, 1:4 respectively. A transparent and fragile fibre was formed in the case of myosin B simply by extruding concentrated solution into buffers, while in the case of soya protein no fibre was formed by this procedure. Instead,

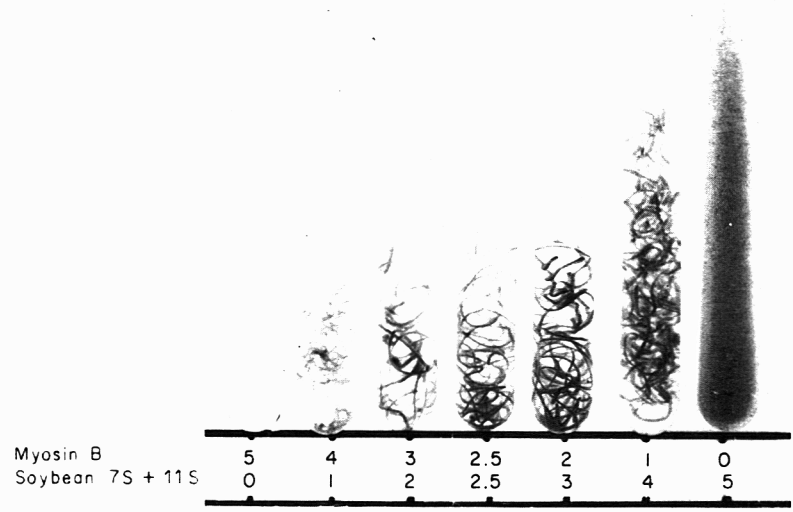

Figure 5. The appearance of protein fibres made from myosin B, soya protein (mixture of 7s and 11s component) and the mixture of myosin B and soya protein. The mixing ratio of myosin B to soya protein is indicated below the bottom of test tubes.

only a turbid protein suspension was observed. In the case of the mixture of myosin B and soya protein, fragile fibres were formed at any mixing ratio of myosin B to soya protein. However, the fragility of the fibre and the turbidity of bathing buffers increased with an increasing amount of soya protein. In addition, the appearance of the fibre was changed from transparent to opaque and the fibre also increased its diameter with increasing the mixing amount of soya protein.

Figure 6 shows electrophoretograms and their densitometric scans of fibres and of proteins suspended in the bathing buffers (see Fig. 4). It is clearly demonstrated that α-actinin disappeared from the electrophoretogram of fibre after the mixing of myosin B and soya protein and appeared in the supernatant liquid of the mixture. This indicates that α-actinin is released from myosin B complex when it binds to soya protein.

Rheological properties of protein fibres

As described in Materials and methods, protein fibres produced by the present procedure were very fragile, but they became strong after they were heated at 70°C for 15 min, so that apparent elasticity modulus (AEM) and breaking force (BF) of the heat treated fibres were examined in order to investigate the functional properties of

proteins. When myosin B fibre was heated to varying temperatures, there was almost no change in both AEM and BF in the range of 0–40°C. However, AEM and BF greatly increased with increasing temperature from 40 to 70°C and at higher temperatures almost no increase was observed in AEM; whereas BF still increased with increasing temperature, although the increasing rate of BF slowed down above 90°C. In the case of

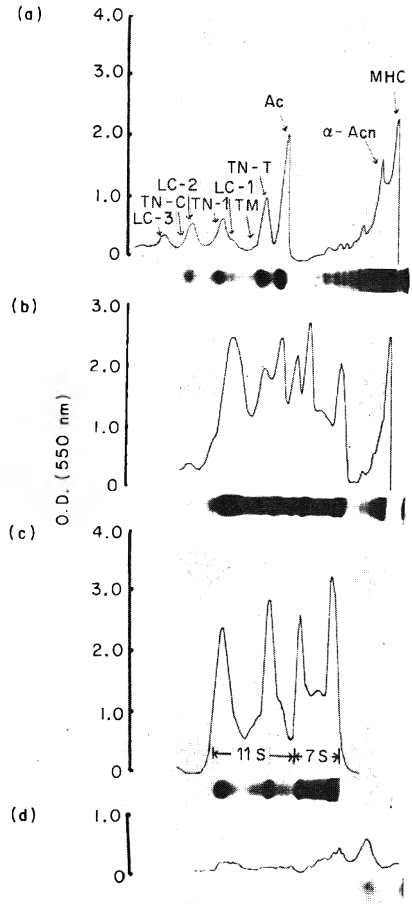

Figure 6. Sodium Dodecyl Sulphate (SDS) polyacrylamide gel electrophoretograms and their densitometric scans of myosin B fibre: the fibre made from the mixture of myosin B and soya protein; the suspension of soya protein; and the supernatant liquid of the fibre made from the mixture of myosin B and soya protein. (a) Myosin B fibre (50 μg); (b) fibre made from the mixture of myosin B and soya protein (125 μg) where the mixing ratio of myosin B and soya protein was 2 : 3; (c) soya protein (75 μg; 1:1 mixture of 7s and 11s component); (d) supernatant liquid of the fibre made from the mixture of myosin B and soya protein (5 μg). MHC, myosin heavy chain; α-Acn, α-actinin; Ac, actin; TN-T, troponin T; TM, tropomyosin; TN-I, troponin I; TN-C, troponin c; LC-1, LC-2 and LC-3, myosin light chains.

the fibre of the mixture of myosin B and soya protein, however, both AEM and BF showed two plateaus in increasing phase at around 70°C and 90–100°C (Fig. 7). The changing pattern of AEM and BF of the fibre made from the mixture of myosin B and soya protein was in accordance with those of heat induced gel of myosin B, and of myosin B-CIF mixture, reported by Haga & Ohashi (1977) who have determined gelling property of the proteins with a rheometer.

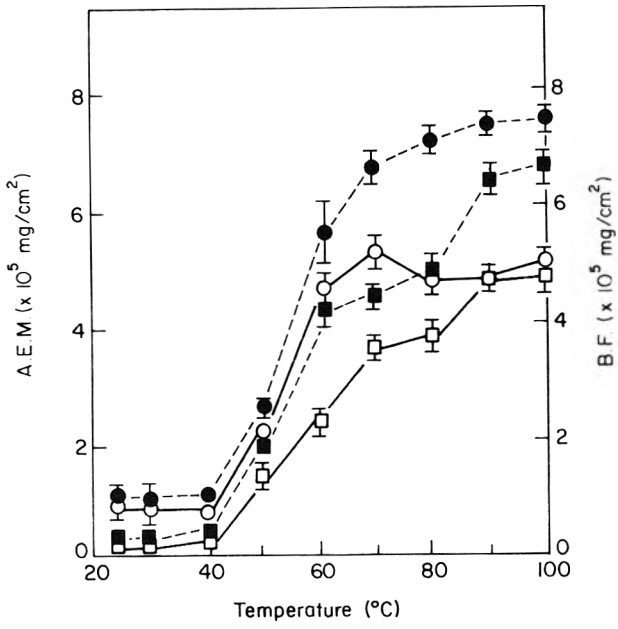

Figure 7. Effect of temperature on AEM and BF of myosin B fibre and the fibre made from the mixture of myosin B and soya protein. Each point shows an average of nine determinations under each condition. Vertical lines represent s.d. at each point. O, AEM of myosin B fibre; ●, BF of myosin B fibre; □, AEM of the fibre made from the mixture of myosin B and soya protein; ■, BF of the fibre made from the mixture.

As shown in Fig. 8, AEM and BF of the heat treated myosin B fibre were greatly increased with increasing protein concentrations, although the increasing rate of these rheological properties gradually slowed down at higher protein concentrations. In the case of the fibre made from the mixture of myosin B and soya protein, on the other hand, the increasing rate of AEM and BF was low up to 15 mg/ml. However, above 15 mg/ml these parameters rapidly increased with increasing protein concentrations.

Figure 9 shows the effect of pH of bathing buffer on AEM and BF of protein fibre. Myosin B fibre shows the maximum values of both AEM and BF at pH 5.2–5.5 and the values of these parameters steeply decreased with decreasing or increasing pH values from this pH range. At extremely low pH values (e.g., pH 1.08), fibre was formed just after extrusion. However, it disappeared rapidly. This may be due to the solubilization of protein. A quite similar result was also obtained in the case of the fibres made from the mixture of myosin B and soya protein, although the values of these parameters were much smaller than those of myosin B fibre.

Figure 10 shows the effect of mixing ratio of myosin B and soya protein on AEM and BF of the resulting fibres. As shown in this Figure, both AEM and BF linearly decreased with increasing the mixing amount of soya protein, except that almost no change was found in BF when the mixing ratio was below 4:1. This result suggests that the addition of small proportion of soya proteins have no adverse effect on the rheological properties of meat products.

The change of rheological properties of protein fibres during storage

Figure 11 shows the changes of rheological properties of protein fibres during storage at 25°C in 0.1 M acetate buffer (pH 5.5) in the native state. In the case of myosin

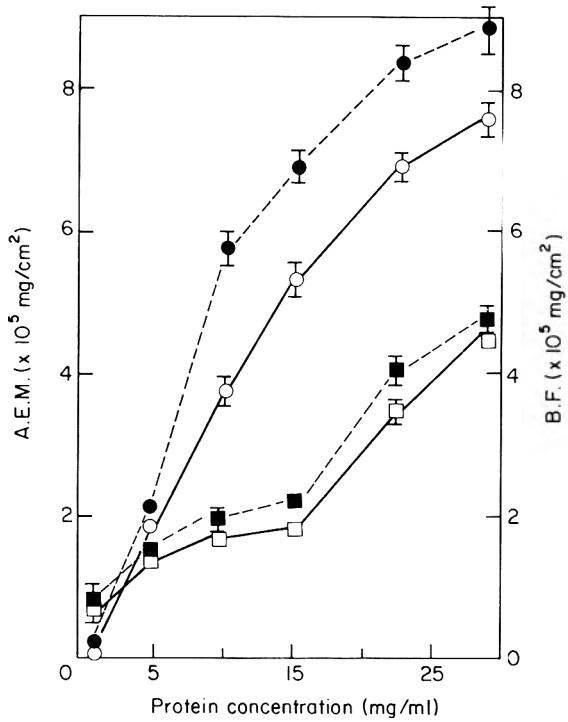

Figure 8. Effect of protein concentration on AEM and BF of protein fibres. Each protein fibre was prepared by extruding varying concentrations of proteins into acetate buffer (pH 5.5). Each point shows an average of nine determinations under each condition. Vertical lines represent s.d. at each point. Symbols are the same as in Fig. 7.

B fibre, AEM and BF substantially decreased during storage of fibre, while in the case of fibres made from the mixture of myosin B and soya protein, no appreciable change was found in those rheological properties. This result indicates that soya protein substantially protects the decrease of the rheological properties of myosin B during storage in the native state before heating.

Scanning electron microscopic observation of myosin B fibre and the fibre of the mixture of myosin B and soya protein

Heating of myosin B fibre at 70°C for 15 min produced an irregularly shaped strand with many hollows like an automobile radiator. On the other hand, the fibre made from the mixture of myosin B and soya protein showed a quite different appearance from that of myosin B fibre; the surface of the fibre was coated with round shaped particles (Fig. 12). In addition the shape of the particles is quite similar to that of soya protein (Wolf & Baker, 1975). This indicates that soya proteins cover the surface of myosin B fibre.

Discussion

It is well known that salt soluble myofibrillar proteins play the most important role in determining the binding quality and the water holding capacity of meat products and fabricated meat products such as beef patties. These products include soya protein. Thus there is a close relationship between solubility and extractability of salt soluble protein of muscle and the quality of meat products. Salt soluble proteins of muscle form

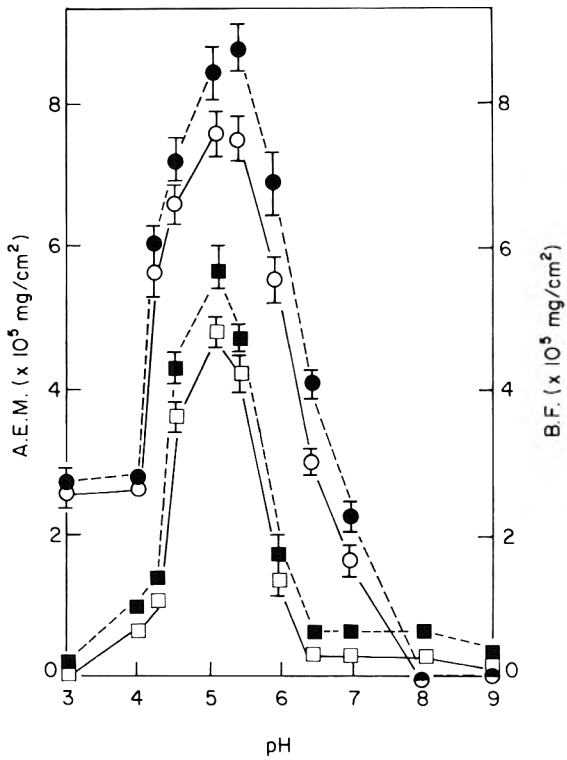

Figure 9. Effect of pH of bathing buffer on AEM and BF of protein fibre. Fibres were prepared in buffers having varying pH values. Each point shows an average of nine determinations under each condition. Vertical lines represent s.d. at each point. Symbols are the same as in Fig. 7.

a gel when they are subjected to heat treatment at temperatures usually used in commercial meat processing plants. On the other hand, soya protein does not form a gel at such temperatures. For instance, the 11s component in a solution containing a high concentration of salt is stable against thermal treatment up to 80°C, while in a solution with a low concentration of salt it aggregates rapidly upon heating. On the contrary, the 7s component is stable in low salt solution upon heating, but it aggregates in high ionic strength solution (Hashizume, Nakamura & Watanabe, 1975). In order to search for the most appropriate condition for the processing of fabricated meat products containing soya protein and to understand the mechanism for the modification of meat quality by soya protein, many studies have been made on the interaction of myofibrillar proteins with soya protein (Haga & Ohashi, 1977; Yamamoto, Kajiyama & Samejima, 1977; King, 1977; Peng *et al.*, 1982a,b). Haga & Ohashi (1977) have shown that the interaction does occur between myosin B and soya protein CIF, resulting in the formation of a gel upon heating when protein concentration is high. They have also found that the most desirable condition for obtaining a gel with strong physical properties is two step heating at 60–70°C and at 100°C. In addition, they have suggested from the findings of gel filtration and SDS polyacrylamide gel electrophoresis of the mixture of myosin B and soya protein that the interaction between myosin B and soya protein does occur even before heating (Haga & Ohashi, 1984a). However, Peng *et al.* (1982a,b) have shown that the interaction between myosin (not myosin B) and soya protein occurs only after heating at 80°C or above. The result shown in Fig. 6 in the present study suggests that α-actinin is released from myosin B complex after mixing of

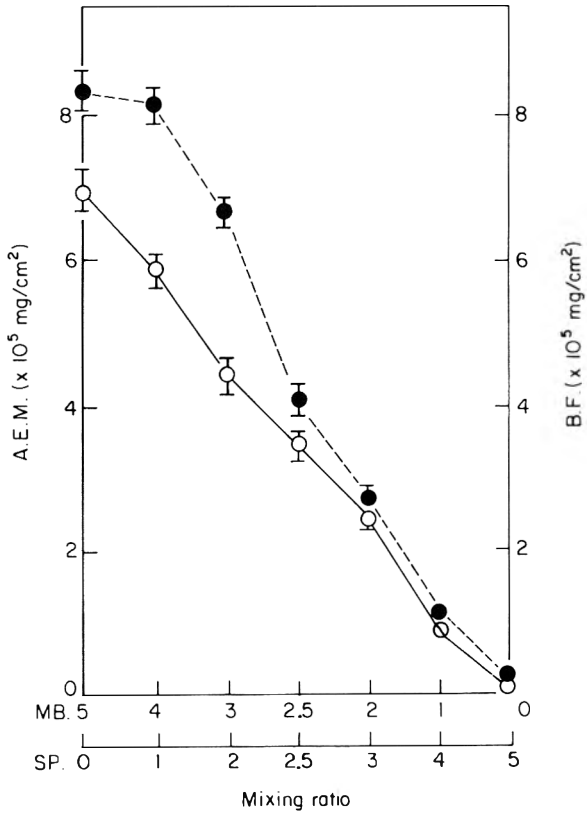

Figure 10. Effect of mixing ratio of myosin B to soya protein on AEM and BF of the resulting fibre. In this experiment, protein concentration of the mixtures at any mixing ratios was 22.5 mg/ml. Each point represents an average of nine determinations under each condition. Vertical lines represent s.d. at each point. O, AEM of the fibre made from the mixture of myosin B and soya protein; ●, BF of the fibre made from the mixture of myosin B and soya protein; MB, myosin B; SP, soya protein.

myosin B and soya protein, indicating that α-actinin is not involved in the interaction between myosin B and soya protein. Since it is well known that α-actinin binds to actin polymer in myosin B complex, the present result suggests that the affinity of soya protein for actin is stronger than that of α-actinin and the binding of soya protein to actin polymer competes with that of α-actinin, so that α-actinin is expelled from myosin B complex (possibly from actin polymer) by the binding of soya protein to actin. Therefore, we can conclude that the binding of soya protein to actin and the binding of actin to myosin occur during the formation of fibre made from the mixture of myosin B and soya protein. As shown in Fig. 5, the diameter of protein fibre increased with increasing the mixing amount of soya protein. This result suggests that soya protein adheres to or surrounds myosin B fibre. The similarity of rheological properties, such as elastic modulus and breaking energy, of fibres made from the mixture of myosin B and soya protein and those of soya protein gel (Haga & Ohashi, 1977) also suggests that the fibre made from the mixture of myosin B and soya protein is composed of myosin B fibre surrounded by soya protein layer. Scanning electron microscopic observation clearly supports this idea (Fig. 12).

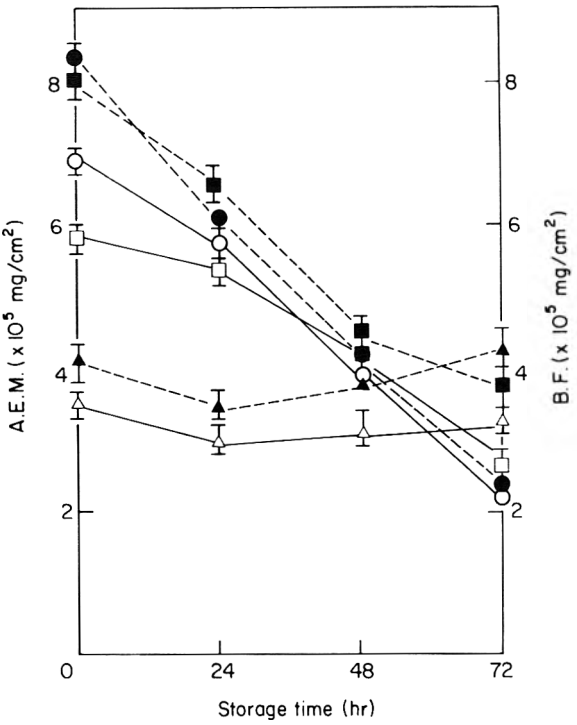

Figure 11. Change of rheological properties of protein fibre during storage. O, AEM of myosin B fibre; ●, BF of myosin B fibre; □, AEM of the fibre made from the mixture of myosin B and soya protein (Myosin B : Soya protein = 4 : 1); ■, BF of the fibre made from the mixture (4 : 1); △, AEM of the fibre made from the mixture of myosin B and soya protein (Myosin B : Soya protein = 1 : 1); ▲, BF of the fibre made from the mixture (1 : 1). Symbols in this figure are the same as in Fig. 10.

The mixing ratio of soya protein to muscle protein is an important factor in the utilization of soya protein for meat products. The results shown in Fig. 10 suggest that the addition of a large amount of soya protein produces meat products having lower rheological properties. Haga & Ohashi (1984b) have also obtained a similar result that the physical property of a heat induced gel depends upon the mixing ratio of myosin B and soya protein 11s component. As shown in Fig. 11, however, the change of rheological properties of myosin B fibre during storage was protected by soya protein. This protective effect may be due to (i) the stabilization of the structure of myosin B fibre by soya protein, or (ii) the inhibition of proteases contaminated in myosin B preparation, responsible for the degradation of protein fibre, by some contaminants of soya protein, such as protease inhibitors. Whichever plays a significant role in protecting the structure of protein fibre, this stabilizing effect of soya protein may contribute to the improvement of the properties of meat products.

The pH is probably the most important factor in modifying the textural properties of protein fibre. As is well known, the isoelectric points of myosin B, 7s and 11s components are about 5.4, 4.9 and 5.0 respectively. (Szent-Györgyi, 1951; Wolf & Briggs, 1959; Koshiyama, 1968). The similarity of isoelectric point and pH dependent rheological changes of protein fibre indicate that the smaller the net charge of the protein the stronger the rheological properties. Therefore, these results suggest that the strength of protein-protein interaction after heat treatment is not determined by salt bonds, but by

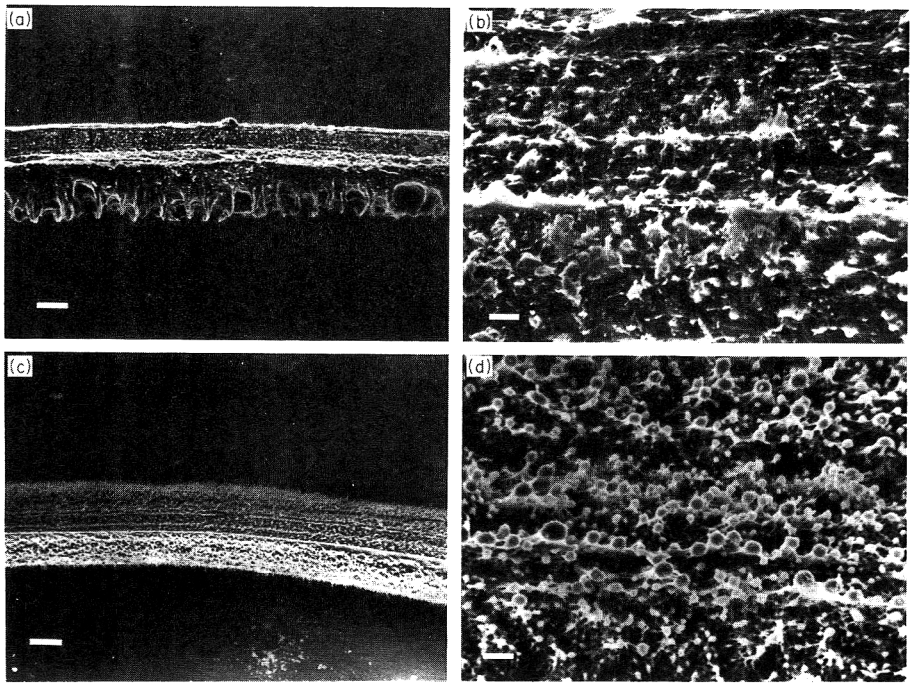

Figure 12. Scanning electron micrographs of myosin B fibre and the fibre made from the mixture of myosin B and soya protein (A), (B) myosin B fibre; (C), (D) the fibre made from the mixture of myosin B and soya protein (weight ratio, 1:1). Bar of A, C represent 200 μm, while bar of B, D represent 20 μm.

other bondings such as hydrogen bonds, hydrophobic bonds and van der Waals forces. Haga & Ohashi (1980) have suggested that hydrogen and hydrophobic bonds play the most important role in the heat induced gelation of myosin B, soya protein CIF and the mixture of them.

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Rheological characterization of microcrystalline cellulose dispersions: Avicel RC 591

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Summary

The development of structure in a microcrystalline cellulose dispersion has been monitored by a series of rheological measurements. This structure is shown to be concentration, shear rate and shear history dependent and conforms to that predicted of an elastic floc. It confers good suspending properties to dispersions of concentration in excess of 0.7%.

Introduction

The area of food hydrocolloids has been dominated by the naturally occurring polysaccharides whose functionality is well known Glicksman (1969), Lawrence (1976), Blanshard & Mitchell (1979). However, due to their inherent inhomogeneity in polymeric form and purity, their characterization has been difficult. A somewhat broader range of functionality, coupled with uniformity, is now available with synthetic gums; an important area being the chemically modified naturally occurring polysaccharides such as cellulose and starch.

Microcrystalline cellulose, which is essentially pure alpha-cellulose, is completely insoluble in water thus making its dispersion extremely difficult. However, a specially hydrolysed form, having hydrophilic properties and incorporating sodium carboxymethyl-cellulose is available which overcomes this problem. It is used as a hydrocolloid binder and texture modifier in a variety of foods and displays many useful properties. The dispersions develop a microstructure which can be characterized by its physical properties, of specific interest here being the rheological attributes of low concentration dispersions of Avicel RC 591.

Materials and methods

Avicel RC 591 NF was obtained from Honeywill and Stein (London). The composition of the powder, as detailed in the technical specification of the FMC Corporation is 89 : 11 parts by weight of alpha-cellulose to sodium carboxy-methyl-cellulose. Average particle size of the rod-like particles, in dispersion is less than 1 μm.

Preparation

It is generally the case that the wide range of functionality achieved within a cellulose dispersion is due to poor dispersion techniques. Consequently this preparatory stage is of prime importance. The mixing technique employed involved the rapid stirring of the dispersion for 30 min at 1500 rpm using a twin-blade propeller mixer. Sample volumes of 100 ml were prepared using distilled water and left for 24 h to fully

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hydrate. Immediately prior to testing, the dispersion was vigorously stirred, in the same manner but for only 15 min before being introduced into the rheometer. The concentration range studied was 0.4–4% w/w.

Method

The rheological technique used was primarily one of cone and plate, thereby ensuring a uniform shear rate throughout the sample volume. This is important when studying a material which exhibits a nonlinear response. Instrument configuration was a 40 mm diameter cone with an angle of 2.4°. In addition, to prevent sample degradation through evaporative losses, a perspex cup attachment was incorporated onto the lower plate assembly. All measurements were made using an Instron 3250 Rotary Rheometer at 24 ± 1°C. The testing procedure was principally one of constant angular rotation though harmonic oscillatory methods were employed in monitoring the time dependence of the viscoelastic moduli. For this latter test, an extended cone-plate configuration was used. Except for the measurements involving the structural development within the dispersions, all samples were pipetted into the rheometer and left for 15 min before testing. The precautions that were taken to prevent water loss by evaporation involved covering the exposed surface of the dispersion with a silicon oil, a procedure which extended the otherwise typical measurement period of one hour considerably. Reference to these and other rheological techniques can be found in the book by Whorlow (1980).

Results

There is evidence of a structural build-up in Avicel dispersions at a concentration of 1.5%. This development was observed by monitoring the storage modulus [$G'(\upsilon)$] at a frequency (υ) of 10 Hz using oscillatory techniques over a period of 18 hr. The sample was subjected to a sinusoidal oscillatory strain of maximum amplitude 0.5 for 10 sec at 300 sec intervals. Measurements of both storage and loss moduli and phase angle were recorded in this linear viscoelastic region. Results in Fig. 1 are given for the initial 4 hr period and were reproducible, irrespective of the duty cycle used. This can be taken as an indication that the measurement is not itself interfering with the structural development within the dispersion. The time dependence of the storage modulus can be shown to obey a power law of the form

$$G'(\upsilon) = 1.12\, t^{0.26}$$

where t is time (min), $G'(\upsilon)$ the frequency dependent storage modulus (Pa). This relationship was observed to hold over an 18 hr period, the maximum measurement period attainable.

It is expected that a dispersion of asymmetric particles would have a viscosity that is both shear rate ($\dot{\gamma}$) and shear history dependent and this was indeed established over a shear rate range of $2.5 \leq \dot{\gamma} \leq 250$/sec. Figure 2 shows the dependence with decreasing shear rate, achieved in a stepwise manner. The dispersion is clearly shear thinning with the viscosity [$\eta(\dot{\gamma})$] though shear rate dependent, not following the more usual power law. By repeating the measurement cycle 2 hr later, it is clear that the dispersion has become more viscous (Fig. 2). Both the original sample and that aged for 2 hr in the rheometer show an increasing non-Newtonian behaviour at low shear rates but this is an effect which can be removed by subjecting the dispersion to a standard pre-test

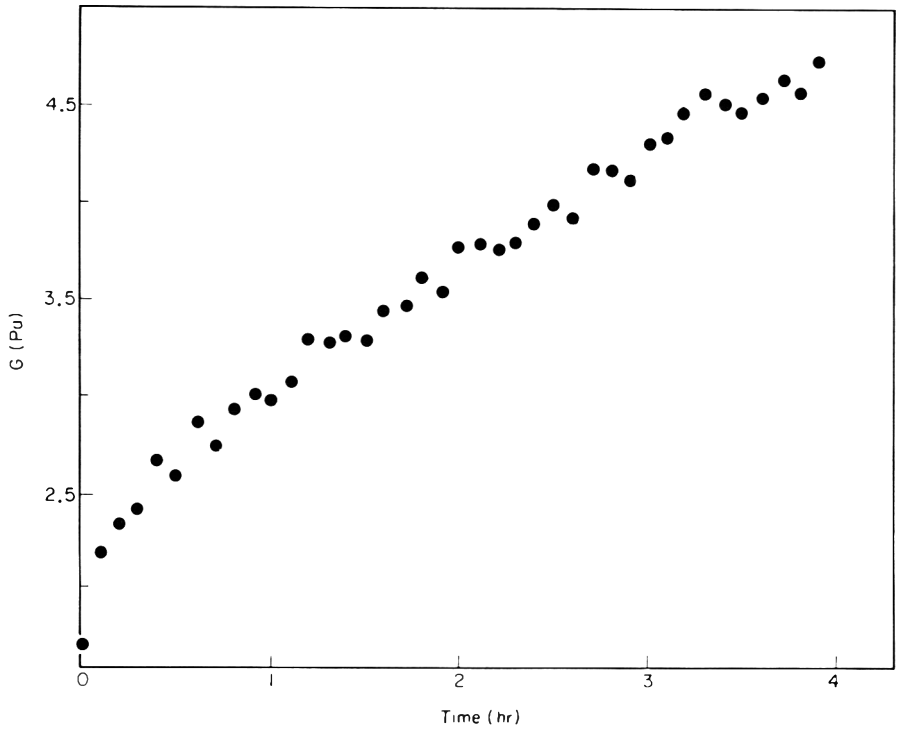

Figure 1. Time dependent rigidity modulus measured at 10 Hz for a 1.5% concentrated dispersion. G in Pa, time in hr.

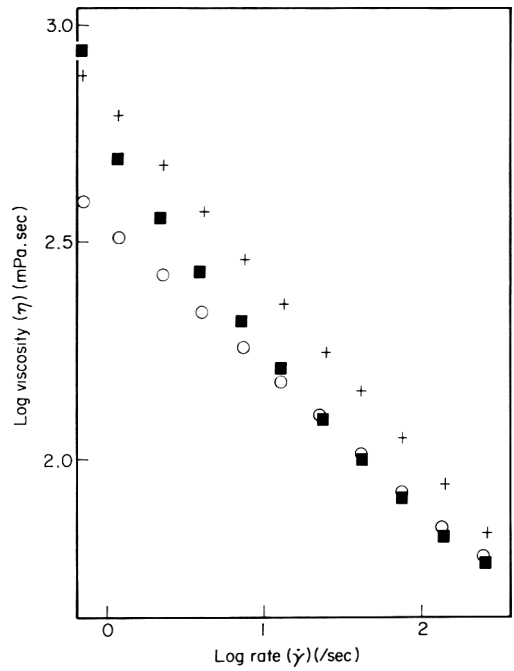

Figure 2. Dependence of log(viscosity) on log(shear rate). 1.5% concentration. O, preconditioned; ■, no preconditioning; +, measured 2 hr later (preconditioned); (η in mPa-sec, $\dot{\gamma}$/sec).

conditioning. This pre-conditioning, subjecting the dispersion to a shear rate of 250/sec for 5 sec immediately prior to testing, gave a power law viscosity dependence (Fig. 2).

$$\eta(\dot{\gamma}) = A(\gamma)^N.$$

where $\eta(\dot{\gamma})$ is the absolute viscosity in Pa sec and N the power index with a value of -0.33. This index decreased to -0.42 after a 2 hr period. This effect is interesting in that it shows that the preconditioning treatment, though detrimental to the structure within the dispersion, does not destroy its development.

It is expected that the dispersions should show a concentration dependent structure and it was this aspect which was investigated by constant rotational techniques. Figure 3 shows the viscosity characteristics of dispersions within the concentration (c) range $0.4\% \leq c \leq 3\%$ at shear rates within the range $0.025 \leq \dot{\gamma} \leq 250$/sec. The dispersions show two distinct regions which are concentration dependent, the critical value being $c \leq 0.75\%$. Below this value the dispersions viscosity obeys a power law dependence on shear rate with the degree of non-Newtonian behaviour increasing with increase in concentration (Table 1). At higher concentrations the dispersions, whilst remaining

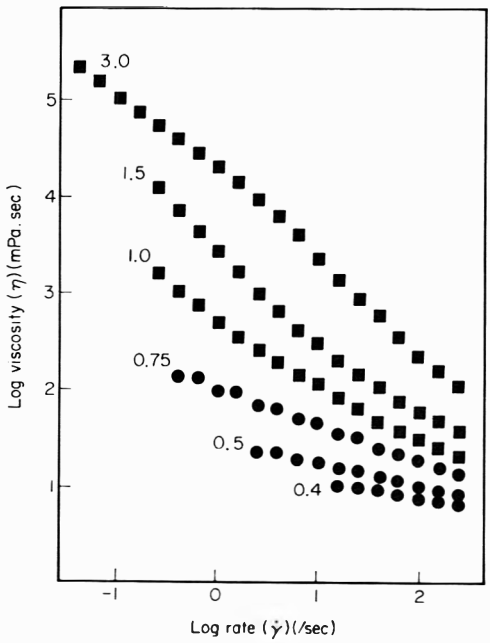

Figure 3. Dependence of log(viscosity) on log(shear rate) for a concentration range. ●, pseudoplastic; ■, structure present; (η in mPa-sec).

Table 1.

Concentration (%)	Index N $[\eta = A\dot{\gamma}^N]$
0.4	−0.15
0.5	−0.23
0.6	−0.28
0.75	−0.35

pseudoplastic, show evidence of a yield value. The systems are also thixotropic but display what is termed 'false-body' thixotropy (Wilkinson, 1960) whereby the structure within the dispersion is not fully broken down under the shear conditions with the consequence of retaining a degree of plastic behaviour.

At higher shear rates ($\dot{\gamma} \geq 10$/sec) the viscosity is also concentration dependent, of the form.

$$\eta = B\, c^M$$

where the index M increases with decrease in shear rate (Fig. 4 and Table 2).

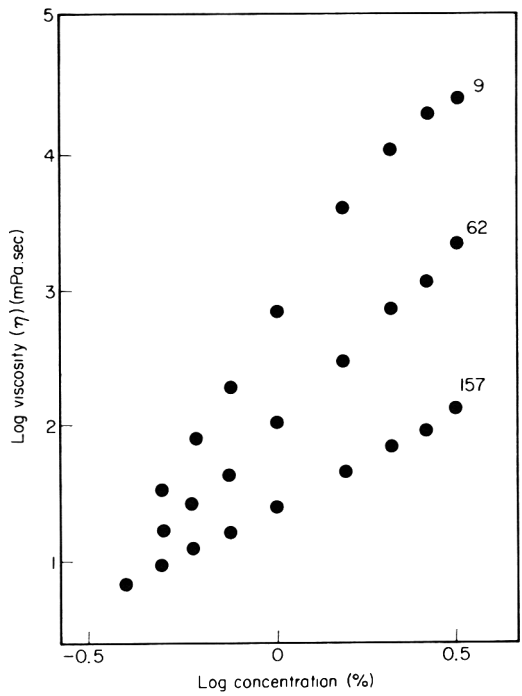

Figure 4. Dependence of log(viscosity) on log(concentration) for a range of shear rates. η in mPa-sec; concentration in %.

Table 2.

Shear rate (/sec) $\dot{\gamma}$	Index M
9	2.6
62	1.7
157	1.4

In limited ranges of concentration and shear rate, dispersions are observed to follow a dependence of the form (Astarita, Marrucci & Nicolais, 1980).

$$\eta(\dot{\gamma}, c) = A\, c^{3}\dot{\gamma}^{-1}$$

at low shear rates.

The results obtained for Avicel dispersions, after preconditioning, do reflect the expected concentration dependence for $\dot{\gamma} \leq 9$/sec and the shear rate dependence for $c \geq 1.0\%$.

Discussion

The ease by which a gross modification to a liquid phase can be achieved by the addition of a particulate phase is well known. However, a minor modification in composition can result in a drastic change in the viscoelastic properties of the dispersion. A similar effect can be produced by subjecting the dispersion to a different shear history and it was this consideration which proved to be of primary importance during the tests.

At all concentrations, the steady shear viscosity is both temporally and shear rate dependent. At $c \leq 0.75\%$, the low shear viscosity limit was not experimentally observed; neither could a yield value be accurately determined. However, dynamic oscillatory tests did show a degree of elasticity, the storage modulus being approximately 30% of the loss modulus, indicative of a weak structure.

The solid like behaviour observed at low shear stresses and shear rates precludes the use of normal viscoelastic theories for the dispersions. During flow, the elasticity is expected to reduce considerably, except for that component inherent in the matrix fluid itself. The elasticity of a 1.5% dispersion was observed to be many orders of magnitude greater than that of the CMC 'solvent', which was barely measurable. This elasticity is consequently due to the formation of an elastic gel. Theoretical analysis of the properties of an 'elastic floc' (Firth & Hunter, 1976; Van de Ven & Hunter, 1977; Hunter & Frayre, 1979) have been undertaken previously, together with experimental evidence that relates the Bingham yield value to the floc volume density (Van de Ven & Hunter, 1979). Data presented here for the Avicel dispersions show that at a concentration of 1.5%, the rigidity modulus is approximately 0.1 Pa and positively dependent on measurement frequency. This is in agreement with the proposed properties of a weak elastic network structure.

A comparison of the Avicel dispersion with that of a CMC solution of equivalent polysaccharide concentration shows that the microcystalline filler increases the high shear rate viscosity by 50%. However, at low shear rates, $\dot{\gamma} < 1$/sec the increase is greater than fifty-fold which can only be due to the network structure formed. From the experimental evidence, it would appear that two different types of structure exist, which develop over different time scales and are differentially disturbed by the preconditioning shear treatment used. However, the structural development of floc formation followed by floc aggregation is not destroyed, merely retarded.

Conclusions

Dispersions of microcrystalline cellulose show thioxtropic and plastic rheological behaviour, the degree of which is shown to be both a function of concentration and shear history. Comparison of data with the model of an elastic floc has shown good agreement. The model predicts that shear history is very important in determining the compactness of the flocs which ultimately influences the dispersions' viscosity but not its elastic properties, provided the maximum shear rate imposed on the sample is above a critical value. This would explain the importance of efficient and controlled dispersion schemes in providing sample uniformity. Additionally, by suitable control of system conditions such as pH and ionic strength, the floc radius and interparticle interaction are

controllable which are essential to the dispersions thixotropic nature and particulate suspension ability.

Acknowledgment

The authors wish to thank Honeywill and Stein for the supply of material.

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Studies on the pearling and milling of sorghum (*Sorghum bicolor*)

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Summary

Twelve per cent pearling was achieved by an under runner disc sheller in a single pass without giving any pretreatment to the grains. The crude fibre, ash and fat contents were reduced from 2.67 to 1.18, 1.5 to 1.1 and 7.8 to 3.0% respectively. Pearling of sorghum improved its cooking qualities. It took 8.5 min for baking chapaties over 12.0 min in the case of whole sorghum grain flours. Pearling of sorghum improved the palatability and ease of rolling of chapaties. The cost of pearling with an under runner disc sheller was Rs.40/t ($4/t) of grain. A mini burr mill was used for making sorghum flour. The average particle size obtained was 0.36 mm. The rise in temperature during milling ranged between 16.0°C and 19.4°C.

Introduction

Sorghum is the most important food crop of rain-fed agriculture in the tropics. In India it is cultivated in 16.12×10^6 ha with an annual production of 11.56×10^6 t. From the nutritional point of view, sorghum has a protein content similar to rice and wheat, and in mineral and vitamin contents it is even better. For human consumption, the sorghum grains are made into coarse grits and cooked in the same way as rice. Sorghum flour is also made and used for making a wide range of traditional recipes, but the sorghum flour has a lower palatability. This is mainly due to its coloured outer bran which contains tannins. The palatability of the flour could possibly be improved by a pearling process which removes a part of the bran and germ. However very little attention was given to improve the quality of sorghum flour as it is considered as a poor man's crop in India. Hence the present investigation was carried out to identify the equipment to achieve a pearling of around 10% without giving any pretreatment to grain and study the effect of pearling on the chemical composition of the resultant product.

Materials and methods

The sorghum variety CSH-5 was procured from the Farm Section, Central Institute of Agricultural Engineering, Bhopal.

Pearling of sorghum

Sorghum was pearled by a batch type dehuller having five carborundum grinding wheels, separated at 2.0 cm and run inside a metal casting. An under runner disc sheller was used which has a hard polisher rubber at the top fixed to the main frame. An emery disc of 30 cm diameter rotates under the polisher rubber at 700 rpm. The gap between the polisher rubber and emery disc was kept at 2.0 mm.

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Milling of sorghum

Whole sorghum/pearled sorghum grains were milled in a mini burr mill. A gap of 1.5 mm was maintained between the two emergy stone discs. The rise in temperature of the flour, the capacity of the mill, the energy consumption per unit of material milled and the average size of flour were recorded. In case of whole grains 3% moisture was also added to loosen the husk and tempered for 16 hr before milling. The flour obtained was sifted by a 70 IS sieve (0.7 mm opening) and suji (semolina) from pearled sorghum was prepared by a hammer mill with a 0.8 mm sieve. The particle size and whiteness of the flour (whiteness meter, model C-3 Klett Electric Laboratory, Japan) were also estimated.

Chemical analysis

The sorghum flour was analysed for protein, crude fibre, ash and fat contents (TPI-1975). All analyses were done in triplicate and the mean values were computed.

Results and discussion

Whole sorghum grains at 9.3, 10 and 12.2% (moisture content on a wet basis) were milled into flour in a mini burr mill. The average particle size and the rise in the temperature of the product were recorded. The data are shown in Table 1. The average particle size obtained was observed to be 0.36 mm. Addition of moisture did not influence the particle size of the flour. The rise in temperature during milling ranged between 16–19°C. The flour was chemically analysed for its ash and fat contents. The results are given in Table 2. It is evident that the fine fractions of flour had more fibre, ash and fat. It may be that the bran layer did not resist crushing and gets pulverized into fine flour. As a result it cannot be removed by sifting. The results are in accordance with Perten (1976), who reported the difference in behaviour of sorghum and wheat flours upon sifting. In the case of wheat bran it is separated as coarse particles upon sieving. This further emphasized the necessity of pearling of sorghum.

Table 1. Milling of whole sorghum by a mini burr mill

Moisture content (%) (wet basis)	Capacity (kg/hr)	Rise in temperature during milling (°C)	Final temperature of flour (°C)	Energy consumption (kW hr/kg)	Size of flour (mm)
9.3	12.0	16.0	43.0	0.034	0.35
10.0	10.5	18.4	48.2	0.050	0.38
12.2	9.5	19.4	49.2	0.056	0.36

Table 2. Proximate composition of unpearled sorghum grain flour

Flour size (μm)	Crude fibre (%)	Ash (%)	Fat (%)
Less than 70 IS sieve (700 μm)	2.67	1.6	7.0
More than 70 IS sieve (700 μm)	1.6	1.2	5.3

Table 3. Chemical composition and physical characters of products from milling of sorghum (12% wet basis)

Product	Protein (%)	Crude fibre (%)	Ash (%)	Fat (%)	Whiteness index	Size of flour (mm)
Whole sorghum	7.8	2.6	1.5	7.0	85.0	0.36
6% pearled sorghum (batch dehuller)	7.5	2.1	1.2	4.0	86.7	0.30
12% pearled sorghum (under runner disc sheller)	7.4	1.8	1.1	3.0	86.7	0.30
Brokens	7.4	0.86	1.2	3.0	—	—
Bran	—	7.6	5.3	11.25	—	—

The sorghum was pearled in an under runner disc sheller. The pearled sorghum grain flour was chemically analysed and the composition is presented in Table 3. There was a reduction in crude fibre, ash, fat and protein contents. The crude fibre, ash, fat and protein contents were brought down from 2.67 to 1.8; 1.5 to 1.1; 7.0 to 3.0 and 7.8 to 7.4% respectively. The decrease in protein content from 7.8 to 7.4% due to 12% pearling is significant at 5% level of significance. The whiteness index of the pearled sorghum grain flour was greater than that of whole grain flour. Increase in the brightness index from 85 to 87% due to pearling is statistically significant at 5% level of significance. Reduction in fat content of the flour improves its keeping quality. Minimizing the fibre in the flour helps in digestibility and further eliminates the coarse feeling to the tongue. Desikachar (1975) also suggested that the sorghum be pearled prior to milling it into flour.

Pearling of sorghum grain was also done in a batch type sorghum dehuller. In this machine, the maximum dehulling efficiency achieved was 65%. The power requirement of the mill ranged from 540 to 670 W. As shown in Figs 1 and 2, at increased dehulling

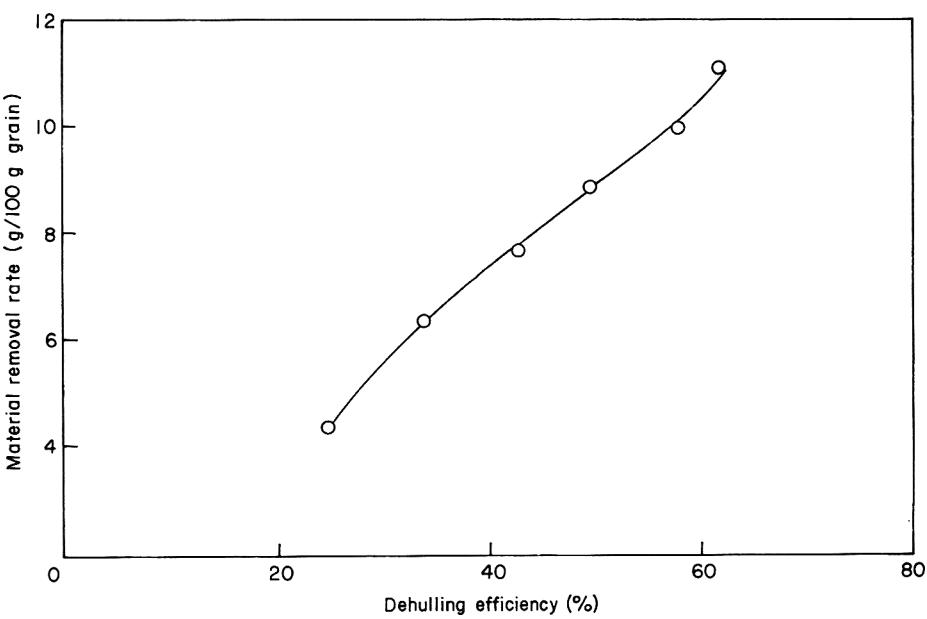

Figure 1. Removal of material from sorghum grain during pearling with batch type pearler.

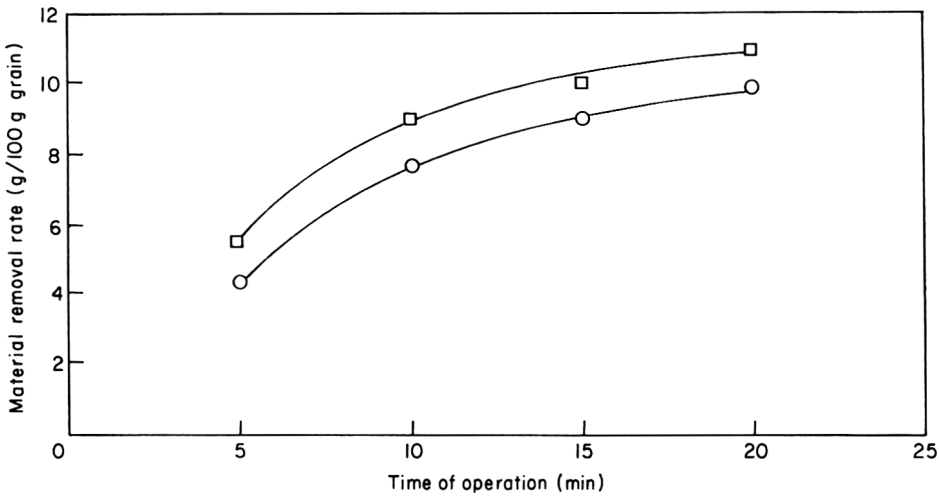

Figure 2. Rate of removal of material from sorghum grain versus time of operation of mill: —□— 1000 rpm; —O— 700 rpm.

efficiency, the material removal rate from grain was also increased. It was observed that after 10 min operation of the mill, material removal from grain became inappreciable. The energy consumption after 10 min of operation amounted to 0.14 kWhr. The capacity of the mill was found to be 30 kg/hr. With the under runner disc sheller 12–14% pearling was achieved in a single pass. The gap between the polisher rubber and emery disc was kept at 2.0 mm. Ten per cent breakage of grains was observed. The dehulling efficiency of the equipment varied from 70–80% as shown in Fig. 3. Material removal rate from grain was 2–14 g/100 g of grain.

Suji (semolina) was prepared from pearled sorghum grain by a hammer mill using a 0.8 mm sieve. The size of suji was 0.42 mm. This could be used in making 'Halwa' and 'Uppama'—which are traditional Indian recipes. A hammer mill with a 0.5 mm sieve was not found suitable for flour preparation.

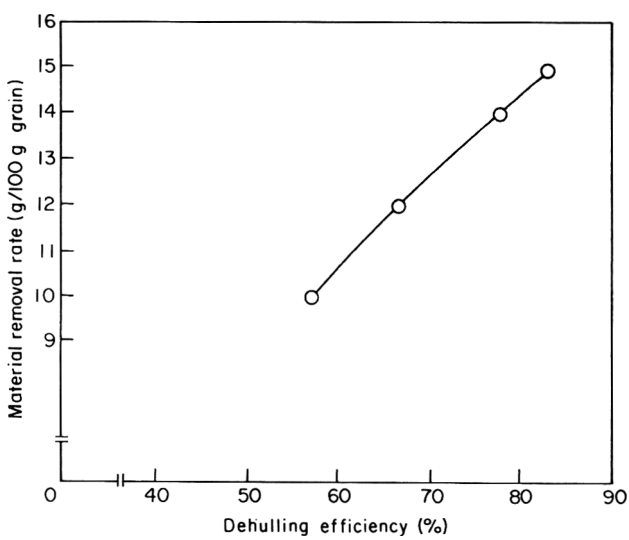

Figure 3. Rate of removal of material from sorghum grain with under runner disc sheller.

Table 4. Water requirement of sorghum flours for making dough, baking time and moisture content of chapaties

Product	Cold water required (ml/100 g flour)	Baking time (min)	Moisture content of chapaties (%) (wet basis)	
			Immediately	After 4 hr
Wheat	70.0	5.50	56.4	32.3
Whole sorghum	85.0	12.0	40.77	32.02
Pearled sorghum	80.0	8.50	38.61	33.95
Wheat (50%) + pearled sorghum (50%)	75.0	7.00	38.00	33.31
Wheat (20%) + pearled sorghum (80%)	80.0	7.50	40.06	33.90

Water requirement for making dough out of whole and pearled sorghum grain flour was determined and presented in Table 4. The data show that the whole sorghum grain flour needs 85 ml/100 g while the pearled sorghum grain flour requires 80 ml/100 g flour to knead into dough, when compared to 70 ml/100 g in wheat flour. The smaller water requirement for pearled sorghum grain flour over unpearled sorghum grain flour is mainly due to its reduced bran and germ contents. The pearled sorghum when blended with wheat flour at 50 and 80% level required 75–80 ml of water per 100 g mixture to knead into dough. The dough so made was divided into 4 equal portions and rolled out into chapaties. They were baked on a hot pan and the baking time was recorded. It took 12.0, 8.50, 5,50 and 7.00–7.50 min for baking whole sorghum grain flour, pearled sorghum grain flour, wheat flour and wheat/sorghum mixtures, respectively. The moisture content of chapaties was found to be 40.7, 38.6 and 36.4% moisture content (on a wet basis) for the above samples. In all the cases the chapaties remained soft even after 4 hr. While rolling of chapaties, it was also noticed that the ease of rolling of pearling grain flour was found better as compared to whole grain flour. The cooking tests further revealed that the cooking time up to 3.5 min could be reduced for 100 g flour if pearled grain flour is used.

Acknowledgments

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Mathematical prediction of leaching losses of water soluble vitamins during blanching of peas

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Summary

A mathematical model was developed to predict leaching losses of water soluble vitamins during pea blanching. For this purpose a literature survey of relevant data on typical D_{eff} values of water soluble vitamins in peas and vegetables was also made. Due to the lack of agreement and reliability of values reported by different workers, a range of values (5×10^{-5}–5×10^{-6} cm²/sec at 77°C) was used for the calculations. A parametric study was then performed for different blanching temperatures and external heat transfer characteristics (water in natural or in forced convection). Blanching of peas at higher temperatures in 'static' water reduced leaching losses. The retention of water soluble vitamins was greater when the size of peas was increased.

Introduction

The effect of blanching on vitamin retention in vegetables has been studied and reviewed by many authors (Thompson, 1982; Lee, 1958; Harris & Karmas, 1975; Selman, 1978; Cain, 1967). It is well known that during water blanching, water soluble vitamins may be lost either due to leaching, thermal destruction, or enzymic oxidation. Recently Hough & Alzamora (1984) developed a mathematical model for predicting thermal destruction of vitamins during pea blanching. They found that the effect of conventional blanching procedures on the thermal destruction *per se* of vitamin C, thiamin and pantothenic acid in peas was negligible. They concluded that reported losses of these vitamins during blanching should be attributed to leaching or enzyme catalysed reactions since thermal destruction had very little importance. The present paper proposes a mathematical model to predict blanching losses of water soluble vitamins during pea blanching. A parametric study was performed for different blanching temperatures and external heat transfer characteristics (water in natural or in forced convection).

Mathematical model

The model determines the blanching time necessary to prevent the enzymic regeneration of peroxidase by means of calculating the food temperature developed during heating and subsequent cooling, and verifying that the appropriate enzymic lethality is

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reached. The following assumptions were made in the development of the model:

(a) the peas are spherical;

(b) peas have a uniform structure and the initial distribution of enzymes and vitamins is uniform;

(c) the thermal transport properties of peas (i.e. thermal diffusivity) are constant throughout the whole process;

(d) the heating and cooling temperatures are constant;

(e) vitamin losses are due to leaching and to thermal degradation, assuming for the last process a first order reaction kinetics. It is to be noted that thermal degradation is included in the model only with the purpose of generalization since it was previously demonstrated (Hough & Alzamora, 1984) that the losses of vitamins in peas due to this mechanism are negligible;

(f) the enthalpic change of the thermal degradation reaction is negligible.

By considering a sphere of radius R, the heat and mass transfer balances result:

$$\frac{\partial T(r,\theta)}{\partial \theta} = \alpha \frac{1}{r^2} \frac{\partial}{\partial r} \left(r^2 \frac{\partial T(r,\theta)}{\partial r} \right), \tag{1}$$

$$\frac{\partial c(r,\theta)}{\partial \theta} = \frac{1}{r^2} \left(\frac{\partial}{\partial r} r^2 D_{\text{eff}} \frac{\partial c(r,\theta)}{\partial r} \right) - kc(r,\theta), \tag{2}$$

$$\text{where } D_{\text{eff}} = D_\infty \exp^{(-E_a/R_g T')} \tag{3}$$

$$\text{and } k = \frac{2.303}{D_{T_r}} 10^{(T-T_r)/z_n}. \tag{4}$$

The corresponding initial and boundary conditions are:

$$c(r,0) = f(r), \tag{5}$$

$$T(r,0) = f'(r), \tag{6}$$

$$\frac{\partial c(0,\theta)}{\partial r} = 0, \tag{7}$$

$$\frac{\partial T(0,\theta)}{\partial r} = 0, \tag{8}$$

$$-\frac{\partial T(R,\theta)}{\partial r} + \frac{h[T_v - T(R,\theta)]}{\lambda} = 0, \tag{9}$$

$$-\frac{\partial c(R,\theta)}{\partial r} + k_c \left[\frac{c'_v - c'(R,\theta)}{D_{\text{eff}}} \right] = 0. \tag{10}$$

The following assumptions are made:

(a) c'_v is assumed to be equal to zero;

(b) at the interface, equilibrium vitamin concentrations in the peas and in the fluid are equal, i.e. $c(R, \theta) = c'(R, \theta)$. This is equivalent to saying that the activity coefficients are the same in both faces, when neglecting the influence of soluble solutes of the peas on the activity coefficient of the vitamin.

The general solution for equation (1) is (Luikov, 1968):

$$T_v - T(r, \theta) = \sum_{n=1}^{\infty} = \frac{2\mu_n}{\mu_n - \sin\mu_n \cos\mu_n} \cdot \frac{\sin\mu_n(r/R)}{rR} \cdot$$

$$\cdot \int_0^R r[T_v - f'(r)] \sin\mu_n (r/R)\, dr \exp(-\mu_n^2 \alpha\theta/R^2), \tag{11}$$

where μ_n is the nth root of the characteristic equation

$$\tan\mu_n + \mu_n/(Bi_h) - 1) = 0. \tag{12}$$

At the beginning of the heating stage it was assumed that the initial temperature distribution in the peas was uniform ($f'(r)$ = constant = T_c). In the cooling stage, $f'(r)$ was taken as the temperature distribution at the end of the previous stage.

The F value of the blanching treatment for enzyme inhibition was computed by the equation:

$$F_{T_r} = \int_0^{\theta_T} 10^{[T(0,\theta) - T_r]/z_e}\, d\theta. \tag{13}$$

In determining the heating time, to satisfy the preassigned F, the roots of equation (13) were found by employing the method of false position to facilitate the convergence.

Having found the correct processing time and then calculated the temperature profile for the whole process (heating+cooling), equation (2) was solved applying the Crank–Nicholson method (Jenson & Jeffreys, 1963). The radius step was taken as $R/10$ and the time step was taken as $\theta_H/100$ for the first ten intervals and then as $\theta_H/10$. Vitamin retention was calculated according to:

$$\frac{cf}{c_o} = \frac{3}{R^3} \int_0^R c(r,\theta_T) r^2 dr, \tag{14}$$

solving the integral numerically by Simpson's method. The equations were programmed on a Texas TI 99 computer.

Heat and mass transfer parameters and kinetic data for process calculation

In order to apply the model it was necessary to know the effective diffusion coefficient of water soluble vitamins in peas, namely D_{eff}. For this purpose a survey of the literature was performed and the results are shown in Table 1, which also shows some activation energy (E_a) values for vitamin diffusion. There are several comments which can be made about the compiled data. They are the following:

(a) very few authors measured D_{eff} values for vitamin diffusion in peas or other vegetables;

(b) the molecular diffusion coefficients (D_{AB}) of soluble vitamins in water are almost equal ($\cong 3\times10^{-5}$ cm^2/sec) for ascorbic acid, thiamin, nicotinamide and piridoxine and their E_a values are in the range 3.6–4.7 kcal/mol;

Table 1. Diffusion coefficients of water soluble vitamins

Vitamin	Diffusion coefficient (cm^2/sec)	Temperature (°C)	E_a (kcal/mol)	System	Reference
Ascorbic acid	2.7×10^{-5}	77	4.7	Water	Schneeberger, Stahl & Loncin (1975)
	9.5×10^{-5}	77	—	Potatoes	Kozempel *et al.* (1982)
	1.4×10^{-4}	85	—	Peas	Lathrop & Leung (1980)
	0.92×10^{-5}	77	10.3	Peas*	Selman (1978)
	1.1×10^{-5}	85	10.7	Peas	Rice & Selman (1984)
Thiamin	2.9×10^{-5}	77	4.4	Water	Schneeberger *et al.* (1975)
	3.6×10^{-5}	77	—	Potatoes	Kozempel *et al.* (1982)
Nicotinamide	2.3×10^{-5}	77	3.7	Water	Schneeberger *et al.* (1975)
	7.9×10^{-4}	77	—	Potatoes	Kozempel *et al.* (1982)
Piridoxine	2.8×10^{-5}	77	3.6	Water	Schneeberger *et al.* (1975)
Riboflavine	3.4×10^{-5}	77	—	Potatoes	Kozempel *et al.* (1982)

*Calculated from the data of Selman (1978) assuming ascorbic acid losses were due to leaching and considering an isothermal process.

(c) some reported D_{eff} values for vitamins showed inconsistencies which are discussed below.

The D_{eff} value of vitamin C in peas reported by Lathrop & Leung (1980) is significantly higher than the corresponding value in water (1.4×10^{-4} cm^2/sec *versus* 3.2×10^{-5} cm^2/sec). The data of Kozempel *et al.* (1982) in potatoes exhibit the same anomaly for thiamin (3.6×10^{-5} cm^2/sec *versus* 2.87×10^{-5} cm^2/sec), nicotinamide (7.93×10^{-4} cm^2/sec *versus* 2.31×10^{-5} cm^2/sec) and ascorbic acid (9.5×10^{-5} cm^2/sec *versus* 2.72×10^{-5} cm^2/sec). An analysis of the data of Lathrop & Leung (1980) for vitamin C diffusion in peas suggested that their values were erroneously calculated. They determined D_{eff} values from the unsteady state diffusion chart for a sphere based upon the fraction of vitamin C remaining in the sample; however, they did not use the chart integrated for the whole sphere, as they should. Moreover, they apparently made a mistake when reading the chart (Rice & Selman, 1984). Kozempel *et al.* (1982) used a leaching model with diffusion in the potato as the rate controlling step to predict the D_{eff} of vitamin C, nicotinamide and thiamin reported on Table 1. However, their model did not take into account that there is a distribution of residence times of potato pieces when considering the precooker as a well-stirred tank (Kozempel, Sullivan & Craig, 1981).

It may be concluded that the available literature indicates a lack of accurate data for the diffusion coefficients for water soluble vitamins in peas (or other vegetables). For this reason it becomes necessary to estimate a value (or range of values) for these kinetic parameters. It may be assumed that the upper value of this range should be that corresponding to molecular diffusion of the vitamins in pure water (D_{AB}), which according to Table 1 is about 3×10^{-5} cm^2/sec at 77°C. The effective diffusion coefficient is related to the molecular one according to (Froment & Bischoff, 1979):

$$D_{eff} = D_{AB} \frac{\epsilon}{\tau} \tag{15}$$

where ϵ is the porosity (approximately equivalent to the water content on a wet basis, i.e. 0.75 for peas) and τ is a tortuosity factor which accounts for the 'real' diffusion path within the pea. Literature data for diffusion of various non-ionic solutes (i.e. sucrose,

glucose, glycerol, butanol) in vegetables or in cellulose model systems indicate that D_{eff} is generally in the order of 0.2–0.7 of the corresponding D_{AB} (Califano & Calvelo, 1983; Brown & Chitumbo, 1975; Stahl & Loncin, 1979; Schwartzberg & Chao, 1982; Loncin, 1978). Thus, it may be safely assumed that D_{eff} values of water soluble vitamins in peas should be somewhere between 5×10^{-5} cm²/sec (approximate value corresponding to molecular diffusion of ascorbic acid, thiamin and piridoxine in water at 77°C) and 5×10^{-6} cm²/sec at 77°C.

Data on the activation energy for vitamin diffusion in peas (or other vegetables) are even more scarce, as also shown on Table 1. From data reported by Selman (1978) for ascorbic acid retention during blanching of peas, a value of E_a about twice as much as for free diffusion in water was obtained. This result agrees with the findings of Loncin (1978) for the diffusion of cyclohexanol in potatoes and of Brown & Chitumbo (1975) for the diffusion of glucose in a densely crosslinked water-swollen cellulose gel (83% water). Consequently, and due to the lack of more reliable data, two values of E_a were selected to describe the temperature dependence of diffusion coefficients of vitamins in peas: 4.5 kcal/mol and 10 kcal/mol.

The values used for the physical and chemical parameters of the model, including enzyme (peroxidase) inactivation, were as follows (Hough & Alzamora, 1984): $F_{100°C}$ = 6.5 min; z_e = 27°C; $\alpha = 1.43\times10^{-3}$ cm²/sec; ρ = 1.07 g/cm³; cp = 0.82 cal/g°C; R = 0.467 cm (unless it is specifically indicated); T_o = 10°C; T_v (cooling stage) = 10°C; $D_{n\,100°C}$ = 60 000 sec and z_n = 26°C for a 'generic' vitamin.

The retention of water soluble vitamins was predicted for two different external heat transfer conditions: water in natural convection or water in forced convection. The heat Biot criterium, Bi_h, was calculated to be 2 and 10 respectively (Hough & Alzamora, 1984). In each case, the mass transfer coefficient, k_c, was evaluated from the heat and mass transfer analogy (Bird, Stewart & Lightfoot, 1963):

$$Nu = 2.0+0.60\,Re^{1/2}\,Pr^{1/3}, \tag{16}$$

$$Sh = 2.0+0.60\,Re^{1/2}\,Sc^{1/3}. \tag{17}$$

Results and discussion

Figure 1 shows the predicted retention (%) of water soluble vitamin as a function of blanching temperature assuming a value of 4.5 kcal/mol for E_a. Figure 2 shows similar results but assuming that E_a is equal to 10 kcal/mol. There are various comments which can be made about these results. Independently of the assumed value of E_a (i.e. 4.5 or 10 kcal/mol), the retention of vitamin increases with increasing blanching temperature. This is due to the larger E_a value for enzymic inactivation. As expected, a higher D_{eff} value produces a lower retention; however, in the range considered (5×10^{-5} cm²/sec > D_{eff} > 5×10^{-6} cm²/sec) this effect becomes less important when the blanching temperature is increased. For example, if the reference diffusion coefficient (D_{eff} at 77°C) is taken as 5×10^{-6} cm²/sec , blanching at 100°C in non-agitated water (Bi_h = 2) will produce 65% vitamin retention; however, if the reference D_{eff} is increased by one order of magnitude (i.e. 5×10^{-5} cm²/sec) this will reduce retention to 45%. It is obvious that this 'one order of magnitude' change in the diffusivity does not produce an equivalent decrease in retention. Figures 1 and 2 also show that leaching losses of vitamins are expected to be lower in non-agitated water (Bi_h = 2) than in agitated water

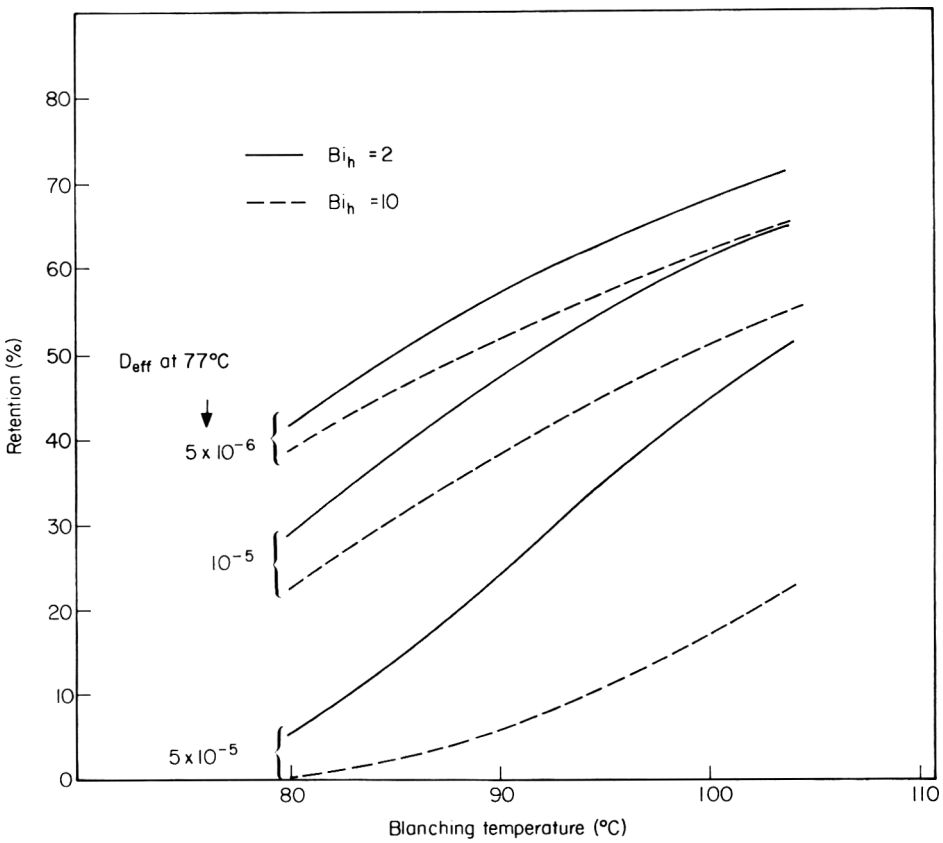

Figure 1. Predicted vitamin retention as a function of blanching temperature (heating + cooling stage); activation energy for vitamin diffusion is assumed to be 4.5 kcal/mol.

(Bi_h = 10). This difference tends to decrease as the value of the reference D_{eff} is decreased; this fact is easily interpreted in terms of the varying relative mass transfer controlling step (internal or mixed).

Figure 3 compares the predicted values from the model of vitamin retention for the whole blanching treatment (heating × cooking stage) with those corresponding to the heating stage alone. It can be seen that little leaching loss of vitamin occurs during the cooling stage. This suggests that if for some reason water cooling of peas becomes necessary, it will not significantly increase leaching losses of vitamins.

In order to compare the range of predicted vitamin losses with actual values, a survey of the literature on vitamin retention during water blanching of peas was made. However, in order to properly compare literature experimental values with predicted ones, the adequacy of blanching procedure utilized in each experiment should be checked. Figure 4 shows the blanching time (heating stage) necessary to obtain a lethality $F_{100°C}$ = 6.5 min in peas of different sizes heated in water (agitated or non-agitated) at 80, 90 and 100°C. The assumed enzymic lethality is that needed to prevent regeneration of peroxidase activity (Hough & Alzamora, 1984). It can be seen that blanching time depends very little on the size of peas and this is due to the short duration of the transient heat transfer period, as compared with the total time needed to inactivate the enzyme.

The results of the literature survey are shown in Table 2, which also indicates

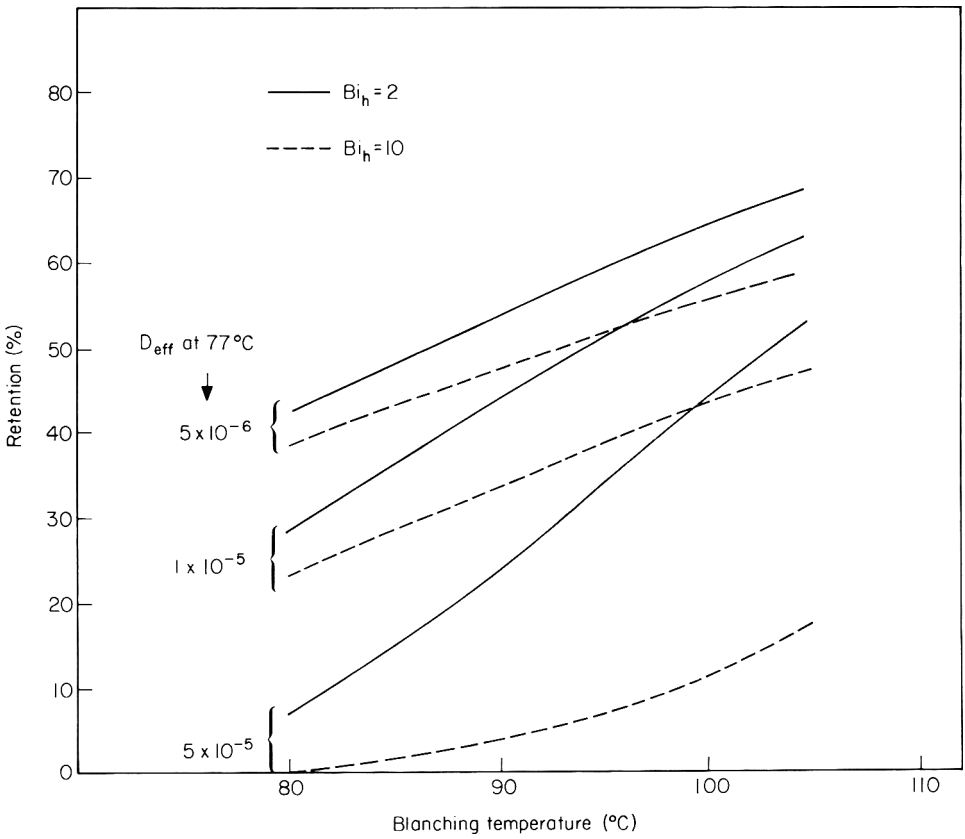

Figure 2. Predicted vitamin retention as a function of blanching temperature (heating + cooling stage); activation energy for vitamin diffusion is assumed to be 10 kcal/mol.

whether or not the blanching treatment was enough to prevent regeneration of peroxidase activity. As shown in this table the blanching time used in most cases is within ±25% of the correct value. Nevertheless, most of the reported experimental values of vitamin retention are within the range 40–70% which is similar to that predicted by the model for some given sets of blanching conditions.

Figure 5 shows the predicted values of vitamin retention as a function of pea size. It can be seen that retention increases significantly as the pea size is increased and this holds for various blanching temperatures and values of the reference diffusivity; also for blanching in non-agitated or agitated water (data not shown). This effect of increasing vitamin retention with increasing size may be explained as follows. The blanching time, as shown in Fig. 4, is very little dependent of pea size, so any increase in size will reduce vitamin losses due to the increased diffusion path.

Conclusions

There are various conclusions which can be drawn on the application of the model which are most useful to minimize leaching losses of vitamins during water blanching of peas. They are the following:

(a) blanching of peas at higher temperatures will reduce leaching losses;
(b) leaching losses will be lower in 'static' water blanching than in agitated one;

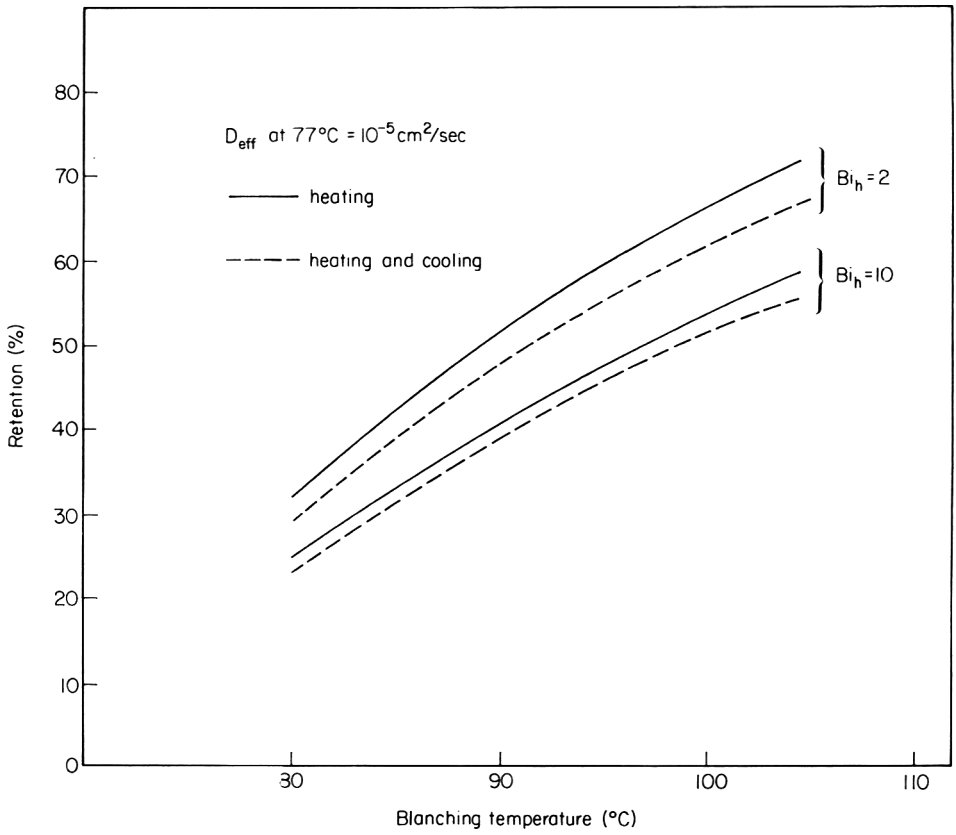

Figure 3. Predicted vitamin leaching loss in the heating and cooling stages of water blanching.

(c) little leaching losses of vitamins occur during water cooling of blanched peas;
(d) leaching losses of peas will be lower when size of peas is increased.

As a consequence of this study it becomes apparent that there is a lack of reliable data on diffusion coefficients of water soluble vitamins in peas (and other vegetables). It is proposed that careful determinations of diffusivities should be made as a preliminary step to further verify the validity of the model, as well as to better optimize vitamin retention during water blanching. It is noteworthy that vitamin concentration may vary within the tissues of the various structures of the pea and that the integrity of the seed coat may significantly affect losses (Selman & Rolfe, 1982); for this reason, experiments are most important.

Acknowledgments

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Nomenclature

α : thermal diffusivity of peas (cm²/sec)
T : temperature (°C)

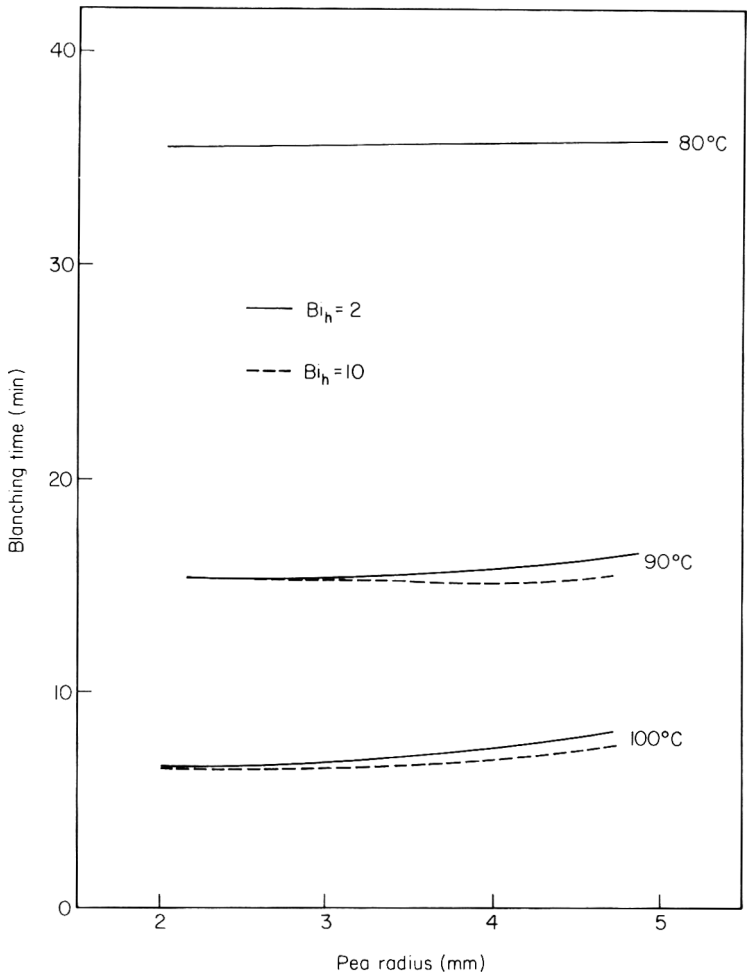

Figure 4. Predicted water blanching times to prevent regeneration of peroxidase activity in peas.

T' : absolute temperature (K)
r : radial coordinate (cm)
R : pea radius (cm)
h : heat transfer coefficient (cal/sec °C cm²)
λ : thermal conductivity of peas (cal/sec °C cm)
Bi_h : heat Biot criterium ($\lambda R/h$)
θ : time (sec)
θ_T : total heating + cooling time (sec)
D_{T_r} : decimal reduction time of the vitamin at $T = T_r$ (sec)
F_{T_r} : enzymic lethality at $T = T_r$ (min)
z : cotangent of the log D *versus* T curve (°C)
D_{AB} : molecular diffusion coefficient (cm²/sec)
D_{eff} : effective diffusion coefficient (cm²/sec)
E_a : activation energy for the diffusion process (cal/mol)
R_g : ideal gas content (cal/K mol)
c : vitamin concentration in the pea (mol/cm³ pea)

Table 2. Literature experimental data on vitamin retention during water blanching of peas

Vitamin	Specifications of peas	Temperature (°C)	Blanching time (min)	Adequacy of blanching*	Type of blancher (%)	Vitamin retention (%)	Reference
Vitamin C	Standard	91	18.5	1.3	Rotary	68	Harris & Karmas (1975)
	Extra standard	91	18.5	1.3		59	
Thiamine	Standard	91	18.5	1.3		69	
	Extra standard	91	18.5	1.3		69	
Vitamin C	Surprise	100	6	0.86		44	
	Lincoln	100	6	0.86		60	
	Charles I	100	6	0.86		45	
Vitamin C	Fancy No. 4 sieve size	93	9	0.75		48	Guerrant *et al.* (1947)
	Standard No. 4 sieve size	93	9	0.75		46	
		93	12	0.75		41	
Thiamine	Fancy No. 4 sieve size	93	9	0.75	Rotary	71	Guerrant *et al.* (1947)
	Standard No. 4 sieve size	93	12	1.0		88	
Riboflavine	Fancy No. 4 sieve size	93	9	0.75		50	
Vitamin C	Alaska, size 4	93.3	8	0.67		59	Wagner, Strong & Elvehjem (1947)
Thiamine	Ungraded	96	8	0.83		76	Clifcorn & Heberlein (1944)

*The adequacy of blanching is defined as the ratio of used blanching time (at a given temperature) to the blanching time actually needed to prevent regeneration peroxidase activity and calculated according to Hough & Alzamora (1984).

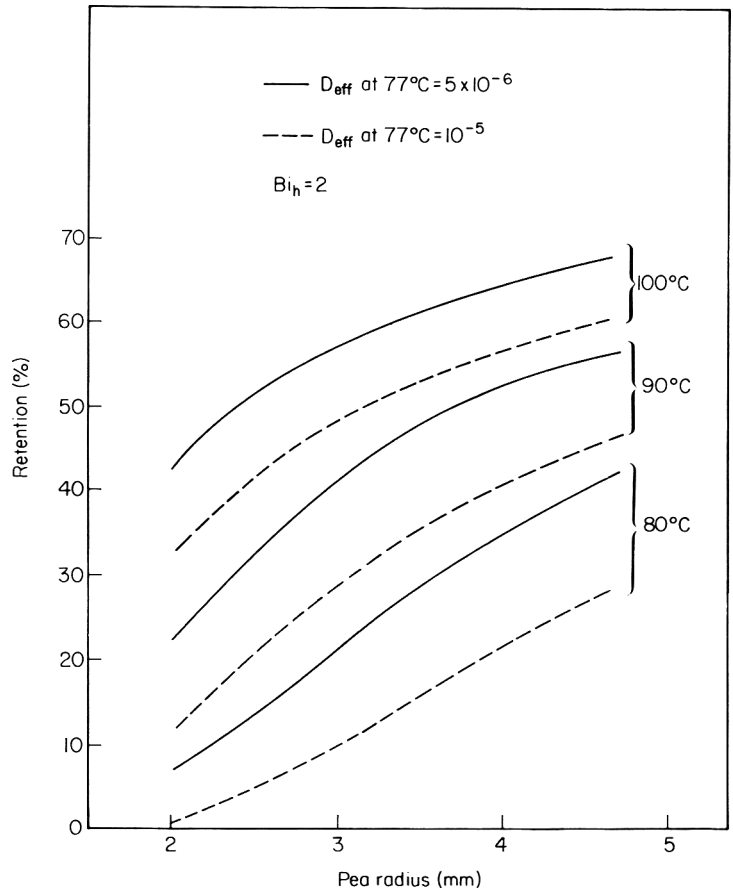

Figure 5. Effect of pea size on predicted vitamin retention during water blanching of peas.

cv : vitamin concentration in the water (mol/cm^3 water)
c' : vitamin concentration in the water ($cv\ \epsilon$, mol/cm^3 pea)
ϵ : porosity of peas
τ : tortuosity factor of peas
k_c : mass transfer coefficient based on concentration (cm/sec)
k : specific constant for vitamin thermal degradation (1/sec)
ρ : density of peas (g/cm^3)
cp : specific heat of peas (cal/g °C)
Re : Reynolds criterion
Pr : Prandtl criterion
Sc : Schmidt criterion
Nu : Nusselt criterion
Sh : Sherwood criterion

Subscripts

v : heating or cooling medium value
o : initial value
f : final value
r : reference value
e : enzyme value
n : vitamin value

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Book Reviews

Physical Properties of Foods. Ed. by M. Peleg and E. B. Bagley.
Westport, Conn.: AVI, 1983. Pp. xiv+532. ISBN 0 87055 418 2. US$49.50.

This book results from an IFT IUFOST Basic Symposium (1982), on the Physical Properties of Foods. In the opening chapter Alina Szczesniak introduces many types of physical properties, such as geometric, optical, thermal and mechanical properties. This appears to set the scene for a comprehensive survey of this important and, up until fairly recently, neglected subject area. However, I was rather disappointed by the coverage, which offered a narrow interpretation of the term 'physical property'. The book deals predominantly with mechanical and rheological properties and offers a very limited amount of information of thermal and electrical properties. However, the Editors do claim that these were covered in an earlier symposium and published as an overview in the February issue of *Food Technology*, 1982.

Articles included are a long and interesting review on electron microscopy (62 pages) and chapters on colorimetry, differential scanning calorimetry and multi-layer emulsions. Chapter 6 deals with the relation of structure to the physical properties of animal material; again properties described relate mainly to rheology and texture. The same applies to the following chapters on horticultural products and baked goods. Chapter 9 deals with the structure and texture of synthetic goods. All these presentations make extensive use of observations from the microscope. Chapter 10, on the physical properties of powders is both interesting and well written and provides a useful review into the problems involved when handling powders. Further chapters on food rheology follow: large deformations in testing and processing of food materials; food dough rheology, structural failure in solid foods and the rheology of emulsions and dispersions. The latter part of the book deals with the physical and chemical properties governing volatilization of flavour and aroma components, expression related products and structure and structure transitions in dried carbohydrate materials. Expression and filtration are dealt with as unit operations; this is of value as expression receives very little attention in most of the engineering text books.

A useful subject index is provided. The chapters, which are of variable length, are well written with clear diagrams and good photographs. A wide variety of topics are covered, not all of which are related to the title of the book, making this worthy of investigation by research or development workers and advanced course students.

M. J. Lewis

Nutritional Standards. By L. J. Minor.
Westport, Conn.: AVI, 1983. Pp. xiii+281. ISBN 0 87055 425 5. US$22.00.

Scarcely had your reviewer read a newspaper article on the demise of the school meal and the development of the 'chips with everything' syndrome in self-service school cafeteria, than he was faced with a book on 'Nutritional Standards' No. 1 in a new Foodservice Standards Series. The text, consisting of nearly 300 pages, is devoted to a collection of papers, aimed at the Food Service Industry, in which a major theme is that

nutrition guidelines for healthier eating, changing food habits and eating patterns are creating a nutrition revolution which will have a considerable impact on future developments. The managers of hotels and restaurants, chefs, dieticians, food manufacturers and organizers of meals eaten outside the home are encouraged by the author to be constantly aware of customer needs as the key to success.

The first chapter summarizes the reasons why food service managers need a basic knowledge of food, the culinary arts, the science of preserving foods, as well as identifying the trend towards lighter healthier eating. In Chapter 2, the importance of food product standards are stated with reference to the food distribution system in the USA. The third chapter reviews food additive standards, allergenic tendencies and diets for hyperactive children. Results from controlled studies in which diets free of additives have been fed to hyperactive children have been inconclusive in terms of any beneficial effect. Nevertheless, parents and teachers alike may still feel there is an opportunity for child control! There is a very useful summary of the functional properties of food additives, their safety evaluation and legislative control. Chapter 4 provides a comprehensive primer on monosodium glutamate (MSG) and introduces the reader to the fact that taste and flavour enhancement are the keys to good nutrition. The author emphasizes that food may be nutritious and have good visual effect, but without flavour, it is neither acceptable nor marketable. The 62 pages of Chapter 5 are well written and referenced. The chapter provides an excellent overview of sensory physiology and flavour analysis and includes a table of GRAS (Generally Recognized As Safe) flavour ingredients along with their usual inclusion rates in foods and drinks.

Chapter 6 on 'Standards in Nutrition' provides a good review of human nutritional requirements, nutrient functions, the composition of foods, the need for roughage and a summary of nutrition labelling developments in the USA. In Chapter 7, the steps in new food product development are briefly summarized together with a basic outline of the socioeconomic changes which have led to the demand for packaged foods which are in non-perishable and convenient states. Chapters 8 and 9 return to expand upon food flavour evaluation in theory and practice. Although the latter chapters provide little in the way of scientific test procedures, they do offer some preliminary insight into taste and odour recognition, flavour fatigue and the effects of time of day and temperature on taste sensitivity.

In conclusion, this book from the US provides the basis for further examination of the skills needed to ensure high quality food service standards. The style of writing is easy to read and may well appeal to those studying catering and those involved in planning and organizing institutional meals.

D. P. Richardson

Topley and Wilson's Principles of Bacteriology, Virology and Immunity, 7th edition. Ed. by Sir Graham Wilson, Sir Ashley Miles and M. T. Parker. (In four volumes.)
Volume 1. General Microbiology and Immunity. Ed. by Sir Graham Wilson and Heather M. Dick.
Volume 2. Systematic Bacteriology. Ed. by M. T. Parker.
London: Edward Arnold, 1983. Vol. 1: pp. xiii×450. ISBN 0 7131 4424 6. £55.00. Vol. 2: pp. xiii+562. ISBN 0 7131 4425 4. £65.00.

The first edition of this famous work appeared in 1929, and it is a considerable achievement for Sir Graham Wilson to have been involved in the writing of seven editions spanning more than 50 years. As pointed out by Wilson and Miles in their preface, they have enlisted over fifty contributors in the preparation of this latest edition, which comprises four volumes, the first two of which were published by the time of the review.

It is first necessary for the present reviewer to declare his interest. I first bought this work in its two-volume 4th edition in 1955 whilst an undergraduate, as the result of its being a recommended text. I believe that the published price was then £8. I wonder how many undergraduates could be persuaded today to buy the four volumes of the present edition at a cost of nearly £270?

The intended audience for this edition is stated to include: 'postgraduate microbiologists, lecturers, research workers, veterinarians, clinicians, epidemiologists and public health workers.'

The nineteen chapters of Volume 1 comprise: 1, History; 2, Bacterial morphology; 3, The metabolism, growth and death of bacteria; 4, Bacterial resistance, disinfection and sterilization; 5, Antibacterial substances used in the treatment of infections; 6, Bacterial variation; 7, Bacteriophages; 8, Bacterial ecology, normal flora and bacteriocines; 9, The bacteriology of air, water and milk; 10, The normal immune system; 11 & 12, Antigen-antibody reactions; 13, Bacterial antigens; 14, 15 and 16, Immunity to infection; 17, Problems of defective immunity; 18, Herd infection and herd immunity; and 19, The measurement of immunity. The second volume contains twenty-eight chapters. After two introductory chapters on isolation, description, identification, classification and nomenclature of bacteria, the following 26 chapters provide descriptions of those groups of bacteria, spirochaetes, chlamydiae, rickettsiae and mycoplasmas which 'may be encountered by medical and veterinary microbiologists'.

The usefulness of a substantial work of this nature (which contains contributions by many very eminent workers) is highly dependent on the index being comprehensive and meticulously prepared. It would seem that the index in this case has not been produced by the named editors. Two consecutive entries in the index to Volume 2 read: 'Colicines, 248' and 'Colicins, 166'. Reference to the two chapters, 6 and 8, which are written by different contributors, reveals that the spelling is indeed different in the two chapters.

If one tests the index by choosing keywords relating to a single topic it can easily be demonstrated to be seriously deficient. To take one example, I searched in vain for chemiosmotic theory, protonmotive force, proton transport or Mitchell. Reference to the index of a text such as 'Microbiology' by Davis, Dulbecco, Eisen and Ginsberg shows that such comprehensiveness can be achieved. In this sense, the current edition of Topley and Wilson's work could be regarded more as a collection of monographs than as a work of reference. For example, while Chapter 30 by M. T. Parker provides an excellent description of *Staphylococcus* and *Micrococcus*, a reader who wished to find out

which organism(s) produced coagulase could only do so by reference to the contents lists at the beginning of each and every chapter. Admittedly, this is pointed out at the beginning of the index: 'most subjects that would normally be entered in the index are included in the contents list at the beginning of each chapter. . .'. In my view this is an error of judgement since encyclopaedic works such as these would frequently be consulted to elucidate matters with which the reader is not already conversant, but to have to refer to the contents lists of up to 105 chapters would prove daunting to most readers. An example of the difficulties which can arise in another way is shown by the statement on page 446 in Chapter 42 referring to the biochemical reactions of *Clostridium* that 'Willis and Hobbs (1959) recommended the use of a glucose gelatin medum . . . in addition to Nagler and fermentation reactions'. The Nagler reaction had first been referred to fleetingly on page 433. A reader unfamiliar with the Nagler reaction and eager for enlightenment will search in vain in that section of the chapter or indeed anywhere *earlier* in the chapter. It does *not* appear in Contents list *or* Index, and we must remain mystified until it is obliquely described on page 451.

These examples and many others too numerous to list here underline the fact that the books are primarily useful for readers seeking essay monographs on the topics defined by the chapter headings. Although Volume 4 is not available to the reviewer, it is stated to contain a 'cumulative general index for Volumes 1–4' which suggests that it is merely a conflated version of the four separate indices. If this be the case, perhaps the editors and publishers could consider the production of a slim volume comprising a fully comprehensive index to Volumes 1–4.

W. F. Harrigan

Measurements in the Rheology of Foodstuffs. By J. H. Prentice.
London: Elsevier Applied Science, 1984. Pp. vii + 191. ISBN 0 85334 248 2. £24.00.

The thirteen chapters of the monograph are preceded by a short introduction in which the author indicates that the book is aimed at the graduate. As a consequence, he points out that it should be regarded as an introduction to, rather than a detailed investigation into, the broad area of food rheology. The following chapters then briefly discuss various aspects of the deformation theory of both solids and liquids from first principles. This is considered essential to an understanding of the overall rheological behaviour which, in turn, should be explainable in terms of its basic structure.

The first chapter on basics introduces the well known models of Maxwell and Kelvin which are discussed at some length before moving onto fluid flow. Up to this point a self evident approach has been used which becomes somewhat confusing and not really acceptable in the more mathematical aspects. Additionally, the occasional reference to diagrams which are considered representative of a range of different effects is undesirable as it merely adds to the confusion. Though there is a preface to the symbols used in the text, it is not complete. The typescript for 'n' and η (eta) are strikingly similar. The chapter on fluids refers to a multicomponent system with the dispersing phase as the 'substrate' and uses the Einstein and more general Brinkman equations to account for the viscosity behaviour. This is a hydrodynamic approach discussing both dilute and concentrated dispersions and their Newtonian behaviour. The development of a more ordered structure in dispersions, namely that of a network, is considered separately in another chapter, but principally for rigid particles.

In any practical situation, experimental data are used to support empirical models such as the familiar power law. The expanded discussion of such models provides the reader with a good basis on which to judge other more sophisticated models.

The chapter on the measurement of rheological properties is by far the longest and concentrates on the structural nature of the material and its identification. The importance of dimension in measuring flow when yield exists as well as inertial effects are discussed. Also considered is the interaction between the flow and the configuration of different types of measuring instrument as well as forced oscillation experiments. The limitations of the measurement naturally lead to the discussion of the reliability of the measurement, the main point to be made being in the role played by the operator. A short chapter on classification of materials merely serves to introduce a more complete appraisal of measurements on some fluids and their interpretation; dairy products and fruit juice are singled out for particular attention with chocolate used to describe the significance of yield values in a product. Plastic fats which possess a structure that readily breaks down have a chapter to themselves with an extensive description of cheese. Some large molecules such as xanthan, guar and aqueous gel phases are covered, though somewhat superficially, since the advances which have been made recently in this area of polysaccharides have been considerable.

The book has achieved a broad and more unified view of food rheology with repeatability and reproducibility being considered more important than absolute accuracy. No references are provided though there is an appendix of further reading which is not exhaustive. A subject index is also provided. The monograph is well presented and generally easy to read with an approach that concentrates on the principles of measurement rather than the food itself.

G. J. Brownsey

Extracellular Enzymes. By Fergus G. Priest. (Aspects of Microbiology, Volume 9). Wokingham: van Nostrand Reinhold, 1984. Pp. viii+79. ISBN 0 442 30588 5. £4.95 (paperback).

Dr Priest has provided a fine addition to the 'Aspects of Microbiology' series and has succeeded in his aim of giving a clear account for advanced students and those working in microbiologically based industries. The book is well written and I found very few typographical errors. Each chapter has a summary and an apparently carefully chosen list of references; the book is summarized, and there is a satisfactory index and a brief glossary.

Extracellular enzymes are of considerable interest commercially but most knowledge of enzyme synthesis and regulation comes from studies of intracellular enzymes. Thus, where specific information on the extracellular enzymes is lacking Dr Priest has drawn on a detailed knowledge of general microbial biochemistry and genetics so that one has a useful summary of protein synthesis and its control.

The essential feature of extracellular enzymes is that they should be passed through a cell membrane. This does not mean that they are always released from the cell; lysosomal enzymes and periplasmic enzymes are also extracellular enzymes. The mechanisms of secretion of extracellular enzymes—that apparent mystery of how protein molecules (and only the 'right' molecules) can get through a membrane when

small molecules cannot—is clearly presented, but in considerable depth so that, for example, one understands the implications of gene fusion studies for future commercial production of intracellular as well as extracellular proteins. The signal hypothesis for export of proteins and experimental verification of it are discussed. It is now clear that a signal 'trailer' portion of polypeptide has to latch on to the membrane so that further translation occurs through the membrane, the 'signal' portion being removed once the whole polypeptide is outside. In some cases passage occurs after translation. Mechanisms of regulation of enzyme synthesis, which provide an important target for manipulation to increase yields, are explained. There are problems for regulation of enzyme synthesis when the substrate is insoluble and outside the cell. The more applied aspects of extracellular enzymes are then considered, mechanisms for selection and improvement of producer strains; increased yields following gene transfer and genetic engineering; and commercial production, extraction, and purification of enzymes.

The book concentrates on extracellular enzymes of proven or likely commercial interest, though the title suggests a broader coverage which might have included consideration of the array of extracellular enzymes produced by *Clostridium perfringens*; the coagulase of *Staphylococcus aureus*; the role of extracellular enzymes in plant pathogenicity and spoilage of harvested vegetables; biodeterioration of building materials; anaerobic digestion of cellulose—in the rumen, in sludge digesters and in landfill. The potential value of greater study of 'unusual' organisms such as myxobacteria and anaerobic rumen fungi might have been emphasized.

The 'Aspects of Microbiology' series is an excellent collection of monographs for which the authors, editors and publishers deserve congratulation. The books are well presented and diagrams are clear and helpful. Type is clear and small (but not too small) so that one gets twice as many words per page (*circa* 600) as in some monographs. I was impressed by the international nature of this book. It was written by an Englishman, working in Scotland, about a subject commercially developed by a Japanese living in America. It was photoset in England, printed and bound in Hong Kong, and published in England by a publisher originating in Holland. Dr Priest's book provides a stimulating survey of his subject *and* an example of international collaboration!

R. W. A. Park

Methods in Food and Dairy Microbiology. By L. R. diLiello.
Westport, Conn.: AVI, 1982. Pp. ix+142. ISBN 0 87055 411 5. US$18.00

This book is intended to be a laboratory manual for technicians employed in the field of quality control and for students studying food microbiology and is written principally for the US market. There are thirty-two chapters which cover a large spectrum of methods/techniques including (i) laboratory materials and equipment, culture media, reagents etc.; (ii) counting methods, laboratory procedures for specific organisms and groups of organisms (including some procedures for foodborne pathogens); (iii) procedures for specific foods, examination of equipment surfaces and recommended sanitary standards and microbial criteria for various products.

The book is well presented with only a few typographical errors noted, but as it is only 142 pages long it will be appreciated that it is written in a concise manner and lacks

much of the experimental detail that would be expected of an instruction manual of this kind. Some chapters are very brief and merely outline experimental procedures e.g. 'Detection Methods for Pathogenic Staphylococci' while another, 'Preparation and Maintenance of Starter Cultures', does not attempt to describe either of the practices stated in that heading.

The culture media, incubation temperatures and experimental procedures recommended for various groups of bacteria or specific organisms, such as *Salmonella, Vibrio parahaemolyticus* and *Staphylococcus aureus,* are often quite different from those favoured in the UK and include use of phosphate buffer as diluent throughout; use of 32°C for presumptive coliform test; absence of pre-enrichment of frozen, dried or heated materials before selective enrichment for various bacteria. There are some basic errors such as reference to 'accuracy' of plate count while meaning 'precision'; implying that psychrotrophs and psychrophiles are similar; stating that coliforms are reliable indicators of faecal pollution of food, milk and dairy products; suggesting a final dilution of 10^{-1} for plating chicken skin swab-rinses and 10^{-2} for fresh and cured meats.

Notable omissions from the text include examination methods for *Clostridium perfringens, Bacillus cereus,* faecal coliforms and *Escherichia coli,* and a plating method for faecal streptococci.

Although it could be argued that many of the examples quoted merely represent differences of opinion, many of the procedures/media etc. described in the book differ in detail not only from those generally used in the UK, but also in many respects from those recommended by the FDA (BAM), APHA and AOAC. The book would, therefore, only add to an already confused state which has developed through proliferation of alternatives, particularly in respect of isolation procedures for foodborne pathogens.

In conclusion, in view of its many deficiencies the book is not considered a suitable laboratory manual for use in the food industry or for food microbiology courses.

A. J. Reynolds

Selection of Technology for Food Processing in Developing Countries. By Domien H. Bruinsme, Wouter W. Witsenburg and Willem Wurdemann. Wageningen: Pudoc, 1983. Pp. xi+199, ISBN 90 220 0873 1. D fl. 15.00.

This book reviews the factors that have to be considered when selecting the most appropriate technology for food processing operations in developing countries. Large automated plants producing high-priced commodities do not necessarily offer the best solution, both for the local community and the country in general, in areas where there may be an intermittent supply of raw materials, transportation and energy problems, low labour costs, and high unemployment.

Development theories, including the modernistic approach ('big is beautiful') and alternative approaches such as 'basic needs', 'appropriate technology' and 'target group approaches' are discussed. In addition, nutritional aspects, economics, social science, food science and food processing engineering all have important contributions to make. Short chapters are devoted to each of these subjects, with each offering a brief synopsis of the important factors, together with a list of further reading. For example, the 'Food process engineering' chapter reviews the type of food to be processed and the unit

operations used; the material is covered in five pages and 13 references are cited. The 'Food science' Chapter covers food spoilage by microorganisms, water activity and food quality (six pages). Therefore it is not a food science or technology text book. Many of the points made are further illustrated with three detailed examples, namely the processing of sugarcane, cassava and maize.

The book is well planned, informative and very readable. There is no index and different type faces are used, which slightly detracts from its appearance. It would be recommended reading for anybody contemplating teaching or working overseas, or being involved with food processing operations in developing countries. The authors obviously have a wealth of experience in the subject area, with examples being drawn from both personal experience and a wide variety of specialized literature.

M.J. Lewis

Developments in Dairy Chemistry—Volume 2, Lipids. Ed. by P. F. Fox London: Applied Science, 1983. Pp. x+430. ISBN 0 85334 224 5. £46.00.

This second volume on 'Lipids' in the series of 'Developments in dairy chemistry' maintains the high standard of the first volume. The eight chapters are contributed by fourteen authors, each recognized as an authority on his subject, from Australia, Denmark, The Netherlands, New Zealand, the UK and USA. The chapters deal with the composition and structure of milk lipids by W. W. Christie; the influence of nutritional factors by J. C. Hawke and W. M. Taylor; the fat globule by T. W. Keenan, D. P. Dylewski, T. A. Woodford and R. H. Ford; physical chemistry of the globule by P. Walstra; physical properties and modification of milk fat by B. K. Mortensen; lipolytic enzymes by H. D. Deeth and C. H. FitzGerald; lipid oxidation by T. Richardson and M. Korycka-Dahl; and nutritional signifiance of lipids by M. I. Gurr. The stated objective is to provide an authoritative reference source for lecturers, researchers and advanced students. Although industrial food scientists are not mentioned, the book can also be regarded as a 'Bible' for them in this field, particularly chapters 6, 7 and 8.

Chapter 1 is concerned with the composition of lipids in the milks of various mammals and the fatty acid composition of lipids in general. In Chapter 2 the authors discuss the blood and the mammary gland as the sources of milk lipids and how nutrition of the animal and biochemical reactions in the rumen can influence the composition of milk fat. Contemporary interest in this subject is centred on the 'fat nature-heart disease' hypothesis and on the everyday problem of the spreadibility of chilled butter. Chapter 3 deals with the long studied and technologically important subject of the milk fat globule membrane. This is the 'seat' of enzymic and other reactions of great importance to the behaviour of milk, and it is unfortunate that its composition varies with the method of insolation. This is not a unique phenomenon in biochemistry. Table 1 summarizes the complexity of the subject and Table 3 emphasizes that the membrane is a miniature biochemical factory. Chapter 4 gives some clues to the behaviour of homogenized milk, cream and butter. The complex and sometimes unpredictable behaviour of cream and butter are discussed in Chapter 5 which deals with the rheology of these products in relation to the proportions of liquid, solid and crystalline fats. It is often difficult to decide when the full flavour of a dairy product becomes a rancid taint. The two main sources of lipases in milk are described in Chapter 6 and the importance

of microbial lipases in refrigerated milk and their thermostability and technological significance discussed. The various factors influencing the oxidation of lipids in dairy products are detailed in Chapter 7, the end result being the balance between the pro- and anti-oxidant factors. This double chapter is the longest in the book, a significant comment on the numerous mechanisms involved. The recent medical study of, and publicity about, the importance of different types of fat in the diet make Chapter 8 of special interest to everyone, especially as western populations obtain about one third of their dietary fat from dairy products. Some extremists urge the consumption of skimmed or low fat milk, oblivious of the fact that if fat is removed from milk it has to be consumed in other forms. Milk fat not only contributes fat soluble vitamins to our diet but is responsible for most of the flavour of all dairy products.

It would be difficult to find any aspect of milk lipids not at least referred to in this book. Even such esoteric subjects as 'pigeon's milk', Refsum's disease and mink milk are mentioned. The numerous references are as up to date as practicable, varying in number from 116 to 454 per chapter. References are numbered except in Chapter 7 which employs the Harvard system thus lending a touch of individuality to the book. Like Volume 1, this book should be in the library of every organization which has a serious interest in dairy chemistry.

J. G. Davis

International Journal of Food Microbiology

An official journal of the International Union of Microbiological Societies (IUMS) and the International Committee on Food Microbiology and Hygiene (ICFMH)

Aims and Scope

International Journal of Food Microbiology publishes full-length original research papers, short communications, review articles and book reviews covering all aspects of microbiological safety, quality and acceptability of foods. Contributions dealing with the following fields are invited: bacteriology, immunology, mycology, parasitology, virology and food fermentation.

The editor will place emphasis on papers dealing with: intrinsic and extrinsic parameters of foods affecting microbial survival and growth, methods for microbiological and immunological examinations of foods, indices of the sanitary quality of foods, incidence and types of food microorganisms, food spoilage, microbiological aspects of food preservation, microbial interaction, and food-borne diseases of microbial origin. Achievements in rapid methods and automation in food microbiology are also included.

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gram(s)	g	second(s)	sec
kilogram(s)	kg	cubic millimetre(s)	mm^3
milligram(s)		millimetre(s)	mm
(10^{-3} g)	mg	centimetre(s)	cm
microgram(s)		litre(s)	l
(10^{-6} g)	µg	millilitre(s)	ml
nanogram(s)		pound(s)	lb
(10^{-9} g)	ng	gallon(s)	gal
hour(s)	hr	milliequivalent	mEq
minute(s)	min	R_F values	R_F

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