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Factors influencing the determination of nisin in meat products

R. G. BELL* AND K. M. DE LACY

Summary

Recovery of nisin from minced meat and meat emulsions was poor and variable. The rate of recovery was little affected by the presence of NaCl and/or $NaNO_2$, particle size, or the meat to extractant (0.02N HCl) ratio, but was significantly affected by the fat content of the meat. For nisin added at 100 iu/g, recoveries at the optimal meat to extractant ratio, 10% wt/wt suspension, ranged from 26% at 3% fat to 76% at 83% fat. At addition levels of 200 iu/g and greater, nisin recovery efficiency was reduced.

Introduction

A reliable analytical procedure is essential if regulations pertaining to the use of any food preservative are to be effectively enforced. The nisin content of foods is determined from acid extracts by a plate diffusion bioassay procedure with *Micrococcus luteus* NCIB 8166 (*M. flavus*) as the test organism (Tramer & Fowler, 1964). Nisin is generally recovered with high efficiency from 20% w/v suspensions of most foods in 0.02N HCl. However, nisin recoveries from meats and meat products are consistently low. This poor recovery is believed to be caused by the strong adsorption of nisin onto meat proteins (Tramer & Fowler, 1964; Fowler, 1981). Although adsorption decreases with falling pH (Ramseier, 1960), meat must still be boiled at an acid pH to liberate the protein-bound nisin. Also, nisin is unstable, resulting in losses during processing and storage (Hurst, 1981). These losses complicate the interpretation of bioassay results and could well explain the observed variability in nisin recovery between products.

Meat and meat products are heterogeneous in composition. For example, salt content, particle size and fat content can vary widely between different meat products. Such heterogeneity could influence the efficiency of the extraction process, as could changes in the meat to extractant ratio. The bioassay procedure, however, has a high degree of precision and can be excluded as a source of variation in nisin determinations. Therefore, under conditions precluding nisin inactivation, product composition and form, and extraction efficiency, would be the principal variables influencing a nisin determination. The present study was undertaken to determine if the meat to extractant ratio, product composition, particle size or nisin concentration influence the recovery of nisin from meat.

Materials and methods

Test organism

Stock cultures of *Micrococcus luteus* NCIB 8166 were grown on Assay Medium slopes at 30°C for 48 hr and then stored at 4°C for up to 1 month. Fresh stock cultures

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were prepared at monthly intervals after the purity of the culture had been checked. Each assay cell suspension was prepared by washing the growth from an Assay Medium plate that had been incubated for 48 hr at 30°C. The growth was washed off with 10 ml of sterile, quarter-strength Ringer's solution. Immediately before use, this cell suspension was diluted in quarter-strength Ringer's solution to give a suspension with an optical density at 650 nm corresponding to approximately 6.0×10^8 cells/ml (Anon, 1974).

Medium

For stock culture maintenance, cell production and bioassay, the Assay Medium developed by Varsanye was used. The medium contained peptone, 10 g; beef extract, 3 g; sodium chloride, 3 g; yeast extract, 1.5 g; unrefined sugar, 1 g; agar, 10 g and distilled water to 1000 ml (Tramer & Fowler, 1964). The medium was heated to dissolve the agar, its pH was adjusted to 7.5 ± 0.1 and then it was sterilized at 121°C for 20 min.

Nisin

Nisaplin (Aplin & Barrett, Trowbridge, England), a standardized nisin concentrate containing 10^6 international units per gram (Mega unit nisin), was used throughout this study. A standard stock solution containing 10^3 iu/ml was prepared by boiling 100 mg of Nisaplin in 60 ml of 0.02N HCl, cooling the solution, and then making its volume up to 100 ml. This stock solution was used to prepare both the assay standards and meat samples containing known amounts of nisin.

Assay procedure

Nisin was extracted by mixing meat samples with 0.02N HCl to make a 10% meat weight to suspension weight (10% w/ws) suspension, which was held at 96°C for 10 min, cooled and then centrifuged to remove particulate matter. The clarified acid extract, which was covered by a layer of solidified fat, was removed with a Pasteur pipette. The nisin content of the extracts was determined by the plate diffusion bioassay procedure of Tramer & Fowler (1964). In this large plate assay, where all samples and standards are represented in quadruplicate, the nisin concentration is calculated from the mean inhibition zone produced by each extract by reference to a five-level standard curve.

Fat analysis

The fat content of meat samples was determined as ether-extractable fat (AOAC, 1984).

Influence of particle size and curing salts

Sodium chloride and sodium nitrite either separately or in combination were added to minced beef at concentrations of 2.5% and 200 ppm, respectively. Each meat-salts mixture and an unamended mince were mixed thoroughly, divided in half, and one half was emulsified in a Waring blender. A 10-g sample of each minced and emulsified mixture was placed in a boiling tube and 1 ml of the nisin standard solution (1000 iu/ml) added to give a concentration of 100 iu/g meat. Nisin-meat mixtures were allowed to interact at 10°C for 1 hr before being extracted as a 10% w/ws suspension in 0.02N HCl for determination of the nisin content. A low temperature, short duration interaction period was used so that chemical losses of nisin, and losses associated with the enzyme nisinase, would be insignificant.

Influence of nisin concentration

Seven 10 g samples of well-mixed minced beef were placed in boiling tubes. Standard nisin solution was added to give final nisin concentrations of 10, 20, 40, 60, 80, 100 and 200 iu/g meat. After 1 hr interaction at 10°C, each nisin-meat mixture was extracted as a 20% w/ws suspension in 0.02N HCl and its nisin content determined.

Influence of meat to extractant ratio

One hundred iu of nisin was added to each of seven boiling tubes containing 0.5, 1.0, 2.0, 4.0, 6.0, 8.0 and 10.0 g of well mixed minced beef. After 1 hr interaction at 10°C, each nisin-meat mixture was extracted in 40 ml of 0.02N HCl and its nisin content determined.

Influence of fat content

Seven 50 g meat emulsions with visual fat contents ranging from 0 to 100% were prepared from visually lean beef steak and beef fat. To aid emulsification, sodium chloride at 2.5% was added to the lean/fat mixtures before they were emulsified in a Waring blender. A 10 g sample of each mixture was placed in a boiling tube and 1000 or 5000 iu of nisin added. After 1 hr interaction at 10°C, each nisin-meat emulsion was extracted as a 10% w/ws suspension in 0.02N HCl and its nisin content determined. The unused portion of each emulsion was retained for subsequent analysis to determine its ether-extractable fat content.

Results

Particle size and curing salts

The determined nisin content was little affected by either emulsification of the meat or by the presence of curing salts (Table 1). The recovery rate, approximately 40%, was slightly enhanced in samples containing 2.5% NaCl and depressed in those containing 200 ppm $NaNO_2$. An interaction of the opposing effects of NaCl and $NaNO_2$ on nisin determination is evident in samples containing both NaCl and $NaNO_2$.

Table 1. Effect of particle size and curing salts on the determination of nisin added to meat at 100 iu/g

NaCl (%)	$NaNO_2$ (ppm)	Nisin (iu/g meat)	
		Minced	Emulsified
0	0	42.5	41.0
2.5	0	44.5	44.5
0	200	41.0	40.0
2.5	200	43.0	42.5

Nisin concentration

Over the range 10–100 iu/g meat, the determined nisin concentration was a constant proportion, about 40%, of that added. However, at the highest added concentration, 200 iu/g, the proportion recovered fell to 32.5% (Fig. 1). This fall in nisin recovery cannot be attributed to extrapolation beyond the linear limits of the standard curve, as the assayed nisin concentrations of extracts fell within the 1.25–20 iu/ml range of the five level standard curve.

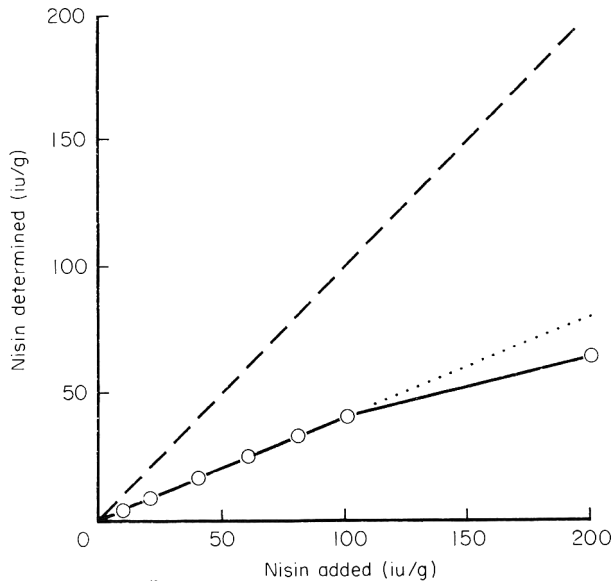

Figure 1. Influence of nisin concentration on the efficiency of the determination of nisin in minced beef. Determined nisin concentration (O) and added concentration (- - - -).

Meat to extractant ratio

The proportion of added nisin detected was highest, 47%, with a meat to extractant ratio of 9.1% w/ws (Table 2). However, over the entire range of meat to extractant ratios examined, the recovery rates differed by only 4%.

Table 2. Effect of extractant to meat ratio on the recovery of nisin from minced beef

Meat : Extractant ratio* % (w/ws)	Nisin (iu/g) Added	Recovered	Recovery rate (%)
1.2	200.0	90.9	45.5
2.4	100.0	44.4	44.4
4.8	50.0	21.9	43.8
9.1	25.0	11.8	47.2
13.7	16.7	7.8	46.7
16.7	12.5	5.4	43.2
20.0	10.0	4.5	45.0

*Weight to weight of suspension.

Fat content

Nisin recovery was directly related to the ether-extractable fat content of the meat samples (Fig. 2). At higher fat contents, recovery efficiency was lower in those samples with nisin added at a concentration of 500 iu/g than in those containing only 100 iu/g.

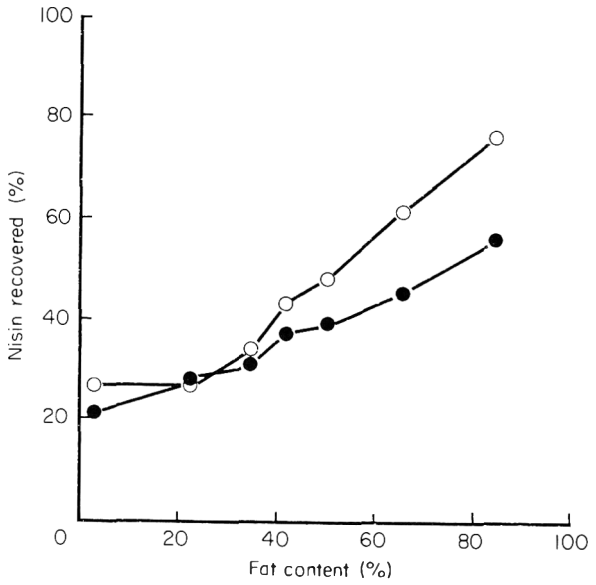

Figure 2. Influence of the ether-extractable fat content of meat emulsions on the efficiency of nisin determination. Added nisin concentrations 100 iu/g (O) and 500 iu/g (●).

Discussion

Nisin concentration as determined from acid extracts is a function of the nisin concentration in the product and the extraction efficiency. At the pH of meat, approximately 5.5, nisin inactivation during a 1 hr interaction at 10°C is not likely to be significant (Tramer, 1964; Hall, 1966). In the present study, therefore, the concentration of nisin at the time of acid extraction would be the same as the added nisin concentration. Thus, the determined nisin concentration represents that fraction of the added nisin that was extracted rather than that portion of nisin remaining after inactivation.

When nisin is present at concentrations that do not exceed the adsorption capacity of the meat surface, the proportion of adsorbed nisin that is extracted will be determined by a partition equilibrium. Nisin recovery was similar from minced and emulsified meat samples containing 100 iu/g, suggesting that the adsorption capacity of neither was exceeded at that concentration. Salt may interfere with nisin adsorption (Bell & De Lacy, 1985), explaining the increased recovery observed in meats containing 2.5% NaCl.

When the adsorption capacity is exceeded, all adsorption sites are occupied; therefore, part of the added nisin would then be associated with the meat surface in a form other than as a monomolecular layer. At the point of saturation the partition equilibrium, and hence the proportion of nisin that can be extracted, is likely to change. The change in recovery rate observed between 100 and 200 iu/g probably reflects such a change in the partition equilibrium. The fact that extraction efficiency fell is perhaps surprising, as the excess nisin molecules might be expected to be less firmly associated with the meat surface than those directly adsorbed. The decreased extraction efficiency

may have been due to excess nisin molecules polymerizing (Gross, 1977) with those already bound to the meat surface. In the high fat emulsions, nisin recovery efficiency was also reduced at the higher nisin application level, which could be another expression of 'super adsorption' to adsorption sites.

The proportion of nisin recovered was virtually independent of the meat to extractant ratio used. Therefore, when low nisin concentrations are anticipated, the extractant volume can be decreased, to ensure a sufficient concentration of nisin in the extract for bioassay without appreciably reducing the accuracy of the determination. Nisin recovery was maximal with a meat to extractant ratio of 9.10% w/ws (4 g in 40 ml), supporting the manufacturer's general recommendation that a 10% w/ws suspension (10 g in 90 ml) be used for nisin determinations in meat products.

The direct relationship between the proportion of added nisin recovered and the fat content of meat emulsions appears to contradict the observed reduction in nisin recovery with saturation of adsorption sites. The increase in nisin recovery may, however, result from fat physically blocking the adsorption sites. An interaction between the opposing effects associated with adsorption site saturation and blocking would account for the smaller increase in nisin recovery associated with the higher nisin application.

The inefficiency and variability of nisin recovery, regardless of their cause, cannot be compensated for by the precision of the bioassay procedure. Although the determined nisin concentration may be analytically suspect for meat products, it may have significance if it represents the unbound active nisin present in a product. However, if antimicrobial activity persists when nisin is no longer detectable (Boone, 1966), the bioassay of nisin in meat products has limited utility.

Conclusions

The nisin content of meat and meat products cannot be reliably determined by bioassay because of inefficient and variable extraction of the antibiotic. Therefore, regulations pertaining to the use of nisin in meat and meat products must be promulgated in terms of permitted addition levels rather than determining residuals.

Acknowledgments

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Effect of frozen storage on protein denaturation in bovine muscle 1. Myofibrillar ATPase activity and differential scanning calorimetric studies

J. R. WAGNER AND M. C. AÑON

Summary

The effect of frozen storage on myofibrillar ATPase activity and thermal transitions in bovine muscle was investigated. Myofibrillar ATPase activity and total enthalpy of denaturation (ΔH) decreased with time of storage. The rate of decrease was lower at $-20°C$ than at $-5°C$ or $-10°C$. Differences in behaviour during storage of muscle after fast or slow freezing could be attributed to differences in ice recrystallization. The observed decreases in area of the first peak seen in the thermograms and Ca^{2+}-myofibrillar ATPase activity show that the myosin head denaturated progressively during storage. The myosin tail also denaturated during storage but the thin filament remained unaltered. Kinetic analysis suggested that the denaturation of the myofibrillar proteins took place through two consecutive first order reactions; an initial rapid reaction followed by a slower one.

Introduction

Frozen storage has been used to minimize the rate of biochemical reactions in muscle, and prevent microbial spoilage. It is currently accepted, however, that myofibrillar proteins are denaturated during the frozen storage of meat (Fennema, Powrie & Marth, 1973; Matsumoto, 1980a). Most of the publications deal with frozen fish muscle (Connell, 1968; Matsumoto, 1980b; Shenouda, 1980); few studies have been performed on muscle from other animal species. Ito, Sung & Fukazawa (1968) and Kang, Ito & Fukuzawa (1983), observed changes in the actin-myosin interaction during frozen storage of rabbit muscle, while Khan & Berg (1967) and Yamamoto, Samejima & Yasui (1977) found changes in both the structure and ATPase activity of myofibrillar proteins of chicken muscle during frozen storage.

Freezing and further frozen storage has also been shown to affect the structure of the myofibrillar proteins in bovine muscle (Awad, Powrie & Fennema, 1968; Rahelić, Pribis & Skenderović, 1974; Carroll, Cavanaugh & Rorer, 1981). However, changes in the biochemical properties of these myofibrillar proteins during frozen storage have not been clearly demonstrated.

The present paper reports the results of studies on the effects of frozen storage, at three different temperatures, on myofibrillar ATPase activity and the thermal transitions (as seen by differential scanning calorimetry) in bovine muscle after freezing at two different rates.

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Materials and methods

Meat sample preparation

Experiments were performed 48 hr post-mortem on semitendinosus muscle from steers. Meat slices (5 cm diameter and 1 cm thickness) were cut with their longitudinal axes parallel to the fibres and were frozen as described previously (Bevilacqua, Zaritzky & Calvelo, 1979; Wagner & Anón, 1985a). Freezing ceased when the meat slices reached −25°C. The local freezing rate of each slice was characterized by its freezing time, t_c (Bevilacqua *et al.*, 1979; Wagner & Añón, 1985a). High freezing rates correspond to a $t_c < 5$ min and low freezing rates to a $t_c > 60$ min. The meat slices were stored in polyethylene bags at −5 ± 1°C; −10 ± 1°C and −20 ± 1°C. When required, the samples were thawed at 4°C overnight.

Preparation of myofibrils

Myofibrils were prepared according to the procedure described by Goll & Robson (1967). The myofibrils were suspended in 0.15 M KCl-0.03M Tris-HCl solution (pH 7.6). Protein concentration was determined by the modified biuret method (Robson, Goll & Temple, 1968).

ATPase activity

Myofibrillar ATPase activity in the presence of Ca^{2+} and Mg^{2+} was determined as described by Hay, Currie & Wolfe (1973). Results were expressed as μmol P_i produced/min/mg protein.

Differential scanning calorimetry (DSC)

Small pieces of muscle (weight *circa* 30 mg) were cut and treated with modified Ringer's solution (0.15 M NaCl, 3 mM KCl and 3 mM $MgCl_2$) and stirred for 4 hr at 4°C. During this period the pH was adjusted at 5.6. Connective tissue was carefully removed with a scalpel (Stabursvik & Martens, 1980).

Accurately weighed samples (15–20 mg) of the treated bovine muscle were hermetically sealed in aluminium pans. As reference, a pan containing 15 μl distilled water was used. The samples were analyzed in a Du Pont Model 910 attached to a Hewlett Packard 7046 B recorder. After DSC analysis the capsules were punctured and the dry matter content determined by drying at 105°C overnight. Temperature calibration was performed using Indium and water and the cell constant E was determined using Indium.

The areas of the observed endotherms were measured with a Morphomat 34 Zeiss image analyzer and the corresponding enthalpies of denaturation were calculated. The areas per unit weight for each peak (partial area/total mg of dry sample) were also determined.

Results and discussion

Studies on ATPase activity

Figure 1 shows the variation of myofibrillar ATPase activity with time of frozen storage at −5°C, −10°C and −20°C for muscle frozen at both high and low rates. It can be seen that after fast freezing (Fig. 1a, c, e) in the presence of either Ca^{2+} or Mg^{2+} the levels of ATPase activity decrease rapidly during frozen storage, the decrease being greater at the higher storage temperatures. In contrast, in slowly frozen muscle most of

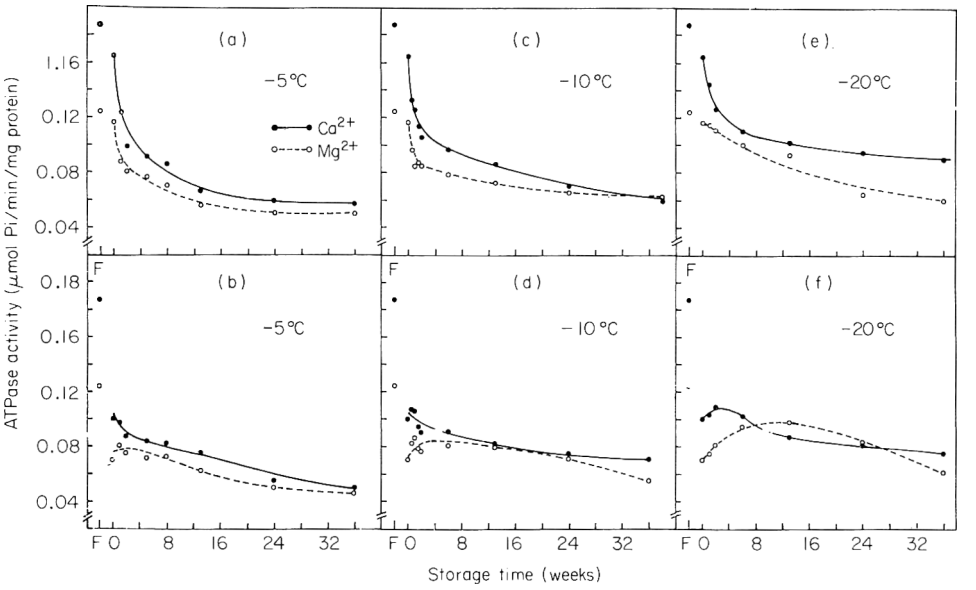

Figure 1. Mg^{2+} (O----O) and Ca^{2+} (●----●) ATPase activity of myofibrils prepared from fresh muscle and frozen muscle stored at −5, −10 and −20°C: (a), (c) and (e) muscle frozen at high freezing rate, (b), (d) and (f) muscle frozen at low freezing rate. F: value corresponding to fresh muscle. Each value is an average of at least four assays (maximum standard deviation: 0.014).

the loss of ATPase activity occurs during the freezing process itself (Wagner & Anón, 1985a). In the presence of Ca^{2+} myofibrillar ATPase activity in the slowly frozen samples decreases slowly during storage at −5°C or −10°C; but at −20°C there is an increase in activity during the first few weeks of storage, followed by a subsequent decrease. In the presence of Mg^{2+}, at all three tested storage temperatures, there is an initial increase of ATPase activity with time; in the slowly frozen samples at −20°C the increase continues until the twelfth week.

The decrease of myofibrillar ATPase activity in the presence of Ca^{2+} indicates an alteration in the active site of the enzyme located in the myosin head. In the presence of Mg^{2+} the increase or decrease of the myofibrillar ATPase activity is due to an increase or decrease in the strength, of the actin-myosin interaction (Ito *et al.*, 1978). Thus, changes in ATPase activity, in the presence of either Ca^{2+} or Mg^{2+}, supplies information about the changes produced in the myosin head (HMM–S1), the portion of the molecule containing both the active site of the enzyme and the site for interaction with actin (Ockerman, 1977).

Differences in behaviour during storage of muscle after fast or slow freezing could be ascribed to the differences in morphology and distribution of the ice crystals formed during freezing. While fast freezing ($t_c < 5$ min) results in the formation of small intracellular crystals, (besides the ice formed in the extracellular space), slow freezing ($t_c > 60$ min) leads to the formation of large extracellular crystals only (Bevilacqua *et al.*, 1979). Upon storage of the muscle at temperatures higher than that reached during freezing (−25°C) thawing of the ice formed and a process of recrystallization both take place. At −25°C the percentage of frozen water in muscle is approximately 90%, while at −5, −10 and −20°C the percentages are 74, 83 and 88%, respectively (Mascheroni & Calvelo, 1974); as a result, the percentages of ice thawed when the temperature changes

from −25 to −5, −10 and −20°C are 16, 7 and 2%, respectively. This, together with the effect of the storage temperature, would explain why storage at −20°C is less harmfull than at −5 and −10°C, but does not account for the differences observed between fast and slow freezing, as the percentage of ice in muscle is related to the temperature reached, and not to the rate of freezing. The differences can be attributed to ice recrystallization; after slow freezing of muscle recrystallization during storage would take place in the extracellular space only, while after fast freezing there is also some intracellular recrystallization (Bevilacqua & Zaritzky, 1982) leading to the formation of large crystals within the fibre, producing distortion. The different location of the recrystallized ice may be the reason for the greater deterioration observed during storage of the fast frozen samples.

Studies by differential scanning calorimetry (DSC)

Thermograms obtained on the rapidly or slowly frozen muscle stored at −5, −10 and −20°C showed differences in the structures of the myofibrillar proteins. Figure 2 shows the differences seen between thermograms of fresh muscle and of muscle stored at −5°C for 24 weeks.

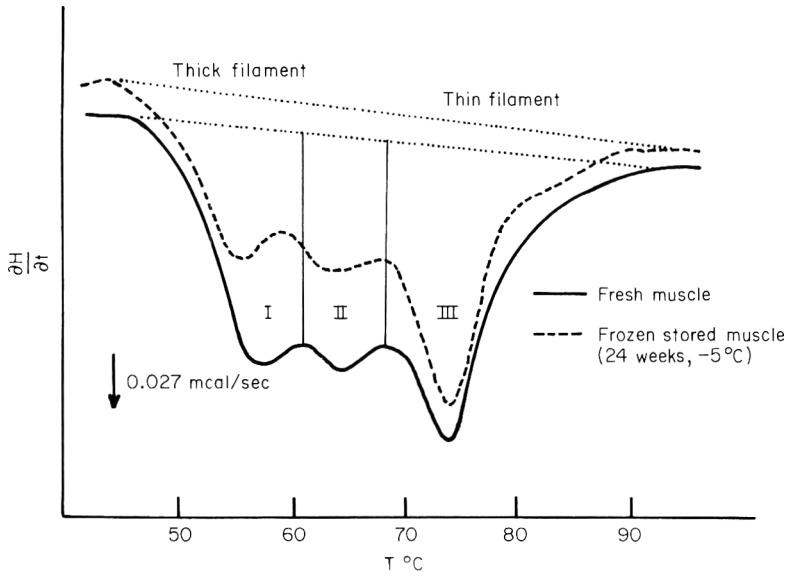

Figure 2. DSC thermograms of bovine muscle treated with Ringer's solution at pH 5.6. Heating rate β = 10°C/min. Dry matter weight from fresh muscle and frozen stored muscle are 3.7 and 4.8 respectively.

As described under Materials and Methods, the samples were depleted of sarcoplasmic proteins and connective tissue; thus the thermograms characterize the thermal transitions of the myofibrillar proteins. The areas and the T_{max} of peaks I and II corresponding to myosin (Wright, Leach & Wilding, 1977; Stabursvik & Martens, 1980) are smaller in the stored muscle, while peak III, corresponding to actin (Wright *et al.*, 1977; Stabursvik & Martens, 1980) remains unchanged. It should be pointed out that according to results obtained in our laboratory (Wagner & Añón, 1985b) peaks I and II correspond to the thick filament (myosin) and peak III to the thin filament

(actin+minor proteins). The total areas of the thermograms were divided into partial areas corresponding to each transition (Fig. 2), thus making possible the calculation of not only the total enthalpy of denaturation (ΔH) but also of the areas of peaks I, II and III. As the peaks associated with the different transitions overlap and their shapes are not known only crude estimates of the areas are possible. However, information can be obtained from these estimations.

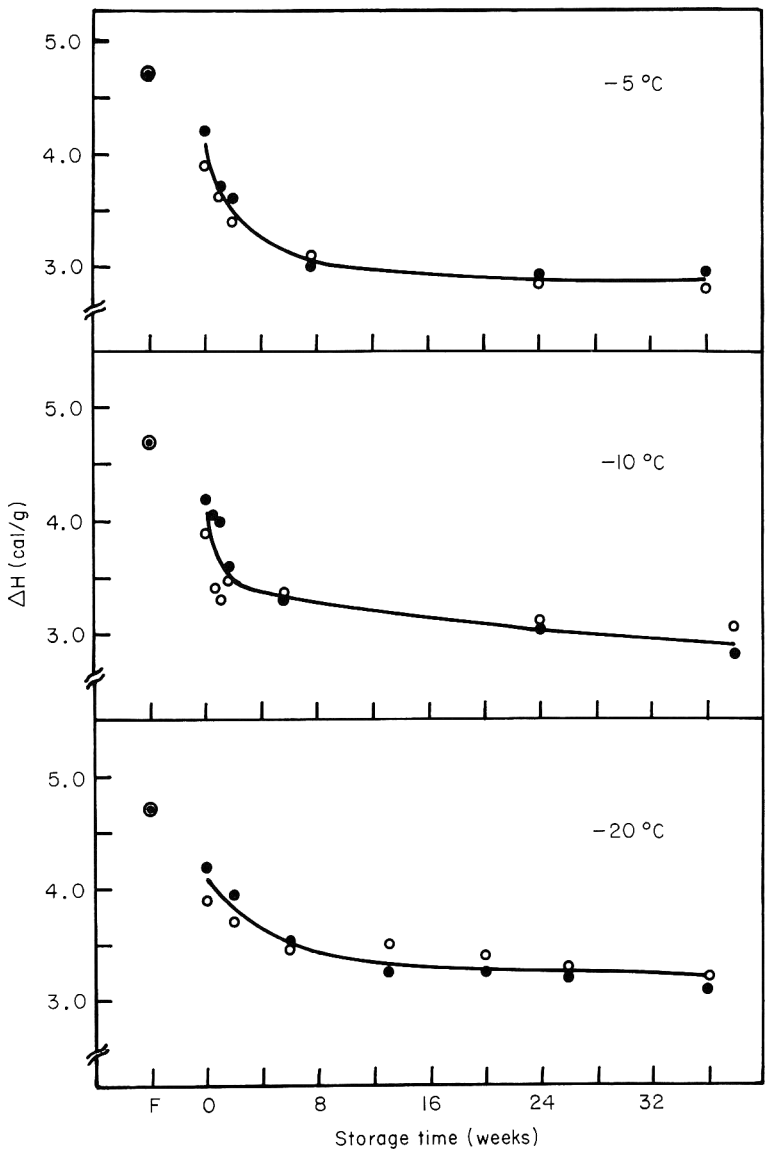

Figure 3. Effect of frozen storage on the enthalpy of thermal denaturation of myofibrillar proteins (ΔH). (●) muscle frozen at high freezing rate, (O) muscle frozen at low freezing rate. F: values corresponding to fresh muscle (◉). Frozen samples were stored at −5, −10 and −20°C. Each value is an average of at least four assays (maximum standard deviation: 0.2).

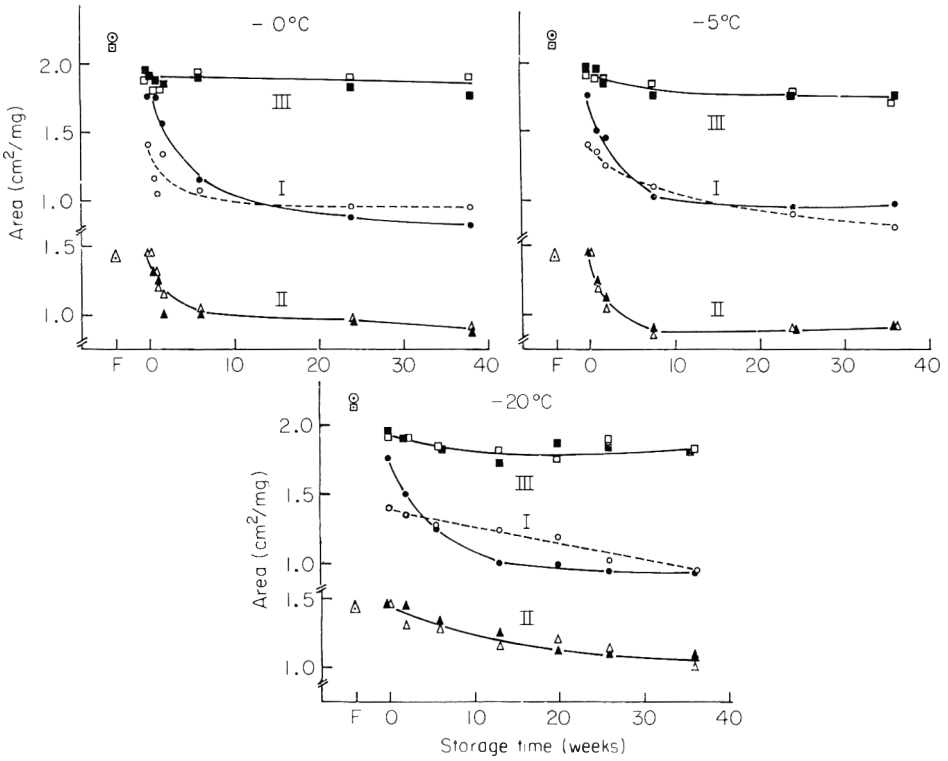

Figure 4. Effect of frozen storage on the areas of peaks I, II and III in the DSC endotherm of frozen bovine muscle. (●, ▲, ■) muscle frozen at high freezing rate. (O, △, □) muscle frozen at low freezing rate. F (⊙, ◬, ⊡) values corresponding to fresh muscle. Frozen samples were stored at −5, −10 and −20°C (maximum standard deviation: 0.2).

The values of ΔH decrease with time of storage (Fig. 3) and, as with ATPase activity, there is a denaturing effect of freezing itself (Wagner & Añón, 1985a). The rate of decrease of ΔH and, consequently protein denaturation, is lower at −20°C than at −5 or −10°C. The areas of peaks I and II also decrease as a consequence of frozen storage, while that of peak III remains practically unchanged (Fig. 4). It can also be observed that peak I is affected by freezing itself, as is the case with ATPase activity (Wagner & Añón, 1985a), while peak II diminishes only upon storage. As with ΔH, at −10°C the specific areas of peaks I and II diminish more slowly than at higher temperatures. The decrease of ΔH values indicates that during freezing and subsequent storage, myofibrillar proteins undergo a progressive denaturation involving bond breaking. The decrease in areas of peaks I and II indicate that these alterations occur mainly in the thick filament (myosin), while the thin filament (peak III) remains unaltered. Under the conditions of the present study (pH = 5.6; μ = 0.16) peak I is believed to be due to thermal denaturation of the myosin head (HMM−SI) and peak II to the denaturation of the myosin tail (LMM) (Wright & Wilding, 1984). Comparison of Figs 1 and 4 shows that the Ca^{2+}-ATPase activity and the area under peak I exhibit similar behaviour; this is to be expected since the active site of the enzyme is located in the myosin head (HMM-SI). Plots of ΔH *versus* Ca^{2+}- and Mg^{2+}-myofibrillar ATPase activities (Fig. 5)

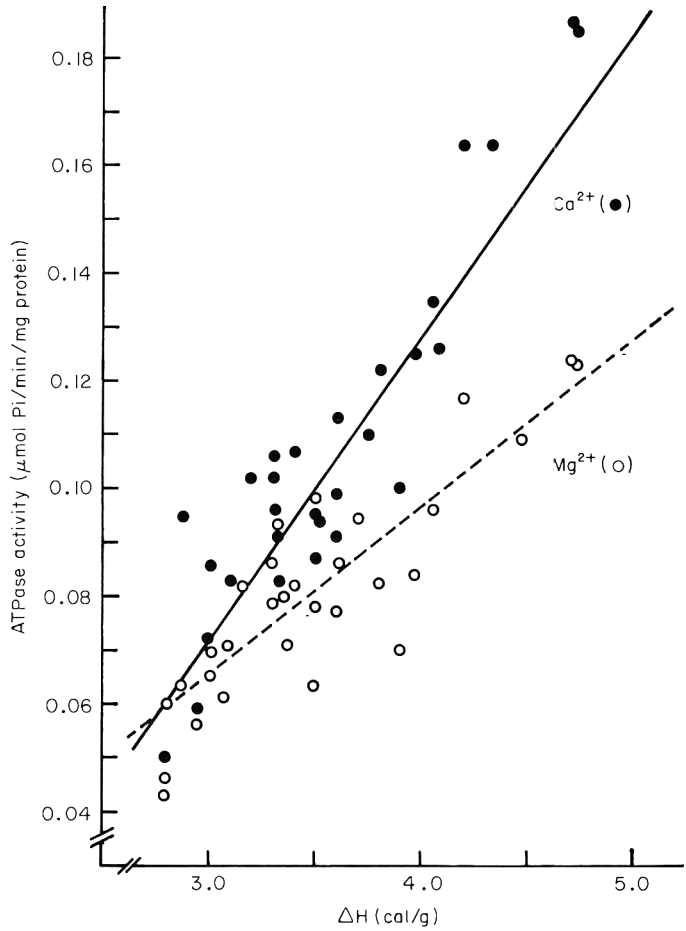

Figure 5. Relationship between myofibrillar ATPase activity in presence of Ca^{2+} and Mg^{2+} and the enthalpy of thermal denaturation of frozen bovine muscle stored at −5°C, −10°C and −20°C: with Ca^{2+}: $y = 0.056x - 0.094$ ($r = 0.86$), with Mg^{2+}: $y = 0.031x - 0.027$ ($r = 0.77$), where r: correlation coefficient.

and the area under peak I *versus* Ca^{2+}-myofibrillar ATPase activity (Fig. 6) were constructed. It is seen that linear relationship between the area under peak I and the Ca^{2+}-myofibrillar ATPase activity support the notion that peak I corresponds to denaturation of the myosin head.

Denaturation kinetics

To study the denaturation kinetics of myofibrillar proteins during frozen storage, myofibrillar ATPase activity in the presence of Ca^{2+} was utilized as a parameter correlating with denaturation of myosin. Assuming first order kinetics with respect to frozen storage time, the natural logarithm of the Ca^{2+}-myofibrillar ATPase activities (for both slowly frozen and rapidly frozen muscle) *versus* time of storage were plotted. It can be seen that the reaction does not proceed as a first order one, but as a reaction of a higher order ($n_t > 1$), most likely as two consecutive first order reactions. Slopes were

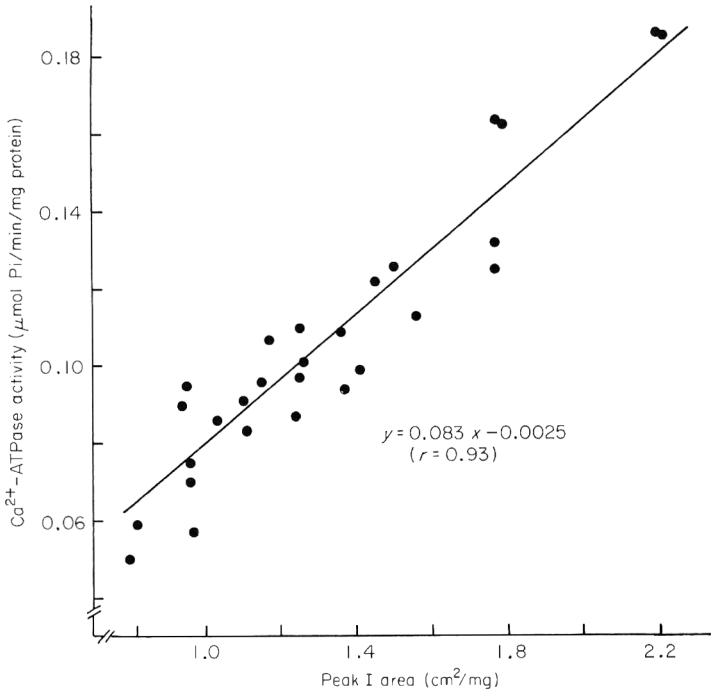

Figure 6. Relationship between myofibrillar ATPase activity in presence of Ca^{2+} and Mg^{2+} and the enthalpy of thermal denaturation of frozen bovine muscle stored at −5, −10 and −20°C. $y = 0.083\,x - 0.0025$ ($r = 0.93$).

calculated by least squares, thus obtaining the rate constants, k (in weeks) of the individual reactions. On this assumption, the process of denaturation takes place in at least two steps: an initial rapid step and a subsequent slower one. The values of the rate constants for each step (in weeks) are (fast freezing −20°C: $k_1 = 0.082$, $k_2 = 0.0076$; −10°C: $k_1' = 0.190$, $k_2' = 0.0157$; −5°C: $k_1'' = 0.245$, $k_2'' = 0.017$, and slow freezing −20°C: $k_1 = 0.0167$, $k_2 = 0.0056$; −10°C: $k_1' = 0.025$, $k_2' = 0.0076$; −5°C: $k_1'' = 0.06$, $k_2'' = 0.016$) as expected. These results show that at −20°C the denaturation reaction is slower than at −5°C and −10°C.

For the calculation of the energy of activation (E_a) corresponding to each step, ln k was plotted against T^{-1} (in K^{-1}) according to the Arrhenius equation; a straight line was obtained, from which the slope E_a was calculated.

The values found for E_a were 10.14 Kcal/mol and 7.74 Kcal/mol (fast freezing) and 10.7 Kcal/mol and 8.68 Kcal/mol (slow freezing) for the first and second step, respectively. These values of E_a are within the range for the energy of activation of recrystallization, E_a = 10.4 Kcal/mol (Bevilacqua & Zaritzky, 1982). Thus it seems possible that recrystallization is one of the causes of denaturation.

The results shown in the present report would indicate that the denaturation of myofibrillar proteins during frozen storage takes place through consecutive reactions. First during freezing (particularly in slow freezing) there is a marked decrease in myosin-actin affinity, which parallels denaturation of the myosin head. During storage

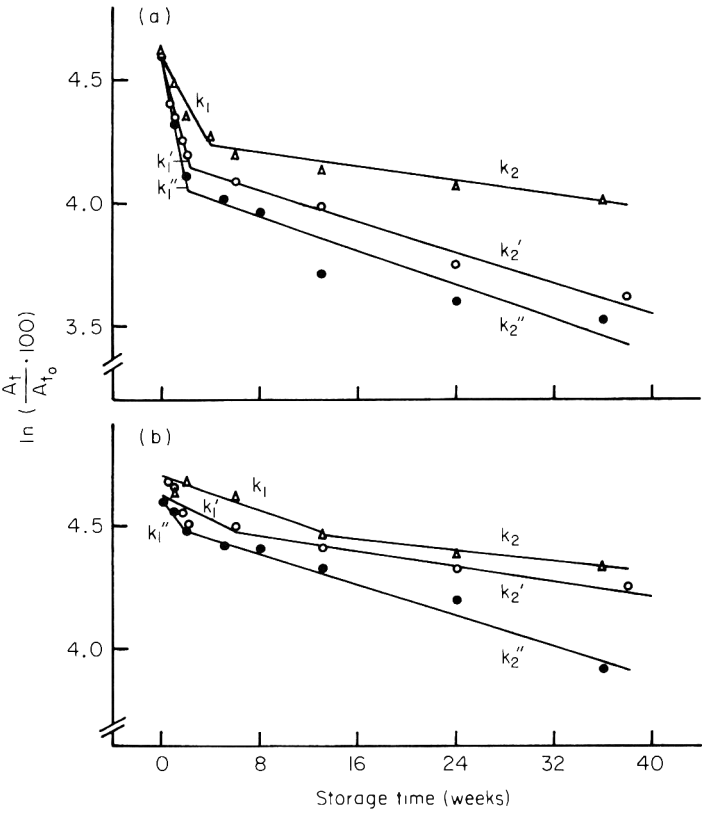

Figure 7. Estimation of the denaturation kinetics of myosin in bovine muscle stored at −5, −10 and −20°C. A_{t_0} and A_t are the myofibrillar Ca^{2+}0ATPase activity of bovine muscle frozen at high (a) and slow (b) freezing rate initially and during frozen storage at −5, −10 and −20°C, respectively. Maximum standard deviation: 0.15.

at least two further stages were detected in rapidly frozen samples; a first stage in which the ATPase activity in the presence of Ca^{2+} and Mg^{2+}, which started to diminish during freezing, keeps decreasing, in addition some denaturation of the myosin tail occurs.

During the second stage (a much slower one) the myosin molecule continues to denature; this parallels an aggregation of denatured proteins, leading to a decrease in solubility and viscosity (this will be dealt with in more detail in a further paper). For the stored samples subjected to slow freezing an increase of the actin-myosin affinity was detected during the first weeks storage especially at −20°C.

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Quantitative immunoassay for soya protein in raw and sterilized meat products

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Summary

An enzyme-linked immunosorbent assay (ELISA) is described for the quantitative analysis of soya protein in meat products. The assay is applicable to raw and sterilized products and is not dependent on soya variety and type of soya ingredient (concentrate, isolate, etc.). Therefore, the assay can be applied without knowledge of product composition, heat pretreatment and other processing conditions. The ELISA is specific for soya; no interference of other product components has been found. The detection limit of the ELISA is 0.5% soya protein. Qualitative analysis by immunoblotting is possible at much lower concentrations. The antibodies used are specific for sodium dodecyl sulphate (SDS) denatured soya protein subunits. The products are extracted with SDS and 2-mercaptoethanol, which guarantees optimum recovery of the protein components. Product extracts can be analysed directly by ELISA without removal of the SDS by using nitrocellulose as a solid phase.

Introduction

Addition of soya protein to meat products has been hampered by legal restrictions in a number of countries. One of the reasons is the lack of suitable methods to determine the soya protein content in consumer products. Preferably, such methods should require a minimum of knowledge of the product to be analysed. Soya protein from a number of different soya varieties is added in many different processing forms: concentrates, isolates, texturates, etc. During processing many meat products are subject to heating. Therefore, the ideal analytical method should be independent of soya variety, type of soya ingredient and heat pretreatment of the product.

Methods for the determination of soya protein in meat products have been reviewed by Olsman & Hitchcock (1980). They concluded that none of the methods was satisfactory when used separately. Two approaches emerged for quantitative determination of non-sterilized products; gel electrophoresis and immunochemical methods. For sterilized products no method had been developed to quantitate soya protein. Electrophoretic methods were improved culminating in the method of Armstrong, Richtert & Riemann (1982), which uses an efficient extraction procedure and an internal standard. Upon sterilization, however, the soya protein escapes detection. At present the enzyme-linked immunosorbent assay (ELISA) developed by Hitchcock *et al.* (1981), is one of the best approaches. In this ELISA, antibodies are used against soya protein which has been 'renatured' from hot urea solution by removing or diluting the denaturant. It is the first assay that can deal with sterilized products. However, quantitative responses with various commercial forms of soya products were not constant. Also for raw and sterilized products the analytical responses differed.

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Another approach to solve the sterilization problem has been reported by Menzel & Glatz (1981). Antibodies against formaldehyde treated soya protein were shown to react with native as well as heated soya protein (125°C). A solid phase radio-immunoassay using these antibodies has been described (Menzel & Hagemeister, 1982) and although the method is claimed to be suitable, among other things, for the determination of soya protein in meat products, no corroborative data are available.

We have now developed an ELISA procedure which can be used for sterilized meat products and which is based on the fact that antibodies can be raised against proteins that have been denatured by sodium dodecylsulphate (SDS). We will also show that heat-denatured and native soya protein are indistinguishable by serological means after SDS-treatment. The ELISA meets all the above described requirements. In addition it has a number of advantages, like low price and simplicity of protocol in common with other ELISA systems.

Materials and methods

Preparation and characterization of immuno-reagents

Glycinin subunits were isolated from soyabean variety 'Express Green' as described earlier (Iyengar & Ravestein, 1981), except for the carboxamidomethylation step, which was omitted. During isolation 0.01 M 2-mercaptoethanol (Fluka AG) was present in all buffers. Subunits were dialysed against water, lyophilized and stored at −20°C.

Antisera were obtained by repeated inoculations of subunits in rabbits. Subunits were dissolved at a concentration of 5 mg/ml in phosphate-buffered saline (PBS; 0.12 M NaCl, 0.003 M KH_2PO_4, 0.01 M $Na_2HPO_4.2\ H_2O$, pH 7.2, 0.02% (w/v) sodium ethylmercuri thiosalicylate (merthiolate from Eli Lilly & Co)) containing 0.75% (w/v) sodium dodecylsulphate (SDS from Serva). The solution was heated at 100°C for 5 min, diluted 1:1 with 0.9% (w/v) NaCl and subsequently emulsified with complete or incomplete Freund's adjuvant (Difco Laboratories). The immunization schedule was as follows: on day 1 and day 4 a total of 2.5 mg protein in complete Freund's adjuvant was injected intramuscularly at four sites. On day 32 again 2.5 mg protein in incomplete Freund's adjuvant was given. If necessary booster injections in incomplete Freund's adjuvant were given every 4 weeks. Part of the animals showed tolerance to soya, probably because soya was part of their diet. Serum was collected at 4 week intervals starting 5 weeks after day 32. Serum was preserved by the addition of 0.02% (w/v) sodium azide and stored at −20°C.

Gel electrophoresis was performed in an SDS-polyacrylamide gel (10% acrylamide, 2.7% bisacrylamide) using a Tris-glycine buffer system described by Laemmli (1970).

For immunoblotting the polypeptides were electrophoretically transferred from SDS-polyacrylamide gel to nitrocellulose (Trans Blot from Biorad) at 10 V/cm for 2 hr at 0°C using the following buffer described by Erickson, Minier & Lasher (1982): 0.025 M Tris, 0.192 M glycine, pH 8.3/20% methanol (v/v), 0.1% (w/v) SDS. Excess protein binding sites on the nitrocellulose were blocked by overnight incubation with 0.2% (w/v) polyvinyl pyrrolidon 350 (PVP, Serva) in PBS. The sheets were exposed for 2 hr to an appropriate dilution of antiserum (typically 1:10000 to 1:2000) in PBS, containing 0.2% PVP and subsequently washed three times with PBS, 0.2% PVP. The immunoglobulins that were attached to the sheet were labelled with iodinated protein A (Amersham). To this end the sheets were exposed for 45 min to a solution containing 0.025 mg/ml protein A and 0.05 mCi/ml ^{125}I label diluted 1:1000 with PBS, 0.2% PVP. Subsequently the sheets were washed twice in PBS, 0.2% PVP, and twice in PBS. The

nitrocellulose sheet was dried and autoradiographed at −70°C overnight using an intensifying screen.

Ouchterlony double diffusion experiments were performed in 1% Agarose A (Pharmacia) in barbital-acetate buffer, 0.043 mol/l Na-barbital, 0.043 mol/l Na-acetate. 3 H_2O, pH 8.2, containing 3% (w/v) polyethyleneglycol-6000 (Merck). After development, the gels were washed, dried onto an agarose gel support medium (Gelbond, FMC Corporation) and stained with Coomassie Brilliant Blue R-250 (Biorad). Subunits A1 to A4 were dissolved at a concentration of 0.1 mg/ml in PBS containing 0.1% (w/v) SDS and heated at 100°C for 10 min prior to Ouchterlony analysis. The eighteen soya varieties mentioned in Fig. 4 were obtained from the Unilever collection. Samples for Ouchterlony analysis were prepared as follows: beans were ground and meal was dissolved, without defatting, at a concentration of 20 mg/ml in PBS containing 1% (w/v) SDS. The samples were heated at 100°C for approximately 10 min and subsequently centrifuged. Aliquots of the supernatants were taken by pipetting through the lipid layer and diluted 1:10 with PBS.

Preparation of standards and samples for ELISA

Hamburgers (raw and pasteurized at 90°C) and canned luncheon meat samples (pasteurized at 90°C) with known amounts of soya protein were kindly provided by Mr W. de Groot (Unimills, The Netherlands). Hamburgers were composed of approximately 80% beef, salt, water and breadcrumbs. Luncheon meat samples were composed of approximately 25% beef, 25–40% pork, 10–20% back fat, 4% potato starch, water, salt, emulsifier and seasonings. The various types and amounts of soya products used are given in Tables 2 and 4. Sterilization was performed by heating the samples at 120°C for 30 min prior to the extraction procedure.

Extraction of meat samples was performed as follows: to each g of meat product 9.2 ml PBS containing 5% (w/v) SDS and 5% (v/v) 2-mercaptoethanol was added yielding a total volume of 10 ml per g product. Due to differences in homogeneity sample sizes were 1 g for luncheon meat and 5 g for hamburgers. Samples were homogenized by means of an Ultraturrax (Janke & Kunkel) and shaken end over end overnight. Next the samples were heated for approximately 10 min at 100°C and subsequently centrifuged to remove insolubles. Supernatants, taken by pipetting through the lipid layer were either subjected to further analysis or stored at −20°C. For analysis, supernatants were diluted either 1:10000 for high soya contents or 1:2000 for low soya contents with PBS containing 0.5% (w/v) SDS and 0.5% (v/v) 2-mercaptoethanol. The concentrations in the final solutions are either 10 or 50 μg product per ml.

Protein sources recorded in Table 1 were checked for possible interferences in the ELISA. The products were dissolved at a concentration of 10 mg/ml in PBS (5% (w/v) SDS, 5% (v/v) 2-mercaptoethanol) and further treated as described for meat products.

Standards were prepared as follows: 10 mg of soya protein (15.38 mg Unico-75 concentrate containing 65% protein; Kjeldahl nitrogen ×6.25) was dissolved in 1 ml PBS containing 5% (w/v) SDS and 5% (v/v) 2-mercaptoethanol). Further treatment was performed as described for meat samples. A 1:10000 dilution of the resulting solution contains 1 μg/ml soya protein. This solution served as 10% standard for the 10 μg/ml product extract and as 2% standard for the 50 μg/ml product extract.

ELISA procedure

Discs 6 mm in diameter were punched from nitrocellulose sheets (Trans Blot from Biorad), wetted with distilled water and transferred to a 24 well culture plate (Becton

Table 1. ELISA signals of non-soya protein sources compared with the 0.0 and 0.5% soya standard

Standard/sample	Protein concentration* (μg/ml)	ELISA signal† (E 492 nm)
0.5% Soya standard	0.25	0.48 ± 0.01
0.0% Soya standard	0	0.08 ± 0.004
Sesame seed	1.0–1.5	0.06 ± 0.01
Sunflower seed	1.0–1.5	0.12 ± 0.03
Groundnut	1.0–1.5	0.18 ± 0.02
Pea meal	~ 2	0.21 ± 0.03
Rape seed	1.0–1.5	0.10 ± 0.02
Broad bean	1.0–1.5	0.13 ± 0.01
Egg	~ 3	0.10 ± 0.003
Skim milk powder	~ 2	0.10 ± 0
Waldron gluten	3.5–4.0	0.17 ± 0.01
Okapi gluten	3.5–4.0	0.17 ± 0.01

*A final dilution of 1 : 2000 for both standards and samples was used in this experiment.

†Means of triplicate determination ± standard deviation.

Dickinson & Co). 0.5 ml of sample or standard was added for 30 min. In order to block excess protein binding sites on the nitrocellulose disc, the solution was replaced with 1 ml 3% (w/v) bovine serum albumin (BSA, RIA-grade from Sigma) in PBS and incubated for 15–20 min. Subsequently the discs were washed twice with 1 ml PBS containing 0.3% (w/v) BSA and 0.05% (w/v) Tween 80 (Koch-Light Laboratories Ltd) (PBS^+). Next 1 ml of an appropriate dilution of antiserum in PBS^+ was added and incubation was performed overnight. The two washing steps were repeated and the discs were incubated for 60 min with 1 ml of an appropriate dilution of protein A-peroxidase conjugate (E.Y. Laboratories Inc.) in PBS^+. Discs were washed three times with 1 ml PBS^+. Finally 400 μl of the following substrate solution was added: 0.1% (w/v) 5-amino-2-hydroxybenzoic acid (Merck) in 0.0048 M $NaH_2PO_4 \times H_2O$, 0.0052 M $Na_2HPO_4 \times H_2O$, 0.0001 M Na_2 EDTA, pH 6.0. Immediately before use 100 μl 0.5% (v/v) H_2O_2 was added to 10 ml of substrate solution. Colour development was allowed to take place for 60 min. Then 250 μl was transferred to a 96 well ELISA-plate (Dynatech) and read at 492 nm by means of a Titertek multiskan (Flow Laboratories). Washings (5–10 min each) and incubations were all performed at room temperature with agitation. Appropriate dilutions of conjugate and antiserum had to be determined for each batch of antiserum. Typical dilutions in this study were 1:500 for protein A-peroxidase conjugate and 1:1600 for antiserum.

Results

Antisera against each of the individual SDS-denatured acidic subunits were assessed by their Ouchterlony titres. Subunits A1 and A2 gave a good and A3 and A4 a weak response in rabbits (results not shown). On immunization, dosages of 2.5 mg protein yielded higher titres than dosages of 0.1 mg. Among the glycinin subunits, A1 was considered to be the best candidate as a soya marker for the reasons explained in the Discussion. The antiserum against A1 has subsequently been used in the immunoassay.

The specificity of the antiserum for glycinin subunits has been studied by immunoblotting (Fig. 1) and Ouchterlony double diffusion (Fig. 2). In Fig. 1 the subunits are studied after denaturation in SDS and 2-mercaptoethanol. The immunoblot demonstrates a strong structural relationship between A1 and A2. Subunit A3 crossreacts weakly and A4 does not react at all with the antiserum. These results are in agreement with those of Moreira *et al.* (1981). We ascribe the high molecular weight bond on the immunoblot of A2 to the dimer of the 38 kD unit. In Fig. 2 SDS denatured subunits are studied in the absence of 2-mercaptoethanol. The Ouchterlony pattern shows multiple precipitation arcs. These probably arise from different association products formed by disulphide crosslinking of subunits. Such crosslinks are plausible because the quaternary structure of native glycinin is stabilized by disulphide bonds (Iyengar & Ravestein, 1981; Staswick, Hermodson & Nielsen, 1981). The extent of crossreactivity of the four acidic subunits is the same as observed in Fig. 1. A spur is present between subunits A1 and A2, indicating A1 specific determinants, in addition to common determinants.

The reactivity of the antiserum with eighteen soya varieties has been studied by double diffusion. All varieties react with the antiserum; Fig. 3 shows complete identity of antigenic determinants. Also in quantitative terms all varieties behave similarly in the double diffusion experiment.

The reactivity of the antiserum on processed soya protein in a meat product has been analysed by gel electrophoresis and immunoblotting. Figure 4a shows electrophoresis patterns and immunoblots of hamburgers containing soya concentrate. On the electrophoretic pattern glycinin subunits are obscured by meat protein bonds. On the immunoblot soya protein additions can be traced unambiguously at concentrations

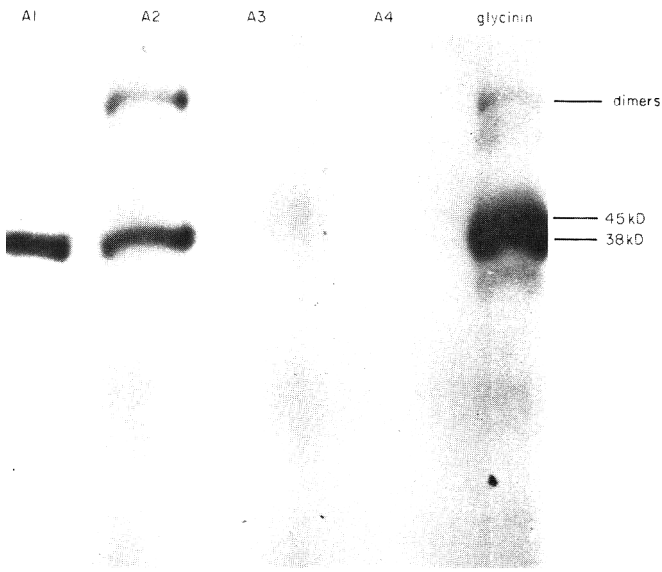

Figure 1. Immunoblotting of total glycinin and the acidic glycinin subunits A1 to A4. The blot was autoradiographed after treatment with anti-A1 serum and ^{125}I-protein A, respectively.

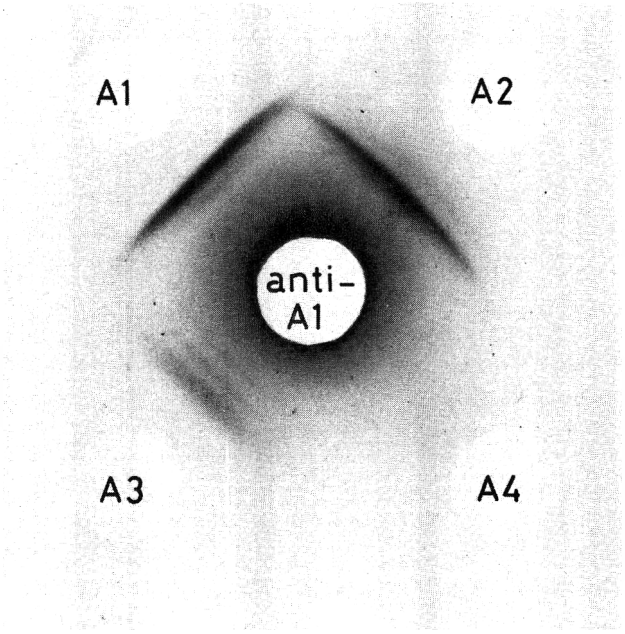

Figure 2. Ouchterlony double diffusion in Agarose of acidic glycinin subunits (A1-A4) against anti-A1 serum. Subunits were analysed at a concentration of 0.1 mg/ml in 0.1% SDS.

down to at least 0.1%. Figure 4b shows the effect of heating at 120°C. The electrophoretic bonding pattern of sterilized products becomes less pronounced as a result of irreversible protein aggregation. The immunoblot shows that glycinin partially aggregates, though to a lesser extent than meat proteins. This causes a decreased intensity of

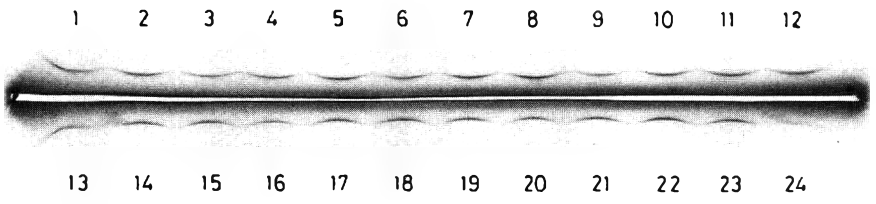

Figure 3. Ouchterlony double diffusion in agarose of soyabean varieties against anti-A1 serum. 1 = Ada, 2 = Altona, 3 = Clay, 4 = Davis, 5 = Forrest, 6 = Hill, 7 = Merit I, 8 = Merit II, 9 = Norman I, 10 = Norman II, 11 = Portage I, 12 = Portage II, 13 = Wilkin, 14 = Caloria, 15 = Gieso, 16 = Amurscaja Zeltana I, 17 = Amurscaja Zeltana II, 18 = Express Green I, 19 = Express Green II, 20 = Okuhara, 21 = Prize, 22 = Steel, 23 = Traverse, 24 = Subunit A1 from Express Green. I and II indicate different crops. Trough was filled with anti-A1 serum Samples were analysed at a concentration of 2 mg/ml in 0.1% SDS.

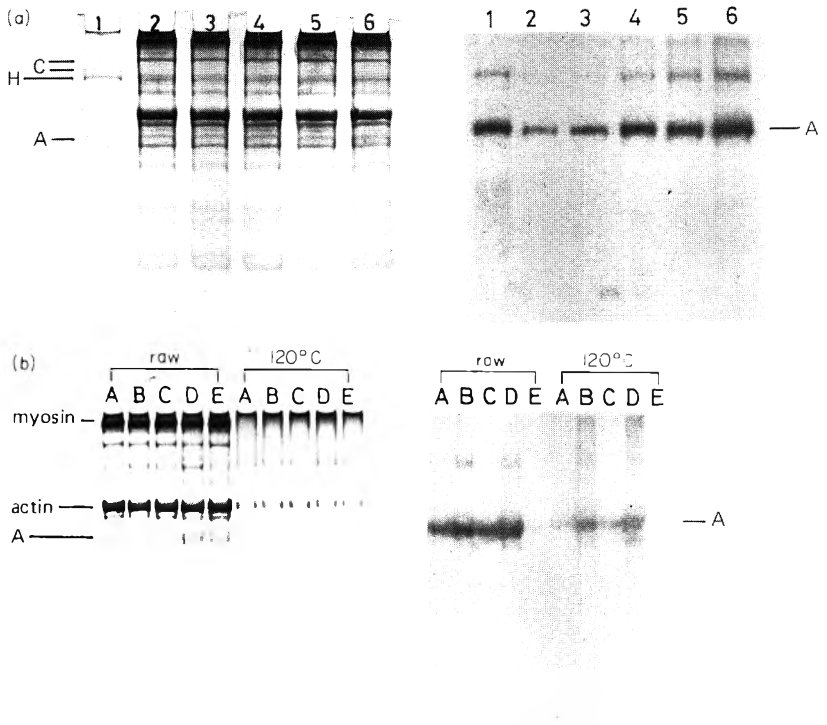

Figure 4. Electrophoretic patterns (left) and immunoblots (right) of hamburgers containing soya protein. (a) Soya concentrate in buffer (1) and various concentrations of soya concentrate in hamburgers: 0.1% (2), 0.25% (3), 0.5% (4), 1% (5), and 2.5% (6). H: hemocyanin, added as a marker according to Armstrong *et al.* (1982). C: conglycinin subunits. By densitometry these subunits are detectable in lanes 5 and 6 of the electrophoretic pattern, which confirms the results of Armstrong *et al.* (1982). A: glycinin subunits A1 and A2. Smearing in the high molecular weight region of the blot (cf. lanes 1 and 6) indicates some degree of glycinin–meat aggregation. (b) Raw and sterilized hamburgers containing soya concentrate and soya isolate. Nomenclature of hamburgers as in Table 2. Smearing in the high molecular weight region of the blot is increased after sterilization as a result of increased aggregation.

the glycinin band and an increased smearing in the high molecular weight region of the blot. The total intensity on the blot, i.e., the intensity integrated over one lane, is to a first approximation the same for the raw and heated product. This shows that the immunoreactivity of the antiserum is not affected by heating the product at 120°C. The blots demonstrate the absence of cross reactivity of the antiserum with meat components. The faint band in the high molecular weight region of the blot indicates an aggregation product, presumably the A2 dimer visible in Fig. 1.

The ELISA system constructed with this antiserum consists essentially of six steps. (Figure 5): (i) the protein constituents of the meat product are denatured and solubilized in boiling SDS in the presence of 2-mercaptoethanol; (ii) the solubilized proteins are allowed to adsorb on nitrocellulose discs; (iii) the excess protein binding sites on the nitrocellulose are blocked with BSA; (iv) the bound glycinin subunits are allowed to react with antibodies; (v) the bound antibodies are labelled with protein A–horse radish peroxidase conjugate; and (vi) substrate is added and the generated colour recorded. Details are described in Materials and methods.

As a standard, soya concentrate (Unico 75) was arbitrarily chosen. Figure 6 shows a

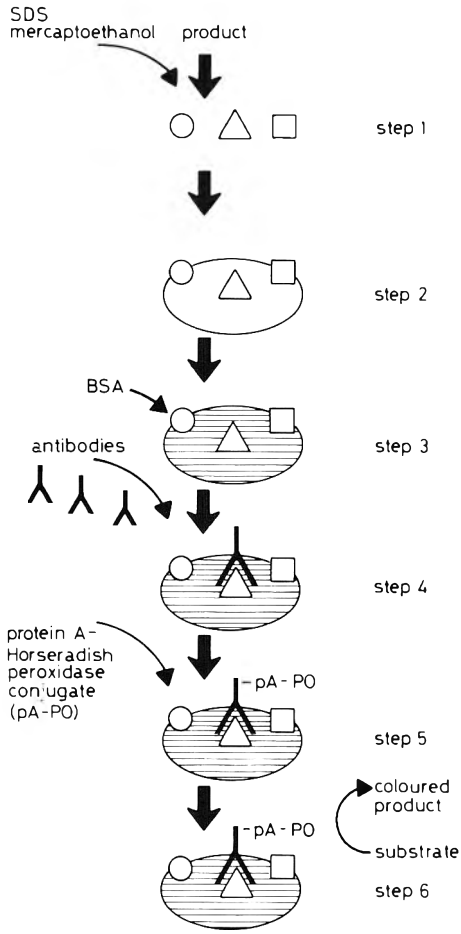

Figure 5. Schematic representation of ELISA procedure.

typical example of a calibration line. The standard solutions contain 0–1 μg soya protein per ml. The product extract contains either 10 or 50 μg product per ml, depending on the sample dilutions chosen (see Materials and methods). Therefore the above range of standards refers to either 0–10% or 0–2% soya protein in the samples. Figure 6 shows a nearly linear dose-response relationship. Therefore, for the analysis of unknowns the standard curves were linearized piecewise. The variance of replicate measurements of standards and samples is approximately constant over the dose range. The coefficient of variation of the optical density readings was on the average 5.8%. From this number the within-assay-error of the soya contents measured in triplicate is estimated (Malan, 1983). This error is about 5%.

The specificity of the ELISA for soya has been tested using extraneous protein sources (Table 1). A number of products have been selected that are either usual constituents of food products or that bear to some extent a phylogenetic relationship with soya. Table 1 shows that the ELISA is highly specific for soya. Non-soya proteins contribute slightly to the ELISA signal only at very high concentrations. In consequence the ELISA can safely be applied at soya concentrations down to at least 0.5%.

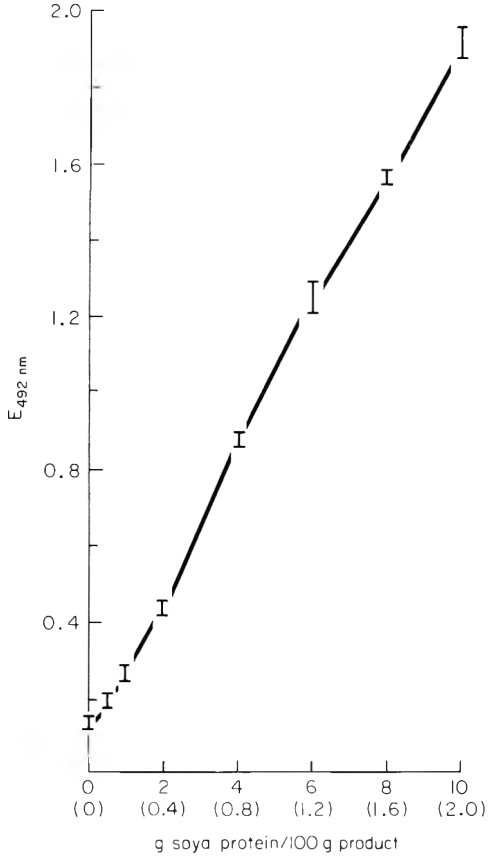

Figure 6. ELISA calibration curve for determination of soya protein. Each point reflects the mean triplicate determinations ± s.d. The 0–2 g protein per 100 g product scale is used for the less diluted product extracts (see text).

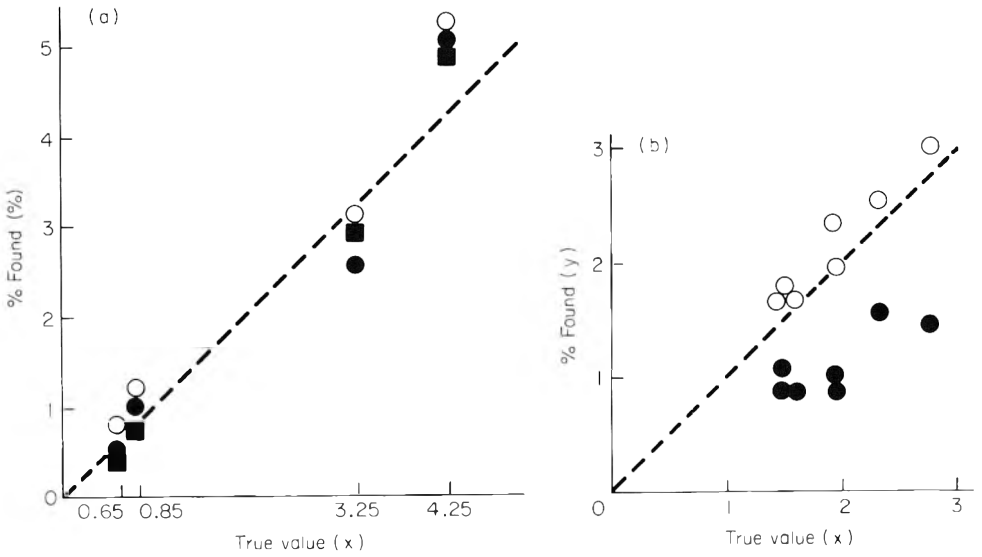

Figure 7. Correlation between actual content and content found by ELISA. (a) Averages over three days for hamburgers, (b) averages over two days and two duplicate extractions for luncheon meat samples. (■) raw, (●) pasteurized, (O) sterilized at 120°C.

Table 2. Percentage of soya protein in hamburgers (g soya protein/100 g product) as determined with ELISA

Sample	Type of soya product used	Actual content	Raw, Day			Pasteurized at 90°C, Day			Sterilized at 120°C Day		
			1	2	3	1	2	3	1	2	3
A	Concentrate	0.65	n.d.	0.5*	0.5*	n.d.	0.5*	0.7*	n.d.	0.7*	0.8*
B	Concentrate	3.25	2.7	2.9	3.0	2.5	2.4	3.0	3.6	2.6	3.0
C	Isolate	0.85	n.d.	0.7*	0.8*	n.d.	1.0*	1.0*	n.d.	1.1*	1.1*
D	Isolate	4.25	5.0	4.7	4.9	5.4	5.0	4.7	5.7	4.7	5.0
E	—	0	n.d.(0.4)	0*,(0.1)	0*,(0.5)	(0.1)	0*,(0)	0.1*,(0.1)	(0)	0*,(0)	0.1*,(0.5)

*Results obtained with 1 : 2000 dilution. For reference sample E in addition the results of 1 : 10 000 dilution are given in brackets. The relative error in soya contents is approximately 5% (see Results).

Data taken on different days.

n.d., Not determined.

Table 3. Effect of sample dilution on ELISA-signal expressed as optical density at 492 nm

	1:10000			1:2000		
Hamburger*	raw	90°C	120°C	raw	90°C	120°C
A	0.17	0.21	0.18	0.46	0.56	0.64
C	0.23	0.27	0.23	0.62	0.75	0.84
E	0.18	0.13	0.17	0.13	0.15	0.16

*Nomenclature of hamburgers, see Table 2.

Table 2 records soya concentrations determined in hamburgers. The recovery of soya protein is, on the average, complete (Fig. 7a). The values measured are independent of the heat-pretreatment of the product. The data obtained from the reference hamburger, which contains no soya, demonstrates that the calibration line for the 0–10% scale is unreliable below 1%. Therefore samples containing less than 1% soya protein are analysed on the 0–2% scale, by using less diluted product extracts (see Materials and methods). This scale gives a better discrimination between low soya samples and blanks, as is illustrated in Table 3. If no knowledge on the soya content is available, samples should be analysed at two different extract dilutions.

Table 4 shows soya contents determined in luncheon meat. Variations of duplicate extractions and day-to-day variation are within acceptable limits. The recovery of soya protein from pasteurized samples is somewhat underestimated for some unknown reason. Sterilized products, however, show a full recovery of soya protein (Fig. 7b). The use of soya concentrate as a standard yields satisfactory results for all types of soya ingredients (see also Table 2).

Discussion

According to a number of investigators (e.g. Olsman & Hitchcock, 1980) immunochemistry is a promising approach for the determination of soya protein in meat

Table 4. Percentage of soya protein in luncheon meat samples as determined with ELISA (g soya protein/100 g product)

			Pasteurized at 90°C, Day				Sterilized at 120°C, Day			
Sample	Type of soya product used	Actual content	1	2	3	4	1	2	3	4
1	—	0	0.2	0.2	0.1	0.2	0.1	0.2	0.3	0.2
2	isolate	1.50	1.0	1.1	1.1	1.1	1.4	2.1	1.5	1.6
3	isolate	2.35	1.5	1.7	1.7	1.5	2.9	2.4	2.5	2.4
4	isolate	1.94	1.1	0.9	1.2	0.9	2.8	2.0	2.5	2.3
5	flour	1.61	0.7	1.1	0.9	0.8	1.6	1.6	1.7	1.7
6	concentrate	1.96	0.7	1.2	0.5	1.1	2.2	1.9	2.1	1.9
7	textured concentrate	2.81	1.3	1.8	1.1	1.6	3.1	3.0	2.9	3.5
8	textured flour	1.51	0.8	0.9	0.8	1.0	1.8	1.6	1.8	2.1

Duplicate extractions were taken on different days, 1:10000 dilutions.

products. This has been confirmed by the ELISA described in this paper. The assay presents a number of important advantages concerning universal use, specificity and sensitivity.

The main advantage of the assay is its applicability to sterilized products. Only the pasteurized luncheon meat samples gave underestimates of the soya content for yet unknown reasons. This problem may be avoided by heating all products at 120°C which restores the antigenic sites (see Table 4). Good evidence for independence of soya variety is shown in Fig. 3. Different crops of varieties from different continents were chosen. The results indicate that no strong dependence on climatological and environmental conditions exist. Similar conclusions hold for different types of soya ingredients. By using concentrate as an absolute standard, the content of soya protein from other processing forms (isolate, flour, etc.) can be determined quantitatively. Even texturates that usually cause problems in detection by other analytical methods (e.g. Molander, 1982) can be analysed satisfactorily.

The ELISA is highly specific for soya. We demonstrated the absence of interference by meat proteins, non-soya vegetable proteins and other meat product constituents by immunoblotting and ELISA. Soya, rape and sunflower proteins show a structural similarity by small angle X-ray scattering (Plietz *et al.*, 1983). Also garden pea (Matta, Gatehouse & Boulter, 1981) and groundnut (Yamada, Aibara & Morita, 1981) contain glycinin-like molecules. Glycinin and legumin subunits of sesame and broad bean form hybrid molecules (Utsumi, Inaba & Mori, 1980; Hasegawa, Tanaka & Tamai, 1981), which is explained by a similarity of their structures. Nevertheless the antibodies do not react with any of these related proteins.

The sensitivity of the ELISA is comparable to other ELISA systems (e.g. Hitchcock *et al.*, 1981). Quantitative analysis of soya protein is possible down to 0.5% in products and can be adjusted to even lower values by using a sample dilution ratio below 1:2000. The assay covers the full range of soya concentrations which is of practical interest. For qualitative purposes, however, the sensitivity is improved considerably by immunoblotting. We could unambiguously detect 0.1% soya concentrate (i.e., 0.065% protein) in hamburgers. The ultimate limit of detection was not assessed because these low concentrations have no practical value.

The size of a representative sample depends on the homogeneity of the meat product. In this study 1 g samples of luncheon meat and 5 g samples of the less homogeneous hamburgers were taken. In order to avoid having to determine the correct sample sizes for each individual meat product, it is advisable to homogenize a quantity of meat product before subsampling for analysis to ensure uniformity of procedure.

The unique properties of the antibodies, that give rise to the above characteristics of the ELISA, are a corollary of the use of SDS. This approach is a further step along the route followed by Hitchcock *et al.* (1981) who used urea as a denaturant. There are two rationales for using SDS. First, it destroys the tertiary structure of proteins and second, it solubilizes proteins with high efficiency. As a consequence of the effects on the tertiary structure, antibodies against SDS-denatured proteins will recognize predominantly continuous determinants that only depend on the primary structure. The discontinuous determinants that exist in proteins with well defined three-dimensional structures will disappear on SDS-treatment. Therefore, processing steps that have a strong influence on the tertiary structure of proteins, like sterilization and texturation, will not affect the reactivity with the antibodies. Indeed, in this study we have demonstrated that the antigenic status of the SDS-denatured glycinin subunits A1 and A2 is

independent of processing conditions. The high solubilizing activity of SDS guarantees complete recovery of soya proteins from products (Tables 2 and 3). Mild extractions necessary for immunoassays based on native proteins give incomplete recoveries, in particular if the food product has been sterilized. Although solubilized, soya protein subunits from sterilized products partly occur as aggregates. For this reason protein separation techniques, like gel electrophoresis, fail for analysis of sterilized products. However, these aggregates do not interfere in the ELISA.

Subunit A1 was considered as the best soya marker for different reasons. It occurs in all soya varieties described so far (Kitamura, Toyokawa & Harada, 1980; Mori *et al.*, 1981; Staswick & Nielsen, 1983). Moreover, the solubility of acidic subunits after heating glycinin is maintained (Yamagishi *et al.*, 1983) and irreversible heat induced reactions with meat proteins occur to a relatively small extent (Peng *et al.*, 1982). The antiserum raised against subunit A1 crossreacts with subunit A2. For quantitative analysis subunits A1 and A2 must constitute a constant fraction of the total protein in all varieties and under all circumstances. Although this is difficult to substantiate the results described in this paper justify their use as marker.

The SDS in the sample necessitates the use of nitrocellulose as the solid phase, because protein-solid phase interactions in the usual microtiter plates are unstable in SDS. Nitrocellulose is becoming increasingly popular as a solid phase in immunoassays (Hawkes, Niday & Gordon, 1982). After sample incubation blocking of excess binding sites is a prerequisite to prevent non-specific binding of the antibodies to the nitrocellulose filters. According to Palfree & Elliot (1982) the protein binding capacity of nitrocellulose, although reduced, is still about 65% at 0.5% SDS, depending on the type of nitrocellulose. Therefore the concentration of SDS in the assay should be kept constant. Remnants of SDS, possibly present in the antibody incubation step do not interfere in the assay. Antibody–antigen interactions are maintained at low concentrations of SDS. Moreover, Dimitriadis (1979) has shown that the disruptive action of SDS can be prevented by non-ionic detergent. In most of the ELISA steps non-ionic (Tween 80) is present in excess of SDS remnants.

The precision of our assay is approximately similar to that of the ELISA on nitrocellulose by Palfree & Elliot (1982) and compares favourably with other ELISA systems reported in the literature. Common to all ELISA systems, our assay exhibits a day-to-day variation which makes daily measurement of standards necessary. For the analysis of large numbers of samples the handling of the nitrocellulose discs is somewhat laborious. However, during the development of the assay new microtitreplate modes, in which the bottom of the 96 wells of a conventional plate has been replaced by nitrocellulose, have been introduced (Millititre filtration plates from Millipore Corporation and the Minifold system of Schleicher & Schuell). This enables the commercially available ELISA instrumentation to be applied to the presently developed soya protein ELISA and makes high sample loads possible.

Acknowledgment

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Effects of additions, irradiation and heating on the activity of Trimethylamine oxide-ase in frozen stored minced fillets of whiting

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Summary

Fish fluid was obtained by centrifuging minced fillets of whiting and used to study the effects of several treatments on TMAO-ase activity during storage at −18°C. Additional experiments were carried out with the whole mince. When the fish fluid or mince was heated for 1 min at 80°C or more, the production of dimethylamine and formaldehyde ceased. Xanthan was the only one of the hydrocolloids studied (pectins, carrageenans, alginates, locust bean gum and carboxymethyl celluloses), which showed a significant reduction of TMAO-ase activity. A low dose of irradiation (300 Krad) did not change TMAO degradation of the fish fluid during subsequent frozen storage.

Introduction

Many gadoid fishes produce large amounts of formaldehyde (FA; about 50 ppm) and dimethylamine (DMA; about 500 ppm) in muscle and intestine tissues during frozen storage (Tokunaga, 1964; Amano & Yamada, 1964; Castell, Neal & Smith, 1970). This production has been attributed to an endogenous enzyme (more concentrated in kidney, pyloric caeca and liver) capable of reducing trimethylamine oxide (TMAO) to the intermediate dimethylaminomethylol which then yields DMA and FA (Harada, 1975). Probably FA contributes to the textural toughening and to the loss of water holding capacity of fish muscle during frozen storage (Connell, 1975; Poulter & Lawrie, 1978). DMA could be a potential precursor of the carcinogenic compound N-nitrosodimethylamine (Matsui, Ishibashi & Kawabata, 1984; Mackie & Thomsom, 1974). The continuing production of DMA in frozen storage may also impair the flavour of fish products.

During the past several years, increasing numbers of new frozen fish products have been introduced which have been pre-cooked before freezing. Among them, pre-fried, battered and bread coated fish and seafood products with a wide range of ingredients, have become well accepted by consumers (Sikorski, Olley & Kostuch, 1976; Hansen, 1980; Lopez-Gravito & Pigott, 1983). As the gadoid fishes play an important role in this industry there is a need for studying practical pretreatments which could prevent the production of DMA and FA in these products during frozen storage. Several attempts have already been made: removal of TMAO and soluble proteins by washing the minces, addition of oxidizing agents and packaging in oxygen permeable materials (Landolt & Hultin, 1981; Reece, 1983; Racicot *et al.*, 1984). Heat treatments have also

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been used to arrest the TMAO-ase activity of frozen stored fish, but the results obtained are not clear in respect to time and temperatures of inactivation (Tokunaga, 1964; Castell, Neal & Smith, 1971; Lall, Manzer & Hiltz, 1975).

In order to avoid fluctuations in DMA and FA determinations fish fluid was studied, which was obtained by centrifuging minced fillets of whiting. The effects of different times of heating at various temperatures, irradiation and additions of several hydrocolloids were examined. The effects of heat treatments on the whole minced fish were also investigated.

Materials and methods

Mince of skinned fillets of whiting (*Merlangius merlangus*) with a high TMAO-ase activity (da Ponte, Roozen and Pilnik, 1985b) was kept in frozen storage at −45°C during 12 months. It was then thawed, centrifuged at 25000 **g** for 30 min and the supernatant liquid (fish fluid) stored for 4 weeks at −80°C.

Skinned fillets of whiting were bought in the local market of Wageningen on 12 December 1984. They were kept on ice and passed twice through a Kenwood mincer model A. 720, using a mincing screen with 4.4 mm holes. The resulting minced fish was divided in portions of 25.0±0.1 g and packed in polyvinyl chloride (PVC) plastic bags (180×300×0.065 mm) in such a way that the minced fish formed a rectangular slab of 178.5×93.5 mm and 1.5 mm thick, on average. The slabs were stored at −80°C for 1 week.

Heat treatments

Fifty-two ml of fish fluid were put in a 500 ml Erlenmeyer flask, in an ice water bath to 0°C, then heated in a microwave oven (Philips, model 2010C) to the desired temperature and immediately afterwards transferred to a water bath at the same temperature as the fish fluid, and held there for different intervals of time. Subsequently the fish fluid was cooled by intensive swirling of the Erlenmeyer flask in an ice water bath. The heat treated fish fluid was then mixed well, divided into 8 ml portions in screw cap tubes and stored at −18°C. Two replicates of each treatment were used to determine the DMA and FA content at various storage intervals. The different heat treatments of the fish fluid are reported in Table 1. The heating and cooling curves of the various treatments were determined with a thermocouple placed in the centre of the Erlenmeyer (Fig. 1).

Table 1. Heat treatments used for the inactivation of TMAO-ase in fish fluid from minced fillets of whiting

Heating time in the microwave oven (sec)	Temperature reached (°C)	Different holding times in the water bath (sec)
8	55	10, 50, 200, 400 and 800
9	60	30, 60, 120, 240, and 480
10	65	10, 30, 90, 180 and 270
11	70	20, 40, 80, 160, and 320
13	80	15, 30, 60, 120 and 240
14.5	85	10, 20, 40, 80 and 160

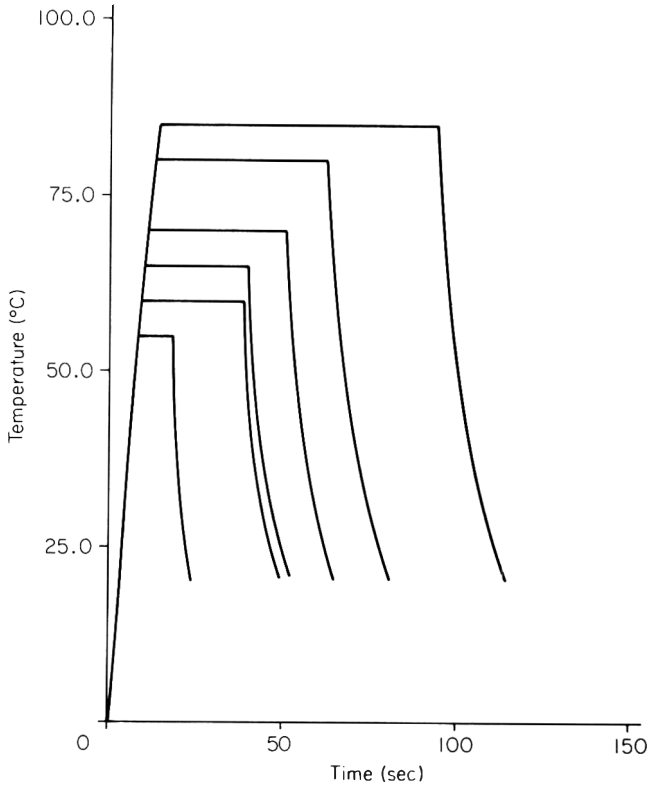

Figure 1. Heating and cooling curves of the different heat treatments as determined for fish fluid from minced fillets of whiting.

The bags with 25.0 g of minced whiting were thawed in an ice water bath and then dipped in a water bath at a desired temperature for 1 min. Immediately afterwards, the bags were cooled by intensive agitation in an ice water bath. Subsequently the bags were blast frozen and stored at −18°C. The various temperatures used in the water bath are shown in Table 2. The heating and cooling curves of these heat treatments were determined theoretically (Fig. 2) using the thermal diffusity of PVC (9.55×10^{-8} m^2/sec), the thermal diffusity of minced fish (1.47×10^{-7} m^2/sec; Aitken & Connell, 1979) and assuming a distance of 0.9 mm for reaching the coolest point of the fish slab. Two replicates of each treatment were used for determining the DMA and FA contents at various intervals of storage.

Additions

Different hydrocolloids were mixed with the fish fluid (5 mg/ml) for 1 hr at refrigerated temperatures (4°C). Afterwards the fish fluids with the different additions were divided into 8 ml portions and stored in screw cap tubes at −18°C. Two replicates of each addition were used to determine the DMA and FA content before and after 46 days in storage.

Hydrocolloids used were: (i) Low esterified pectin (L.P.) and High esterified pectin (H.P.) from Obi-Pectin (Bischofszell, Switzerland); (ii) Potassium Kappa carrageenan (HF 55758-63) and Iota carrageenan (HF 33895-96) from Copenhagen Pectin Factory

Table 2. Effect of various heat treatments on TMAO-ase activity in mince of whiting during frozen storage

Heat treatments (1 min at T°C)	Days at (−18°C)	Production of DMA-Nitrogen		Production of FA	
		μg DMA-N/g mince	(%)*	μg FA/g mince	(%)‡
Blank	0	38.0±1.5	100	9.2±0.5	100
	21	93.3±3.0	100	28.6±2.0	100
	45	147.7±7.5	100	43.3±3.5	100
50	0	37.3±0.2	98	5.9±0.4	64
	21	87.7±4.8	91	22.0±1.5	83
	45	139.7±5.9	93	34.3±0.3	83
55	0	38.4±2.0	101	5.1±0.3	55
	21	82.8±0.3	80	19.0±1.4	72
	45	119.4±1.8	74	27.6±1.2	66
60	0	39.7±0.5	104	5.0±0.2	54
	21	80.2±2.6	73	16.6±1.3	60
	45	109.3±4.5	63	25.2±0.3	59
65	0	39.0±2.1	103	3.6±0.3	39
	21	72.7±2.7	61	13.3±0.8	50
	45	103.1±0.6	58	23.4±1.8	58
70	0	36.7±1.7	97	3.0±0.2	33
	21	54.7±3.5	33	9.4±1.0	33
	45	77.0±1.9	37	13.6±0.6	31
75	0	38.5±0.9	101	2.6±0.1	28
	21	43.8±0.1	10	5.3±0.1	14
	45	54.8±1.7	15	7.1±0.4	13
80	0	36.9±1.4	97	2.5±0.2	27
	21	37.9±1.8	2	3.2±0.3	4
	45	41.7±1.6	4	3.4±0.2	3
85	0	37.8±0.5	99	2.6±0.2	28
	21	37.2±0.6	−1	3.4±0.3	4
	45	40.3±0.5	2	3.5±0.2	3
90	0	36.2±1.5	95	2.6±0.2	28
	21	36.8±1.7	1	2.8±0.2	1
	45	39.3±2.1	3	2.9±0.3	1

*$\frac{\text{DMA-Nitrogen of the treat. at } t \text{ time} - \text{DMA-Nitrogen of the treat. at 0 time}}{\text{DMA-Nitrogen of the Blank at } t \text{ time} - \text{DMA-Nitrogen of the Blank at 0 time}} \times 100\%$.

‡$\frac{\text{FA of the treat. at } t \text{ time} - \text{FA of the treat. at 0 time}}{\text{FA of the Blank at } t \text{ time} - \text{FA of the Blank at 0 time}} \times 100\%$.

Ltd. (Denmark); (iii) Lambda carrageenan, Viscarin 402, (Batch 321403), from Marine colloids Division, FMC Corporation (Springfield, New Jersey, U.S.A.): (iv) Kelcogel HV, Kelcosol and Kelcocolloid HVF (different types of alginates) from Kelco/AIL London (U.K.); (v) Xanthan gum, Keltrol F (KTLF-67203A), from Kelco (New Jersey, U.S.A.); (vi) Locust bean gum (L.B.G.), Meyprodin 200, from Meyhall Chemical AG (Switzerland); and (vii) Carboxymethyl cellulose (CMC), Akucell AF 705, AF 1505, AF 2205 and AF 2805 from Enka bv, Industrial Colloids (The Netherlands).

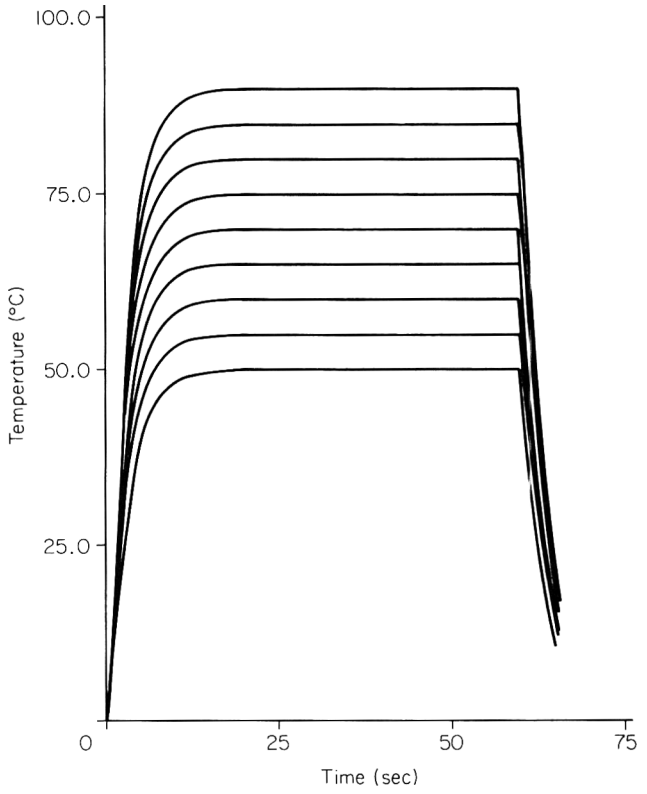

Figure 2. Heating and cooling curves of the different heat treatments as determined for the coolest point of slabs of minced fillets of whiting packed in PVC bags.

Irradiation

Screw cap tubes with 8 ml of fish fluid were gamma irradiated (cobalt 60, 300 Krad, −20°C) in the Pilot Plant for Food Irradiation, Wageningen, and then stored at −18°C. Two replicates were used for determination of DMA and FA during storage.

DMA and FA determination

Five percent trichloroacetic acid extracts of mince or fish fluid were used for the determination of DMA and FA. DMA was determined by the colorimetric method of Dyer & Mounsey (1945). The free fatty acid (FA) content was determined by the procedure outlined by Castell & Smith (1973) using the Nash reagent (Nash, 1953).

Statistical analysis

Differences were analysed by Duncan's multiple range test at a probability level of 1% (Stell & Torrie, 1982).

Results and discussion

Heat treatments

During heating and cooling of both the fish fluid and mince the heat transfer proceeded rapidly (Figs 1 and 2). The fish fluid was heated from 50 to 85°C in about 7 sec and the mince in 12 sec.

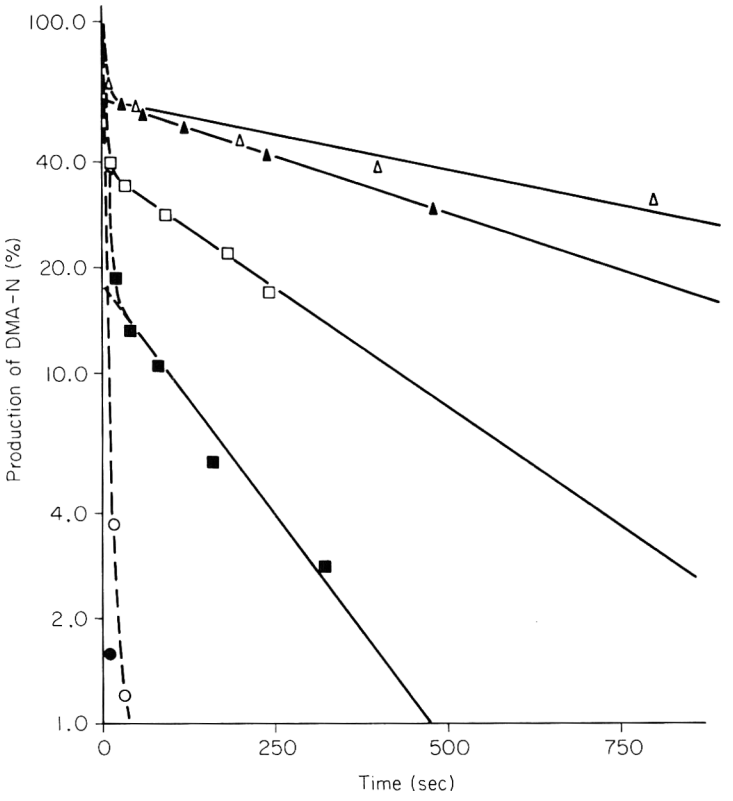

Figure 3. Thermal inactivation of TMAO-ase in fish fluid from minced fillets of whiting. Production of DMA as a function of time and temperature: △ 55°C, ▲ 60°C, □ 65°C, ■ 70°C, O 80°C and ● 85°C.

The thermal inactivation curves of TMAO-ase in respect to the production of DMA and FA in the fish fluid are presented respectively in Figs 3 and 4. In both cases the thermal inactivation curves consist of initial steep straight lines, which seem to be the result of very quick (almost instantaneous) inactivation rates. Then the curves show small transition parts and final straight lines with flat slopes, which indicate relatively moderate rates of inactivation.

At present, little is known about the exact structure of the TMAO-ase molecule and its localization in the fish muscle (Parkin & Hultin, 1981; Lundstrom, Correia & Wilhelm, 1982). The fish fluid is a complex mixture of soluble proteins, suspended particles and minor organic and inorganic compounds. It is possible that differences in heat stability of TMAO-ase are due to differences in thermostability of isoenzymes or of complexes of proteins and TMAO-ase. Also TMAO-ase aggregation caused by the heat treatment may protect the enzyme (Schleusener, 1982).

The effect of various heat treatments on the TMAO-ase activity of the mince of whiting during frozen storage is reported in Table 2. Comparing these values with the curves in Figs 3 and 4 it seems that TMAO-ase is somewhat more thermostable in the mince than in the fish fluid. Perhaps a higher proportion of TMAO-ase is complexed in the fish muscle than in the fish fluid thus protecting it from heat denaturation.

Heating reduced the free FA content of the samples determined by the Nash reagent

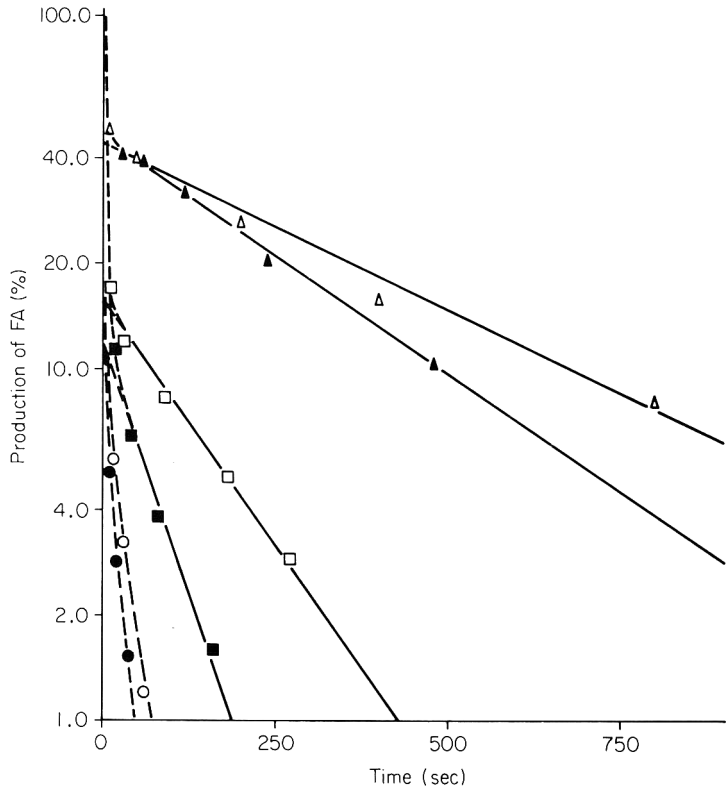

Figure 4. Thermal inactivation of TMAO-ase in fish fluid from minced fillets of whiting. Production of FA as a function of time and temperature. See Fig. 3 for symbols.

(Nash, 1953). This may be due to heat accelerating the FA reactions. It seems also that heated mince and heated fish fluid increased their capacity to bind FA during storage at −18°C and therefore the percentages of FA found were somewhat lower than the ones of DMA (Table 2 and Figs 3 and 4). Similar results were reported by Castell & Smith (1973).

Reports about thermal inactivation of TMAO-ase are difficult to interpret. Tokunaga (1964) found that heating of muscle tissue of Alaska pollock for 30 min at 40°C caused 20% decrease in FA production after 4 weeks at −17 – −19°C. This production disappeared almost totally when heating was carried out at 50°C. Heating of fillets and minced flesh of silver hake up to 60°C had little or no effect on the rate of DMA formation during subsequent storage for 1 month at −10°C (Lall *et al.*, 1975). Heating of silver hake mince to 80°C was found to stop the DMA formation totally. Sevensson (1980) concluded that thermal inactivation of partially purified TMAO-ase from kidney tissue homogenates of cod and blue whiting followed first order reaction kinetics. In our experiments, the thermal inactivation curves seem to be the resultants of two first order reactions. It can be concluded that holding at 80°C or higher for 1 min is sufficient to arresting TMAO-ase activity in minced fillets of whiting. At lower temperatures a quick partial inactivation can be obtained but eliminating the remaining TMAO-ase activity will require much longer holding times.

Additions

In previous reports (da Ponte, Roozen & Pilnik, 1985a and b: da Ponte *et al.*, 1985c) we described the effect of various hydrocolloids on the stability of frozen minced fillets of whiting and cod. Some of the hydrocolloids used (Kelcosol, Xanthan, Iota and Kappa carrageenan) diminished the production of DMA and FA during storage at −18°C. In the present experiments only xanthan reduced the DMA and FA contents of the fish fluid stored at −18°C (about 86% of the blank, Table 3).

Table 3. Effect of additions of different hydrocolloids on the TMAO-ase activity of fish fluid of whiting during frozen storage

Additions	Days in frozen storage (−18°C)	μg DMA-Nitrogen per g of fish fluid	μg FA per g of fish fluid
Blank	0	75.9 ± 1.6	24.2 ± 0.1
	46	158.0 ± 3.4*	81.7 ± 1.6*
K-carrageenan	0	74.9 ± 2.0	23.9 ± 0.3
	46	154.5 ± 4.0*†	79.2 ± 1.1*
I-carrageenan	0	74.7 ± 2.7	23.5 ± 0.4
	46	157.6 ± 3.6*	82.4 ± 0.6*
λ-carrageenan	0	74.6 ± 1.6	23.6 ± 0.2
	46	155.9 ± 4.7*	80.4 ± 1.0*
Xanthan	0	74.5 ± 2.3	23.3 ± 0.1
	46	145.0 ± 4.1†	70.1 ± 0.1†
CMC 705	0	75.5 ± 0.1	23.2 ± 1.1
	46	158.1 ± 2.1*	79.4 ± 1.4*
CMC 1505	0	75.1 ± 0.4	24.0 ± 0.1
	46	154.1 ± 2.5*†	79.3 ± 0.1*
CMC 2205	0	74.4 ± 0.6	24.6 ± 0.8
	46	152.7 ± 4.4*†	79.3 ± 0.8*
CMC 2805	0	75.3 ± 2.0	23.6 ± 0.4
	46	156.5 ± 2.4*	79.6 ± 0.8*
L.B.G.	0	73.8 ± 1.3	24.8 ± 0.5
	46	157.5 ± 0.3*	80.2 ± 1.4*
L.P.	0	75.0 ± 2.0	24.9 ± 0.3
	46	154.7 ± 4.0*†	78.4 ± 0.7*
H.P.	0	72.4 ± 1.3	24.4 ± 0.1
	46	157.3 ± 2.2*	78.4 ± 0.7*
Kelcogel	0	72.6 ± 0.0	24.3 ± 0.3
	46	160.0 ± 0.1*	80.9 ± 3.0*
Kelcosol	0	73.9 ± 1.1	24.7 ± 0.6
	46	158.5 ± 3.0*	79.8 ± 2.8*
Kelcocolloid	0	74.3 ± 0.5	24.9 ± 0.1
	46	157.0 ± 1.3*	80.1 ± 1.7*
L.B.G. + Xanthan	0	74.2 ± 0.6	23.4 ± 0.8
	46	156.0 ± 4.0*	78.2 ± 2.7*

*† Values in the same column with the same superscripts were not significantly different ($n = 2$; $P < 0.01$).

Irradiation

The bacterial load of mechanical deboned fish minces can increase rapidly unless strict hygienic procedures are used. Raccach & Baker (1978) showed that mechanical deboning of either frames or headed and gutted fishes increased the bacterial load by a factor of ten. Minced fish, even of good initial quality, has very short shelf life at chill temperatures.

Radiation could be employed for decreasing the number of viable microorganisms present in the fish minces and consequently it could increase the shelf life of this raw material for the manufacture of fish products. Studies on the cleavage of TMAO in fish flesh showed that irradiation enhanced the production of FA and DMA in gadoid fishes (Tozawa & Amano, 1969a and b). In these studies, doses of 600 Krad and more were used.

In our experiments we determined the effect of a lower irradiation dose (300 Krad) for the production of DMA and FA in the fish fluid stored at −18°C. Irradiation did not show any noticeable effect, also not during subsequent frozen storage (Table 4).

Table 4. Effect of irradiation on the production of DMA and FA in fish fluid of whiting during frozen storage

	Not irradiated		Irradiated	
Days in frozen storage (−18°C)	μg FA/g fish fluid	μg DMA-Nitrogen/g fish fluid	μg FA/g fish fluid	μg DMA-Nitrogen/g fish fluid
0	28.9 ± 1.0	69.4 ± 2.4	28.9 ± 0.5	72.1 ± 1.1
21	53.7 ± 0.1	108.1 ± 0.9	49.6 ± 0.3	103.8 ± 0.5
35	62.8 ± 0.0	121.5 ± 0.1	60.0 ± 0.5	118.3 ± 1.3
53	69.3 ± 0.2	135.9 ± 2.1	67.7 ± 0.9	133.1 ± 2.3

Conclusions

Heat treatment of frozen gadoid mince could overcome some of the undesirable changes in texture and water holding capacity attributed to FA production by TMAO-ase. In our experiments, it was found that heating of mince of whiting up to 80°C or more for short periods of time reduced drastically the production of DMA and FA during subsequent frozen storage. At lower heating temperatures it took much more time. This information may be useful for the pre-frying of ready-to-serve fish products which are becoming increasingly popular. The addition of xanthan caused about 14% reduction of DMA and FA production during frozen storage. A low dose of irradiation (300 Krad) did not enhance the formation of DMA and FA in the fish fluid.

Acknowledgments

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Silage from tropical fish 3. Lipid behaviour

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Summary

Acid silages were prepared from silverbelly (*Leiognathus* spp.) at 30°C with 3% (w/w) of 98% formic acid. Lipid oxidation takes place actively during ensilation as demonstrated by changes in fatty acid composition, TBA value and peroxide value. Lipid extraction, with chloroform : methanol or isopropanol, or the addition of an antioxidant before ensilation can limit the build up of lipid oxidation products. Lipid extraction also suppresses the autolysis of proteins in a defatted silage compared with a normal silage when measured by total soluble nitrogen or soluble NPN. The solubilization of collagen appears to be unaffected by lipid extraction perhaps reflecting the non-enzymic nature of this process. The limited autolysis in defatted silages may be beneficial in restricting the release of free amino acids capable of reacting with lipid oxidation products resulting in a lower nutritional value for silage based diets.

Introduction

The fate of the lipids in fish silages has received less attention than that of the proteins, a surprising fact given that changes in the lipids can have far-reaching nutritional and organoleptic consequences. For example, the products of lipid oxidation have been implicated in the poor performance of poultry fed silage-based diets incorporating tropical fish (Kompiang, Arifudin & Raa, 1979).

Lipids may degrade either by hydrolysis to yield free fatty acids (FFA) or the polyunsaturated fatty acids (PUFA) may oxidize. In silages prepared from fatty species such as mackerel, herring and sprats the FFA (as oleic acid) may reach 20% of the lipid content after 1 year at 23°C (Tatterson & Windsor, 1974). The increases have been attributed to the action of lipases, to the presence of the organic acids and to release of FFA at acid pH from water-miscible salts (Reece, 1980).

Oxidative changes have been followed in cold-water fish silages by Tatterson & Windsor (1974) who observed decreases in the iodine value of silage lipids during 1 year's storage. Johnsen & Skrede (1980) measured peroxide values (PV) in cod viscera silages and the values found were taken to indicate considerable oxidation of the lipids. However, the change in PV with time was not monitored so the results are of limited value as it is known that PV measure only the concentrations of intermediates in oxidative reactions. Disney *et al.* (1978) found that in dried tropical fish silage-maize/cassava mixtures stored under tropical conditions the PV increased after 1 month but after 3 months fell to values below the initial levels.

The nutritive value of tropical fish silages has been improved by lipid extraction and boiling (Kompiang *et al.*, 1979) and one might expect that the addition of antioxidants would also improve the quality but this has not been fully investigated. The present

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study was carried out to monitor the extent of lipid oxidation during ensilation of the tropical fish silverbelly and to study the effect on lipid oxidation of modifying the silage by lipid extraction or by antioxidant addition. The autolysis of proteins was also followed.

Materials and methods

The method of preparation of an unmodified silage has been described earlier (Hall *et al.*, 1985a) and such silages are referred to here as 'normal' silage. Three batches of the tropical fish silverbelly (*Leiognathus* spp.) were used to produce normal and modified silages.

Batch 1: Silages prepared from fish subjected to chloroform: methanol extraction

Freshly minced fish was separated into liquor and sediment by centrifugation (Hall *et al.*, 1985a) and the lipid in the sediment extracted with chloroform:methanol (C:M) according to the method of Bligh & Dyer (1959). Traces of solvent were removed from the sediment by gently heating on a water bath and the separated liquor, also freed of any lipid, added back to the sediment together with water equivalent to that removed from the sediment in the extraction process. Formic acid (98%) was added at 3% (w/w) and the defatted silage and a normal silage were incubated at 30°C for 14 days and sampled at 0, 4, 7, 10 and 14 days and the TBA value of the whole silage determined by the distillation method of Tarladgis *et al.* (1960). The hydroxyproline, total soluble nitrogen (TSN) and non-protein nitrogen (NPN) contents of the liquor were determined as previously described (Hall *et al.*, 1985a).

Batch 2: Silages prepared from fish subjected to isopropanol extraction or incorporating antioxidant

Freshly minced fish was extracted with isopropanol (IPA) in the ratio 1:5 (w/v) and traces of solvent removed by gentle heating on a water bath. The lipid free liquor from an active 4 day old silage was added to the defatted mince (1:10 liquor:mince) and formic acid (98%) added to give a pH of 3.75. A normal silage prepared from this batch of fish was divided into two portions to one of which was added butylated hydroxy anisole (BHA), in a little methanol, at 200 ppm (0.02%) of the lipid content of the silage. The three silages were incubated as described above and sampled at 0, 3, 7, 10 and 14 days. At each sampling the TBA value of the whole silage was determined according to Tarladgis *et al.* (1960) and the PV of C:M extracted lipid determined according to Banks (1937). The TSN and hydroxyproline contents of the liquor were determined as described above. The fatty acid composition of the silage lipid was also determined at 0 and 14 days.

Batch 3: Silages containing antioxidant

A normal silage and silage with added BHA (0.02% of the lipid) were prepared and the TBA value and PV determined at 0, 3, 7, 10 and 14 days. After 21 days the soluble hydroxyproline, soluble NPN and TSN were determined. The fatty acid composition of the silage lipid was determined after 0 and 21 days.

Determination of fatty acid composition

This was determined on the methyl esters of lipid extracted by the method of Bligh & Dyer (1959). A known weight of lipid was dissolved in chloroform (about 5 mg/ml),

1 ml was evaporated to dryness and 5 ml of methanol: c. sulphuric acid (95:5 v/v) was added. The mixture was heated at 70°C for 2 hr, 5 ml of water added and the aqueous solution was extracted with 3×2 ml of petroleum spirit (40–60° BPR). The collected extracts were washed with 5 ml of saturated sodium bicarbonate, evaporated to dryness and the residue taken up in 5 ml of iso-octane. Aliquots (2 μl) were subjected to gas chromatography with operating parameters: carrier gas argon (20 ml/min); column 2.1×2 mm i.d. packed with 3% SP2310/2% SP2300 on 100/120 mesh chromosorb W-AW; initial temperature 190°C for 2 min then rising at 2°C/min to 220°C; a flame ionisation detector was used. Identification of esters was by comparison of retention times with those of standard fatty acid esters.

Results and discussion

Effect of chloroform: methanol extraction

Extraction with C:M lowered the lipid content of the silage to about 0.1% (wet weight), when determined during ensilation by C:M extraction, compared with 2% (wet weight) in a normal silage produced from the same raw material.

Changes in TSN and soluble NPN are shown in Fig. 1. Both normal and defatted silages showed the typical changes in the rates of autolysis (Hall *et al.*, 1985a) but far less ocurred in the defatted silage. Although NPN represented a large proportion of the soluble nitrogen in both silages it was a far lower proportion in the defatted silage (61% compared with 81%). Changes in soluble hydroxyproline (g/16 gN), derived from collagenous tissue, are shown in Fig. 2. Here the release of hydroxyproline was apparently greater for the defatted silage but as the normal silage had a higher TSN content the actual amount released was similar for both silages. Thus far less proteolysis

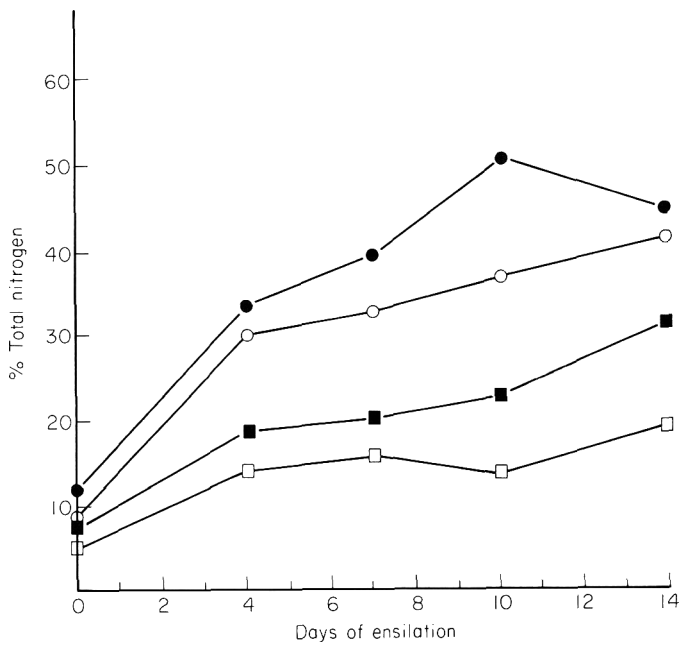

Figure 1. Changes in the TSN and soluble NPN (% of total silage nitrogen) during ensilation: normal silage TSN (–●–●–); normal silage soluble NPN (–O–O–); C : M defatted silage TSN (–■–■–); C : M defatted silage soluble NPN (–□–□–).

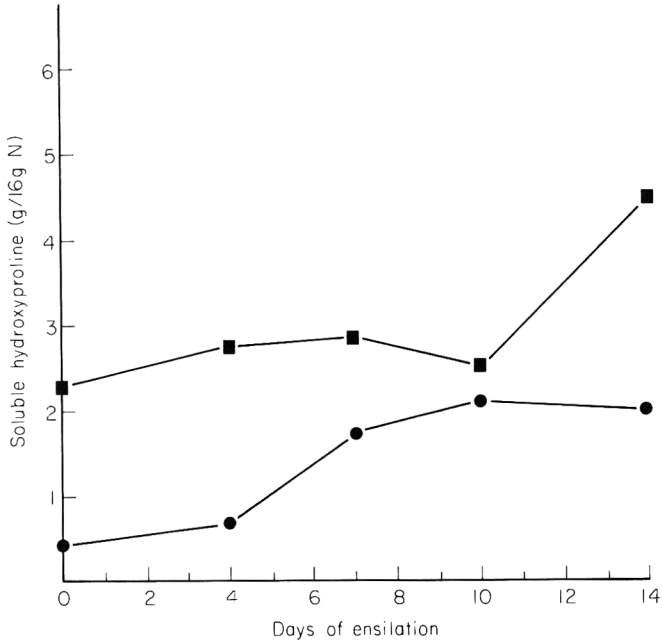

Figure 2. Changes in soluble hydroxyproline (g/16 g soluble nitrogen) during ensilation : normal silage (–●–●–): C : M defatted silage (–■–■–).

occurred in the defatted silage than the normal one although similar amounts of collagen were solubilized. If, as seems likely, the liquor in the present study contains active proteases then the limited autolysis achieved may be due to the denaturing effect of the C:M extraction process. Because little enzymic breakdown of collagen occurs during ensilation of silverbelly (Hall *et al.*, 1985a) its release will be less affected by the extraction process hence the observed effects on hydroxyproline solubilization.

Changes in TBA value are given in Table 1 and show that there was a rapid decline in the normal silage whilst the defatted silage had lower initial values which changed little on storage. The major reactant in the TBA determination is malonaldehyde (MA) and the decline in the normal silage could reflect its reaction with amino acids and proteins after which it cannot be detected by the TBA test (Buttkus & Bose, 1972). The TBA values found in the defatted silage might arise from the residual lipid, after C:M extraction. The chief source of MA is the oxidation of PUFA (Dahle, Hill & Holman,

Table 1. Change in TBA value during ensilation of a normal silage and a C:M defatted silage*

Days of ensilation	Normal	Defatted
0	22.2 (0.4)	2.5 (0.1)
4	0.5 (0.1)	5.5 (0.1)
7	6.1 (0.2)	3.1 (0.3)
10	7.7 (0.5)	3.1 (0.1)
14	5.1 (0.3)	2.5 (0.4)

*Results are quoted as mean (standard deviation) of duplicate determinations.

1962) which would be present in the residual lipids as these presumably include the tightly bound phospholipids found in membranes. Johnson & Skrede (1980) suggested that residual lipid after solvent extraction may be more susceptible to oxidation than untreated lipid in silages.

Isopropanol extraction

Raa & Gildberg (1976) showed that the sediment from an 8 day old silage, if extracted with IPA, could be further solubilized when suspended in the liquor of a freshly prepared silage. As chloroform: methanol extraction appears to inhibit ensilation (Fig. 1) extraction with IPA was studied. This lowered the lipid content of the silage to 1% (wet weight) when determined by C:M extraction during ensilation which suggests a milder treatment than C:M extraction. The addition of liquor from a 4 day old silage was done to ensure the presence of active proteases in the silage. Allowance was made for the presence of TSN and soluble hydroxyproline from this source in measuring autolysis in this silage (Figs 3 and 4 respectively). Essentially the same behaviour was found here for the normal silage and defatted silage as in the C:M investigation indicating that the milder extraction procedure was ineffective in preventing the inhibition of autolysis observed.

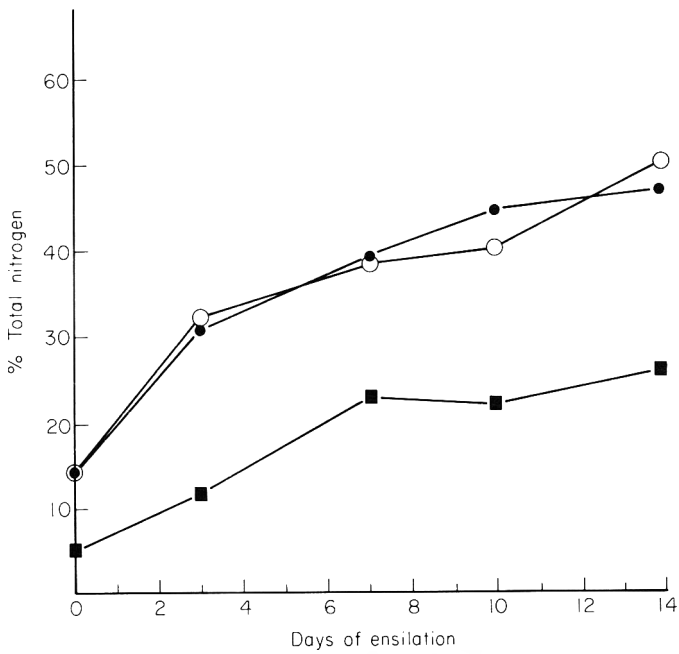

Figure 3. Changes in TSN (% of total silage nitrogen) during ensilation : normal silage (–●–●–); normal silage +BHA (–O–O–); IPA defatted silage (–■–■–).

It has been suggested that extensive autolysis is not necessary or even desirable to produce a silage with good nutrient value (Hall, Ledward & Lawrie, 1985b). All that is required is sufficient liquefaction of the fish tissues to allow mixing with a carbohydrate source before drying to produce a poultry diet. Therefore, lipid extraction before ensilation should improve the quality of the silage while still permitting sufficient autolysis to occur.

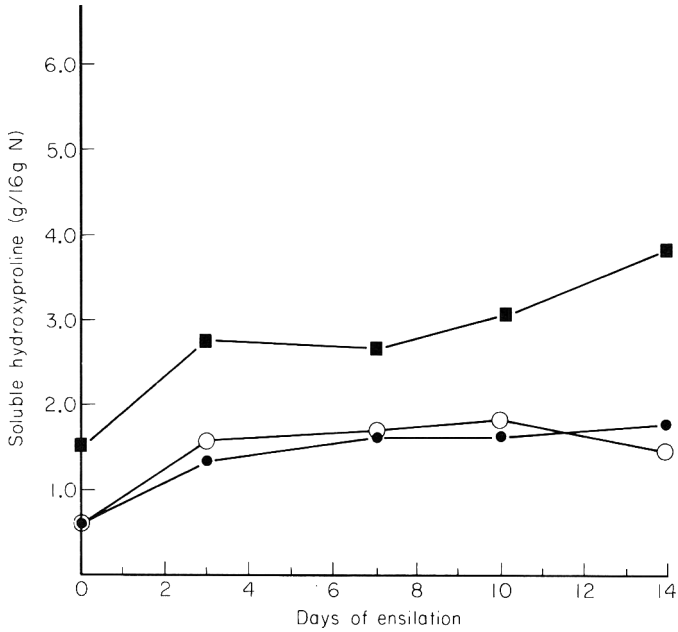

Figure 4. Changes in soluble hydroxyproline (g/16 g soluble nitrogen) during ensilation : normal silage (–●–●–); normal silage + BHA (–O–O–); IPA defatted silage (–■–■–).

Changes in TBA value and PV are given in Table 2. The normal silage shows a gradual if variable decrease in both indices during ensilation. The defatted silage shows higher TBA values and PV compared with the normal silage on prolonged storage. The greater proportion of residual lipid after IPA extraction, compared with C:M extraction, potentially susceptible to oxidation could account for the elevated values found for the IPA treated samples and the decrease in that treated with C:M. The difference between the TBA values of batches 1 and 2 could also reflect the variability of the raw

Table 2. Change in TBA value and PV (milliequivalents/kg of lipid) during ensilation of a normal silage, normal silage + BHA and an IPA defatted silage*

	Normal		Normal + BHA		Defatted	
Days of ensilation	TBA	PV	TBA	PV	TBA	PV
0	19.5	304.4	19.5	304.4	19.1	10.2
	(0.8)	(22.8)	(0.1)	(22.8)	(0.4)	(0.4)
3	20.3	65.8	5.9	18.6	19.5	88.3
	(0.5)	(2.5)	(0.1)	(2.1)	(0.4)	(3.2)
7	13.2	25.5	5.4	17.6	19.5	n.d.
	(0.5)	(2.3)	(0.1)	(1.9)	(0.8)	
10	21.1	48.5	5.5	16.6	16.7	75.9
	(1.6)	(1.6)	(0.6)	(3.2)	(2.8)	(3.0)
14	10.1	39.1	2.4	12.5	16.4	82.5
	(0.5)	(6.5)	(0.2)	(0.5)	(0.9)	(10.2)

n.d. = Not determined.

*Results are quoted as mean (standard deviation) of duplicate determinations.

material and emphasizes the need for both TBA values and PV to be measured over a period of time, not in isolation, to have meaning.

Changes in the fatty acid composition of the silage lipid are given in Table 3. For ease of interpretation the identifiable saturated acids (SA), monounsaturated acids (MUA) and the PUFA have been grouped together. Although not adding up to 100%, they represent a substantial proportion of the components extracted by C:M and separated by gas chromatography. The change in the proportion of these groups for a normal silage after 14 days is in agreement with the proposal (Labuza, 1971) that SA are not oxidized so their proportion increases, MUA oxidize slowly so their proportion increases slightly and PUFA oxidize rapidly so their proportion decreases. The lipid extracted from the 14 day defatted silage is that left behind by the IPA extraction and could have been susceptible to oxidation (Johnsen & Skrede, 1980). However, the pattern of oxidation is not directly comparable with that of a normal silage since the composition of a day 0 defatted silage is unknown.

Table 3. Fatty acid composition (% of total acids) of a normal silage, a normal silage+BHA and an IPA defatted silage after 0 and 14 days ensilation.

Fatty acid*	Day 0	Normal Day 14	Normal+ BHA Day 14	Defatted Day 14
C14:0	6.14	7.52	12.44	7.13
C16:0	14.76	16.31	24.99	24.07
C16:1	9.76	9.57	7.08	8.48
C18:0	9.68	10.91	10.02	13.43
C18:1	9.00	11.38	12.66	14.99
C18:2	1.56	1.66	1.21	1.56
C18:3†	0.87	0.87	1.21	Nil
C18:3‡	0.18	0.38	0.92	Nil
C18:4	1.32	1.05	1.09	0.96
C20:1	2.43	2.32	2.41	2.88
C20:4	5.61	4.18	1.81	2.25
C20:5	0.79	0.64	0.15	0.17
C22:1	5.98	5.25	4.88	5.47
C22:5	1.19	2.15	1.10	1.97
C22:6	9.09	7.07	3.71	4.86
Total saturated	30.57	34.73	47.65	44.63
Total mono-unsaturated	27.22	28.51	27.03	31.82
Total poly-unsaturated	20.62	17.98	11.02	12.13

*The acids are identified by the number of carbon atoms in the molecule: number of double bonds, the carboxyl carbon would be C1.

†γ Linolenic acid with double bonds at C6, 9, 12.

‡η Linolenic acid with double bonds at C9, 12, 15.

Effect of antioxidant addition

The addition of BHA to a normal silage, in batches 2 and 3, had no effect on autolysis. The typical behaviour is shown for batch 2 in Figs 3 and 4 for TSN and soluble hydroxyproline respectively. A similar trend was shown in batch 3 and after 21 days

final levels were: TSN 48.6 and 48.7 (% of total nitrogen); NPN 39.7 and 42.6 (% of total nitrogen); soluble hydroxyproline 1.59 and 1.59 (g/16 g soluble N) for the normal and normal+BHA silages respectively.

Changes in TBA value and PV for the silages+BHA are given in Table 2 (batch 2) and Table 4 (batch 3). In both cases the TBA value and PV declined to values less than those found for normal silage. The addition of an antioxidant to an actively oxidizing system cannot destroy peroxides or their breakdown products or ameliorate their destructive effects but may prevent further build up of both these reactive species (Labuza, 1971). Changes in fatty acid composition (Tables 3 and 5) indicate similar behaviour in both silages+BHA compared with the normal silages. Thus, the oxidation of PUFA appears to be greater in the silages+BHA and the proportion of SA increases markedly compared with the normal silages. Phenolic antioxidants (such as BHA) act by stopping the free radical chain mechanism of lipid oxidation (Labuza, 1971) so it is consistent with this action that evidence of PUFA oxidation (see Tables 3 and 5) is associated with low TBA values and PV (see Tables 2 and 4). Changes in the proportions of each group of acids for the normal silages in batch 2 (Table 3), and batch 3 (Table 5) are similar for PUFA and MUA but there is no increase in SA for batch 3 during ensilation.

Table 4. Change in TBA value and PV (milliequivalents/kg of lipid) during ensilation of a normal silage and a normal silage+BHA*

	Normal		Normal+BHA	
Days of ensilation	TBA	PV	TBA	PV
0	16.8	43.5	16.8	43.5
	(1.9)	(1.6)	(1.9)	(1.6)
3	15.6	101.7	3.0	34.4
	(0.1)	(7.2)	(0.2)	(2.2)
7	13.6	63.3	2.2	31.8
	(0.2)	(7.2)	(0.1)	(0.6)
10	9.5	98.6	1.9	20.5
	(0.3)	(2.4)	(0.2)	(0.2)
14	9.9	83.9	1.5	23.9
	(0.1)	(10.1)	(0.1)	(4.2)

*Results are quoted as mean (standard deviation) of duplicate determinations.

Conclusions

Lipid oxidation takes place actively during ensilation proceeding in parallel with autolysis. This though does not lead to increased TBA values suggesting that the MA formed may condense with the proteins and amino acids in the mixture lowering their nutritional value (Gardener, 1979). The build up of lipid oxidation products can be limited by the addition of an antioxidant or lipid extraction before ensilation. Both treatments have drawbacks in that the antioxidant must be added before oxidation is advanced and lipid extraction must be thorough as the residual lipid may be very susceptible to oxidation. Autolysis is limited in the defatted silages although this does not affect the liquefaction of fish tissues and may well be beneficial (Hall *et al.*, 1985b).

Table 5. Fatty acid composition (% of total acids) of a normal silage and a normal silage+BHA after 0 and 21 days ensilation.

Fatty acid*	Normal		Normal+BHA
	Day 0	Day 21	Day 21
C14 : 0	7.69	9.44	18.03
C16 : 0	21.32	20.10	22.27
C16 : 1	8.81	10.56	11.19
C18 : 0	9.56	9.04	8.92
C18 : 1	9.62	7.95	8.01
C18 : 2	1.12	1.43	0.15
C18 : 3†	0.31	0.59	0.15
C18 : 3‡	0.09	0.59	0.11
C18 : 4	0.96	1.15	0.61
C20 : 1	1.54	1.73	1.57
C20 : 4	4.86	3.93	3.44
C20 : 5	0.44	0.61	0.15
C22 : 1	6.63	6.58	6.21
C22 : 5	2.74	1.87	Nil
C22 : 6	10.27	7.28	Nil
Total saturated	38.57	38.58	49.22
Total mono-unsaturated	26.60	26.82	26.98
Total poly-unsaturated	20.79	17.45	4.61

*The acids are identified by the number of carbon atoms in the molecule: number of double bonds, the carboxyl carbon would be C1.

†γ Linolenic acid with double bonds at C6, 9, 12.

‡η Linolenic acid with double bonds at C9, 12, 15.

In small scale operations in developing countries production of a dried poultry diet without complicated manipulations or use of expensive additives is desirable. To this end, further studies should be devoted to understanding the interplay between lipid oxidation products and protein breakdown products under the conditions of diet production. The nutritional consequences of such reactions should also be investigated.

Acknowledgments

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Macromolecular changes associated with the heat treatment of soya isolate

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Summary

The denaturation temperatures of both the 7S and 11S proteins present in a 70 PDI soya flour and an isolate prepared from it, increased by over 30°C as the water content of the system decreased from 90 to 20%. In dilute dispersions (> 70% water), the precipitate formed on heat treatment was almost totally soluble in 3.0% sodium dodecyl sulphate (SDS), irrespective of the heating temperature (20–120°C). However, in more concentrated systems, heat treatment at 120°C for 1 hr caused increasing amounts of SDS insoluble protein to form as the water content decreased. Such aggregates were also insoluble in beta-mercaptoethanol (ME), but soluble in mixtures of SDS plus ME.

It is suggested that at low concentrations, heat treated soya proteins are primarily aggregated by hydrophobic interactions but that, on decreasing the water content of the system, disulphide bond formation becomes a significant factor in stabilizing the aggregate.

Introduction

Soya protein in the form of soya isolate, concentrate, flour or grits is usually used as an ingredient in several food products, and as such its chemistry and reactivity in both model and real food systems has been widely researched. Heat treatment is known to bring about denaturation of the two major proteins, the 7S and 11S fractions, at about 80 and 100°C, respectively, in 10% dispersions (Hermansson, 1978). It is also well established that for several proteins, such as collagen (Finch & Ledward, 1972) and myoglobin (Hagerdal & Martens, 1976), the temperature of the denaturation process increases and the enthalpy decreases with decreasing water content. It is possible that the denaturation characteristics of the soya proteins exhibit a similar concentration dependence.

In the extrusion processing of soya flour or grits, the moisture content of the feed is usually between 20 and 40% and the systems are subjected to high temperatures, pressures and shear rates. There is little information on the changes undergone by the macromolecules in these systems, although it is normally assumed that following heat denaturation the proteins are aligned in the barrel of the extruder where they aggregate to yield, on expulsion from the extruder and the consequent evaporation of superheated water, a textured product.

The present study was undertaken to gain some insight into the denaturation and aggregation behaviour of soya proteins subjected to heat processing at different moisture contents, results that should aid our understanding of the types of reactions undergone by such systems during extrusion.

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Materials and methods

Soya isolate was prepared as outlined by Wolf (1970) from a 70% PDI flour to give a final dispersion containing about 6% protein.

Heat processing

The original dispersion (about 6% protein) was subdivided and, after packing into cans and sealing, the aliquots were heated for 1 hr by immersion in water baths at the appropriate temperature (60, 80 and 100°C), or by processing in a static retort for 1 hr (120°C). One sample received no heat treatment and samples of this, after freeze drying, were processed at 120°C at varying moisture contents (5–90%). After processing, the isolates were recovered from the solutions by freeze drying. All consisted of about 85% protein and about 5% water.

Scanning calorimetry

This was carried out on a Perkin Elmer DSC 2 with dispersions, doughs or powders of the flour and isolates containing from 5 to 94% moisture. The heating rate was 5°C/min. The moisture content of the original material was taken into account in calculating the moisture content of each system.

Solubility studies

The solubilities of the isolates obtained on freeze drying the 6% dispersions after heating at 60, 80, 100 and 120°C, were determined in distilled water, 3.0% sodium dodecyl sulphate (SDS), and 1.0% mercaptoethanol ME plus 3.0% SDS by shaking 2.0 g of the powder with 98 ml of solution for 16 hr at 20°C. After centrifugation at 38000 g, the nitrogen content of the aqueous phase was determined by Kjeldahl analysis.

The solubilities of isolates subjected to processing at 120°C for 1 hr at a range of moisture contents (5–90%) were determined in both 3% SDS and 1% ME at a ratio of 1 part isolate to 50 parts solvent (w/w).

Results

As expected two endothermic peaks were seen on the thermograms of the unheated isolate and flour (Fig. 1), corresponding to the 7S and 11S fractions of the protein. The best estimate of the denaturation temperature of a protein is given by the onset temperature of the peak (Finch & Ledward, 1972) but as thermograms of foodstuffs are generally complex this parameter is usually difficult to determine. Thus it has become general practice to quote the peak temperature, T_m, as the denaturation temperature (Poulter *et al.*, 1985) and this convention was adopted in the present work.

It is seen from Figs 2 and 3 that T_m is little affected by the moisture content of the system up to an isolate, or flour, concentration of about 40%, at which level T_m for both the 7S and 11S fractions increases quite sharply.

Thermograms obtained of 50% solutions of the isolates preheated to 60, 80, 100 and 120°C indicated that at 80°C the 7S fraction was totally denatured but the endothermic peak associated with the 11S fraction was only lost on heating to 120°C for 1 hr. Heating at 60°C caused little apparent change in the thermograms. As expected from Fig. 3, on heating the dry powder (about 5% moisture) at 120°C for 1 hr the endothermic peak associated with the 11S fraction was still seen on subsequent calorimetric analysis.

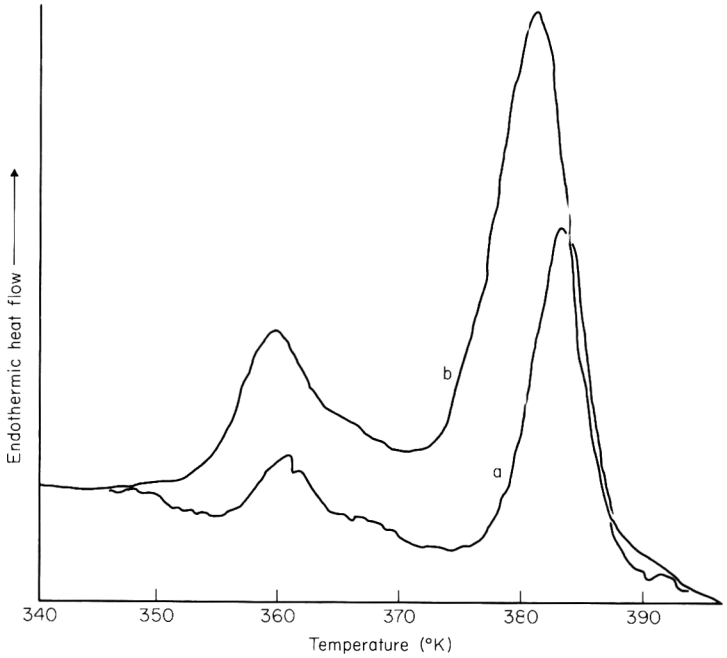

Figure 1. Thermograms of (a) 13.6 mg of a 50% dispersion of the 70PDI soya flour at a sensitivity of 0.2 mcal/sec and (b) 28.4 mg of a 50% dispersion of the isolate prepared from it at a sensitivity of 0.5 mcal/sec.

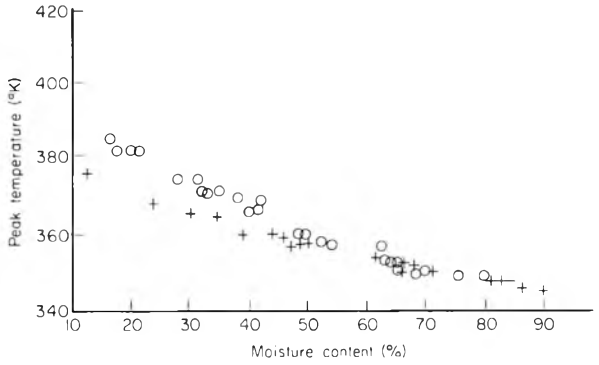

Figure 2. The effect of the moisture content of the system on the peak temperature, T_m, for the 7S proteins of 70PDI soya flour (O) and the isolate prepared from it (+).

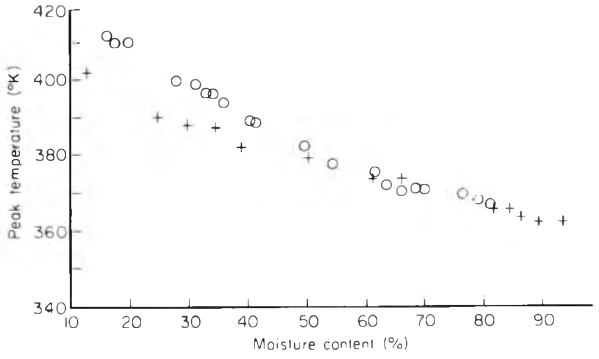

Figure 3. The effect of the moisture content of the system on the peak temperature, T_m, for the 11S proteins of the 70PDI flour (O) and the isolate prepared from it (+).

Solubility studies

It is seen from Table 1 that as the 6% dispersions of the isolates were heated there was a progressive loss of water solubility with increasing heat treatment. This loss in solubility was not observed in solutions of 3% SDS (and 3% SDS plus 1% ME) (Table 1). Thus, even after heat treatment at 120°C for 1 hr, the precipitate formed in 6% solutions of the isolate is still totally soluble in 3% SDS. It is known though that soya extrudates formed at 120°C are only partially soluble in 3% SDS although they are almost totally soluble in mixtures of 3% SDS and 1% ME at a protein content of 2% (Sheard, Ledward & Mitchell, 1984). Thus, the effect of the moisture content of the isolate during heating at 120°C on its subsequent solubility was investigated.

Table 1. Percent solubility in distilled water, 3.0% SDS or 3.0% SDS+1.0% ME of a laboratory prepared soya isolate after heat treatment at 60, 80, 100 or 120°C for 1 hr*

Processing temperature (°C)	Water	3% SDS†	3% SDS+1% ME†
20	95.4±0.2	99.3±0.1	95.3±1.0
60	63.4±0.4	96.6±0.3	94.1±0.1
80	33.2±0.3	93.7±0.3	92.2±0.6
100	21.6±0.4	87.1±0.7	92.2±0.6
120	23.7±0.1	92.5±0.3	96.6±0.1

*During heat processing the protein content of the solutions was about 6%.

†Each value is the mean±standard error of three determinations.

Such results are shown in Fig. 4 where it is seen that following heating at moisture contents in the range 10–90%, the solubility of the protein, in both 3% SDS and 1% ME, decreases as the moisture content decreases until a moisture content of about 20% is reached, at which point the solubility increases quite dramatically.

Discussion

It has previously been shown that following processing under typical conditions, a soya extrudate can be almost completely solubilized in 3% SDS plus 1% ME suggesting that no non-disulphide covalent links are involved in the formation of the aggregate (Sheard *et al.*, 1984). The present work though has shown that on heating 6% solutions of undenatured isolate at up to 120°C all the bonds leading to aggregation are SDS labile.

SDS is assumed to rupture hydrogen bonds and hydrophobic interactions, and these presumably stabilize the aggregates formed when these dilute solutions are heated. Thus, even at 120°C few, if any, disulphide crosslinks are formed in these dilute systems (Table 1). This observation also appears to be true up to isolate concentrations of about 30–40% (Fig. 4). However, as the moisture content increases still further there is a significant increase in the concentration of SDS resistant linkages (Fig. 4) and as it is known that at these protein concentrations soya extrudates are almost totally soluble in mixtures of SDS plus ME, it would suggest that disulphide linkages start to reach a

significant concentration in the aggregate at these water contents. It is of interest that the observed increase in disulphide bonding in the aggregates becomes apparent at similar moisture contents to those at which significant increases in stability of the native protein are seen i.e. 30–40% water. The similarity of the profiles seen for the flour and isolate suggest that water is the limiting factor in the denaturation and not the protein content, which is 1.5–2 times greater in the isolate than the flour systems at equivalent water contents.

As might be expected, solubility in ME is always less than in SDS and decreases sharply with decreasing water content of the system (Fig. 4), indicating increased formation of non-disulphide linkages at lower water content.

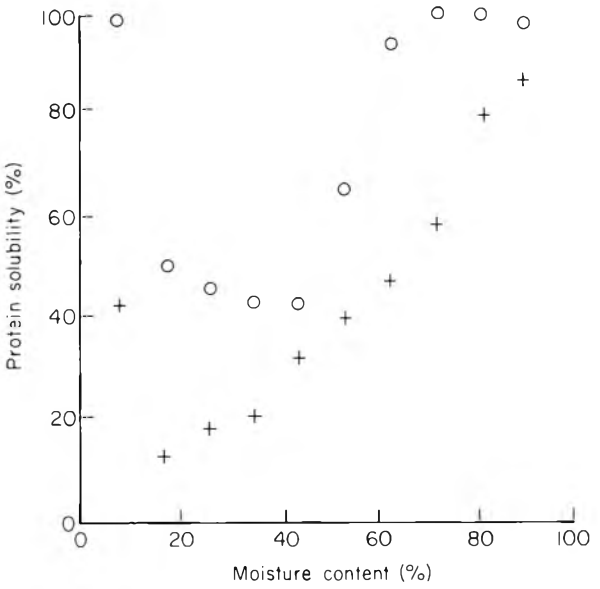

Figure 4. Nitrogen solubility in 3.0% SDS (O) or 1.0% ME (×) of a laboratory prepared soya isolate after heat treatment for 1 hr at 120°C in the presence of varying amounts of water.

Figure 4 shows that the dry powder heated at 120°C is still relatively soluble in both SDS and ME and this may well be related to the observation that the 11S fraction is not apparently denatured under these conditions. This suggests that denaturation of the proteins is a necessary prerequisite leading to insolubility. However, even after heating 6% solutions at 60°C some loss of water solubility is seen (Table 1) suggesting that some conformational and aggregational changes take place on prolonged heating at temperatures below that leading to denaturation. This requires further study.

It is interesting to note that T_m for the proteins appears to be more dependent on the water content of the system in the flour than the isolate, suggesting that the carbohydrate-water carbohydrate-protein interactions play a significant role in the transitions.

In the present study only the temperature and the moisture content of the systems have been varied and thus care must be exercised in relating these results to the changes taking place during such processes as extrusion cooking where other variables are involved. However, the marked dependence of the denaturation temperature of the

proteins on the moisture content of the systems and, more importantly, the types of linkages formed between the denatured molecules may well explain, at least to some extent, the wide variations in hydration and textural properties of extrudates formed from feeds of slightly different moisture contents and of the feeds of the same moisture content at different temperatures (Sheard, Mitchell & Ledward, 1985).

Acknowledgment

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Extrusion of corn grits containing various levels of hydrocolloids

J. A. MAGA AND O. O. FAPOJUWO

Summary

Fourteen hydrocolloids, locust bean, agar, guar, alginate, tragacanth, carrageenan, xanthan, pectin, gelatin and Methocels A4M, E4M, F4M and K4M were dry blended with corn grits at levels of 0.1, 0.5 and 1.0%. Twenty percent moisture was added and each product extruded in a Brabender Model PL-V500 laboratory extruder at temperatures of 50, 75, 100, 125 and 150°C with a 1:1 screw operating at 100 rpm. Locust bean, agar, guar, alginate, arabic and Methocels E4M, F4M and K4M significantly lowered torque at an extrusion temperature of 50°C, but did not in general produce significant reductions at higher extrusion temperatures. In contrast, tragacanth, carrageenan, xanthan, pectin, gelatin and Methocel A4M did not significantly reduce torque over the concentrations and conditions evaluated during the extrusion of corn grits. All the Methocel derivatives lowered yield at low extrusion temperatures. No gum significantly increased yield over the entire extrusion temperature range evaluated. Therefore, certain gums can significantly reduce torque without influencing yield during the extrusion of corn grits.

Introduction

Energy costs are rapidly increasing and becoming a larger portion of the production costs in food processing. However, with extrusion food processing, a certain portion of energy costs can be controlled (Harper, 1981). The studies by Harmann & Harper (1973) and Bruin, Van Zuilichem & Stolp (1978) have shown that power consumption (or torque) can be decreased during extrusion by increasing temperature and moisture content. However, it is not practical to depend on these alterations for decreasing total energy cost as it may sometimes be necessary to extrude at lower temperature and moisture content to obtain desired functional properties. Therefore, it is necessary to investigate other ways of lowering energy requirements during extrusion.

Hydrocolloids (gums) have as one of their major properties the ability to impart viscosity to a fluid system. The type of viscosity or flow behaviour of various hydrocolloids differs, and it appears that some hydrocolloids can alter viscosity in such a way as to decrease torque (Smith, Mitchell & Ledward 1982; Boison, Taranto & Cheryan, 1983; Berrington *et al.*, 1984). All these groups have reported that alginate, under certain extrusion conditions, did modify the torque required to extrude soy flour. Boison *et al.* (1983) and Berrington *et al.* (1984) also reported on using certain cellulose derived hydrocolloids in association with the extrusion of soy flour. In addition, Berrington *et al.* (1984) evaluated pectins, guar, locust bean and carrageean, but under limited extrusion conditions. Glicksman (1984) has reported that hydroxypropyl

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cellulose derivatives have the ability to increase output by lowering energy requirements. Also, Ganz (1973) patented the use of hydroxypropyl cellulose in extrusion applications.

Therefore, the primary objective of this study was to evaluate a wide variety of commercially available hydrocolloids for their potential to influence torque over a wide range of conditions during the extrusion of corn grits.

Materials

Commercially available dehulled and degerminated yellow corn grits were used as the base material. Samples of agar, alginate, arabic, carrageenan, gelatin, guar, locust bean, pectin, tragacanth and xanthan hydrocolloids were obtained from a major commercial supplier. The cellulose derived hydrocolloids, Methocel A4M, E4M, F4M, and K4M which possess similar viscosity properties but differ in methoxyl and hydroxypropyl residues, were obtained from Dow Chemical Co., Midland, Michigan, U.S.A.

Pre-extrusion blending

Corn grits were dry mixed with either 0.1, 0.5 or 1.0 weight percent of each of the fourteen hydrocolloids for 10 min in a Paterson-Kelly model LB-P-8 twin shel. blender, followed by the 20% addition of 20°C tap water and mixed for another 20 min.

Extrusion conditions

A Brabender Plasticorder Extruder Model PL-V500 was used. The barrel had a diameter of 19.05 mm with a 20:1 length to diameter ratio and was rifled with eight 0.79×3.18 mm longitudinal groves. A 1:1 compression ratio screw was used along with a 1.98 mm diameter by 1.27 cm length die. The unit was equipped with a variable speed drive, a tachometer, and a balance-type torque indicator. The barrel was equipped with electrically-heated, compressed air-cooled collars controlled by thermostats to maintain desired feed, mix and die temperatures to ±2°C. Runs were performed at barrel temperatures of 50, 75, 100, 125, and 150°C. Screw speed was maintained at 100 rpm for all runs. In addition, for each temperature series a non-hydrocolloid control was run.

Experimental design and statistical evaluation

The hydrocolloid/temperature combinations evaluated represented 215 individual extrusion runs which were repeated a total of three times thus resulting in a total of 645 individual runs conducted over a four week period. The experimental design is summarized in Table 1. Torque values were recorded directly from the extrusion unit, combined for each of the three replications and the data subjected to least significant difference (l.s.d.) analysis with $\alpha = 0.05$.

Table 1. Variables evaluated*

Variable	Level
Hydrocolloids (14 different)	0, 0.1, 0.5, 1.0% w/w
Extrusion temperature	50, 75, 100, 125, 150°C
Screw configuration/speed	Constant (1:1, 100 RPM)
Die size	Constant (1.98 mm)
Feed moisture	Constant (20% w/w added)

*With three replicates represents 645 runs.

Results and discussion

Torque

Figure 1 summarizes the effect of different concentrations of various hydrocolloids on torque during the extrusion of corn grits at different extrusion temperatures. In general, increasing extrusion temperature decreased torque. This observation agrees with the results obtained with the results of other groups (Harmann & Harper, 1973; Bruin *et al.*, 1978).

Among the fourteen hydrocolloids studied, only locust bean, agar, guar, alginate, arabic and Methocels E4M, F4M, and K4M significantly lowered torque during extrusion. All of these gums showed significant reduction of torque only at the low extrusion temperature of 50°C except Methocels E4M, F4M and K4M which reduced torque up to an extrusion temperature of 100°C.

Agar, alginate, arabic and Methocels E4M, F4M, and K4M reduced torque significantly, but only at the highest level of addition (1%). Locust bean and guar on the other hand significantly lowered torque at the lower levels of addition. Tragacanth, carrageenan, xanthan, pectin, gelatin and Methocel A4M did not significantly reduce torque at all levels of addition and at all extrusion temperatures.

It is interesting to note that Methocels of E4M, F4M and K4M, which contain increasing amounts of hydroxypropyl groups, did lower torque, whereas A4M did not. Thus, it appears that the hydroxypropyl group is needed for methylcellulose-based gums to lower torque during the extrusion of corn grits.

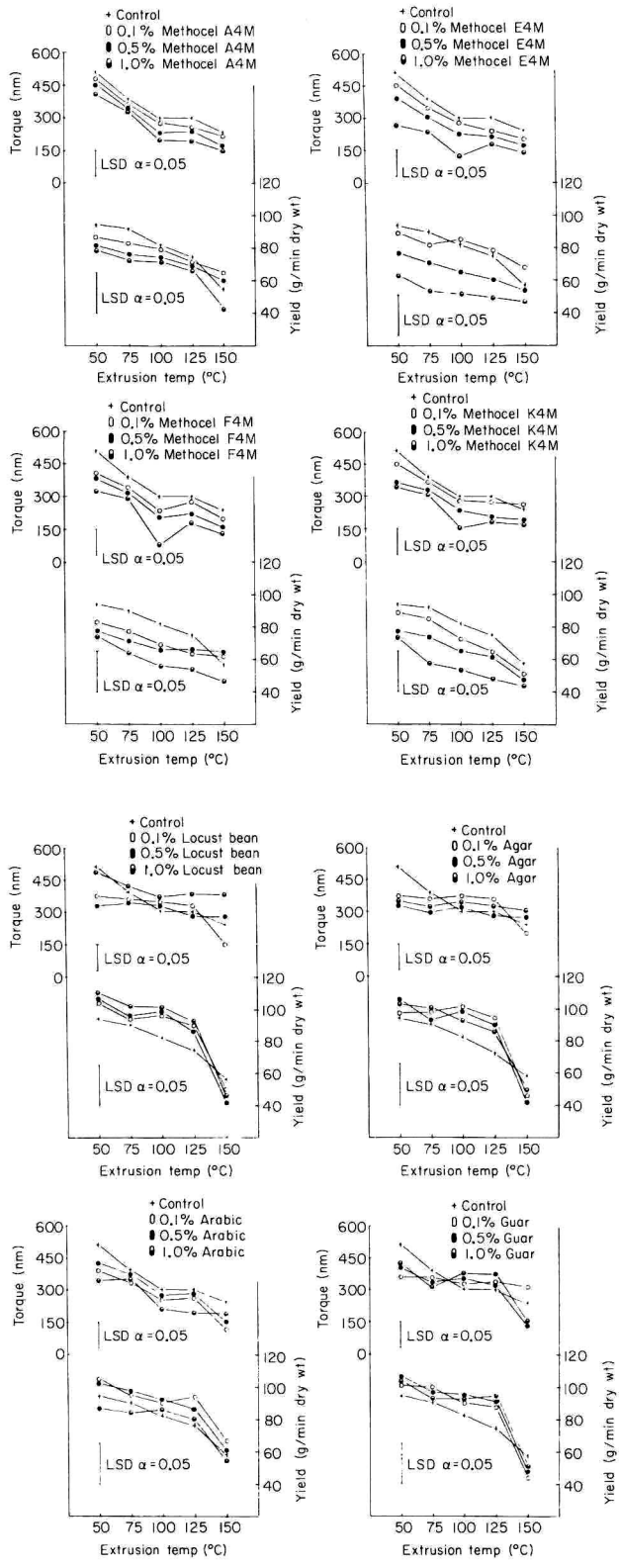
Results obtained in this study for alginate and Methocel A4M are different from those reported by Boison *et al.* (1983). This difference might be due to the differences in the materials that were extruded in the two studies. The defatted soy flour extruded by Boison *et al.* (1983) obviously contained a higher protein content than the corn grits that were extruded in this study. Thus it appears that the ability of a hydrocolloid to lower torque during extrusion may depend on its interaction with the constituents being extruded. Further studies will be necessary to study the specific interactions of hydrocolloids with proteins, carbohydrates and lipids during extrusion to understand this interaction more fully.

Yield

Data on yield are also presented in Fig. 1. Increasing extrusion temperature generally decreased yield. Addition of Methocels A4M, E4M, F4M and K4M decreased yield while a slight but not significant increase in yield was observed for the other hydrocolloids. Thus the advantage of torque reduction by the Methocels appears to be nullified by their resultant decrease in yield. Therefore, the energy saved by reduced torque may still have to be supplied to achieve equal yield as when these gums are not added.

Since locust bean, guar, arabic, alginate and agar lowered torque and did not decrease yield, they may have application in lowering power requirements during the extrusion of corn grits at low extrusion temperatures where they appear to be most effective.

Several other factors need to be calculated and/or evaluated before the overall contribution of gum addition to extruded corn grits can be predicted. For example, the cost of added gum *versus* the reduction in production cost due to lower energy requirements needs to be considered. In addition, the method of gum addition needs to be evaluated. In this study, the simplest form of addition (dry blending) was utilized.


+ Control
0 0.1% Methocel A4M
0.5% Methocel A4M
1.0% Methocel A4M
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0 0.1% Methocel E4M
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1.0% Methocel F4M
+ Control
0 0.1% Methocel K4M
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1.0% Methocel K4M
+ Control
0 0.1% Locust bean
0.5% Locust bean
1.0% Locust bean
+ Control
0 0.1% Agar
0.5% Agar
1.0% Agar
+ Control
0 0.1% Arabic
0.5% Arabic
1.0% Arabic
+ Control
0 0.1% Guar
0.5% Guar
1.0% Guar
Torque (nm)
Yield (g/min dry wt)
LSD α=0.05
Extrusion temp (°C)

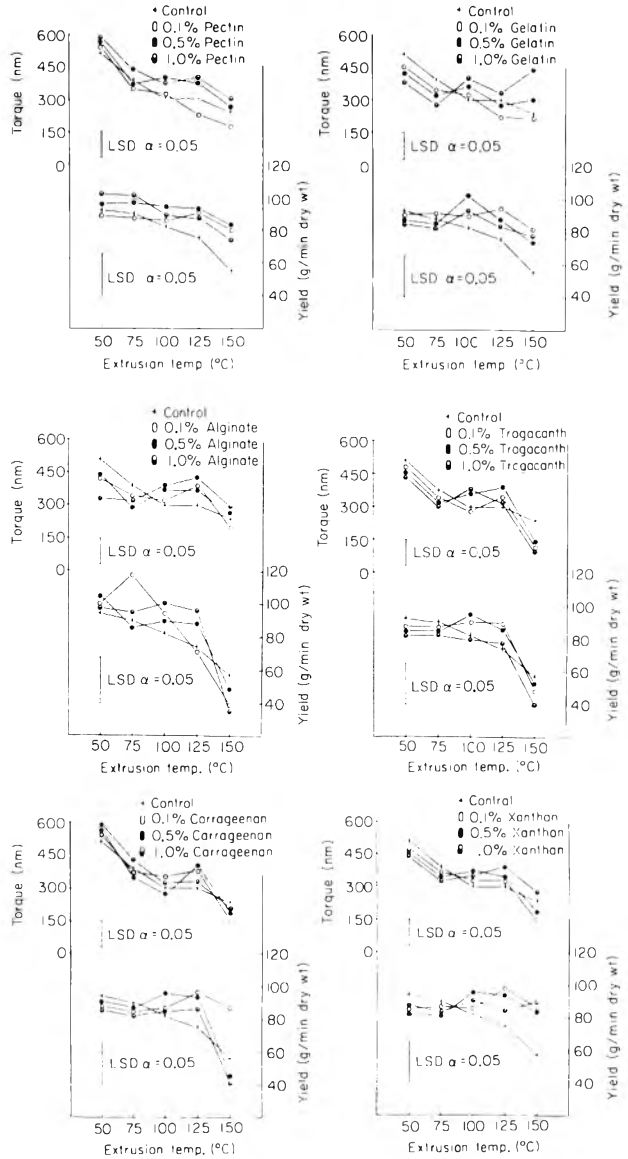

Figure 1. Influence of extrusion temperature and hydrocolloid type/amount on torque and product yield.

Perhaps different results would have occurred if the gums were rehydrated or reconstituted prior to extrusion. Also, the effect of added gums on the physical, chemical and functional properties of the extruded product needs to be studied.

Results obtained in this study indicate that certain hydrocolloids can lower torque and thereby lower energy requirements during extrusion. It appears that the usefulness of a particular hydrocolloid in lowering torque may depend on several factors including the type of product being extruded and the temperature of extrusion. An understanding of the interactions of hydrocolloids with food constituents during extrusion and the effects on the properties of the extruded product should make it possible to choose the correct gum for a particular extrusion process. Work continues in our laboratory in this direction. Use of hydrocolloids to lower power consumption during extrusion may be of significant economic importance to food industries utilizing the extrusion process.

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Shrinkage in canned mushrooms treated with xanthan gum as a pre-blanch soak treatment

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Summary

Vacuum treating freshly harvested mushrooms with a 1% xanthan gum solution (XVT) containing 0.25% sodium metabisulphite (SMBS) prior to blanching and canning gave a lower shrinkage value than for corresponding samples vacuum treated with water, or those canned by conventional means or the 3S process. A combination of chill storage (2–4°C) for 1–3 days coupled with 1% XVT was found best and gave even lower total canning losses (chill storage loss + blanch loss + retort loss); these were 6% lower than in the 3S process and 11.5% lower than for mushrooms canned by the conventional procedure. These data suggest that xanthan gum has considerable potential for reducing shrinkage in canned mushrooms. The XVT canned samples had an excellent colour and an acceptable texture. Further storage tests over a 9 month period with xanthan treated canned mushrooms showed than an SMBS level of 0.1% in the pre-blanch soak solution maintained an excellent colour in the canned product. No in-can mushroom shrinkage took place during the 9 month storage test.

Introduction

Shrinkage during processing is a major problem for the mushroom canner and has been the subject of considerable research in a number of countries (Beelman, Kuhn & McArdle, 1973; Gormley & MacCanna, 1980; Singh *et al.* 1982). Studies on the effects of different strains (Gormley & MacCanna, 1980) and the use of the 3S and a modified 3S process (Gormley & Walshe, 1982) on reducing shrinkage in canned mushrooms have been carried out in this laboratory recently. It was found that the 3S process in combination with other treatments was very effective in reducing shrinkage in canned mushrooms although there were some colour (slight browning) and texture (slightly too tough) problems. In tests at Kinsealy Research Centre in 1983 the incorporation of xanthan gum into mushrooms using a vacuum soak prior to blanching greatly reduced blanching losses in mushrooms for freezing (Gormley, 1984). This in turn led to the present study which investigated shrinkage in canned mushrooms treated with xanthan gum as a pre-blanch soak treatment.

Xanthan gum is a microbial polysaccharide produced in sugar fermentation medium by the bacteria *Xanthomonas campestris*. It is a heteropolysaccharide composed of the monomer units mannose, glucose and glucuronic acid and has a molecular weight of several millions. It is a permitted food additive in Ireland.

In the study described below there were three distinct experiments. Experiment 1 involved comparing the effect of xanthan gum (0.5 or 1.0%) applied as a pre-blanch soak treatment on mushroom blanch and retort losses; the data were compared with those for mushrooms canned by the standard procedure and also by the 3S process. The

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effect of length of chill storage between harvesting and canning on the performance of xanthan gum in reducing shrinkage was assessed in experiment 2. Experiment 3 was a long term trial and assessed the effects of different levels of sodium metabisulphite (SO_2) on canned mushroom colour and texture and also on changes in drained weight in canned xanthan gum treated mushrooms over a 9 month period.

Materials and methods

White strain (*Agaricus bisporus*) commercially grown mushrooms were used for all tests. The xanthan gum (Keltrol, Kelco/AIL International) and sodium metabisulphite (SMBS) were applied to the mushrooms as a solution. Mushrooms in the solution were subjected to a vacuum of 75 kPa for 0.5 hr. The samples were then washed, weighed, blanched (5 min in boiling water with 1.5% citric acid), cooled in water for 5 min and canned in 1.5% brine in plain cans (300×408) with lacquered ends at 121°C for 0.5 hr. The samples were assessed 2–3 weeks after canning, for drained weight (after 4 min), whiteness (Hunter L on five mushrooms—2.5 cm aperture) and shear press value (standard test cell, 100 g of whole mushrooms). Departures from this procedure (where they occur) are given below. Samples were compared with those (the control) canned by the standard procedure, which was the same as that above except that no pre-blanch soak treatment was given, and also with those prepared using the 3S process (Gormley & Walshe, 1982).

Experiment 1: Xanthan gum versus *3S treatment* versus *control*

The effect of xanthan gum (0.5 or 1.0% solution), applied as a pre-blanch vacuum soak treatment, on mushroom blanching and retort losses was compared with data for mushrooms canned by the standard procedure (control) and by the 72 hr 3S process (Gormley & Walshe, 1982). Batches of mushrooms (1.2 kg) each were vacuum treated with water, 0.5% xanthan gum or 1% xanthan gum solution on day 0 (day of harvesting) as described above. Each treatment also contained 0.25% SMBS. These treatments were compared with a control (water wash only—no vacuum treatment) and with mushrooms treated by the 72 hr 3S process; the soak solution for the 3S process also contained 0.25% SMBS. There were three replicates for each treatment and the canned mushrooms were evaluated as outlined above.

Experiment 2: Effect of chill storage/xanthan treatment

Experiment 1 was conducted using day 0 (harvested and processed the same day) mushrooms. However, in commercial practice mushrooms tend to be older post-harvest when processed. In experiment 2 batches of mushrooms were held for 0, 1, 2 and 3 days at 2–4°C prior to canning in order to study the effects of such a delay on overall weight loss and on canned product colour and texture. The term overall weight loss comprises weight loss during chill storage coupled with blanching and retort losses. In addition to the four time treatments there were three processing treatments, i.e., vacuum soak in water (WVT), vacuum soak in 1% xanthan gum solution (XVT), and the 72 hr 3S treatment. The level of SMBS in all water/solution soak treatments was 0.25% and blanching and retorting conditions were as outlined above. There were two replicates for each treatment, giving a 4×3×2 situation or 24 portions; lot size prior to storage and processing was 1.2 kg.

Experiment 3: Long term colour and shrinkage test

This test commenced in May 1984 and concluded in February 1985. It was felt that the SMBS level of 0.25% used in the soak water/solutions in experiment 1 and 2 was rather high and so it was decided to look at the effect on canned mushroom colour of using levels of 0, 0.05, 0.10 and 0.25% in the 1% xanthan gum solution. This experiment also afforded the opportunity of studying any further changes in mushroom shrinkage in-can over an extended period. The test involved the use of day 1 (i.e., chill stored for 24 hr at 2–4°C) mushrooms and the experimental design was four SMBS levels, four testing dates and three replicates. The canned samples were evaluated on 18 May, 7 July and 30 November in 1984 and finally on 15 February 1985 for drained weight, colour and shear value as outlined above. The samples were also tested for SO_2 content and pH value; 200 g portions were pureed and were placed in a Monier Williams apparatus for SO_2 analysis (Pearon, 1962). pH was measured on the liquor drained from the mushrooms in each can. It became apparent during the storage period that the higher levels of SMBS were causing some de-tinning. This was quantified in physical terms on the November and February tests dates by inverting the drained cans over the 5 cm aperture of the Hunter meter and measuring reflectance (L) values.

Results

Experiment 1: Xanthan gum versus *3S treatment* versus *control*

Vacuum soaking gave a larger water/solution uptake than the soak treatments of the 3S process (Table 1). The 1% XVT gave the smallest ($P < 0.001$) blanch loss followed by the 3S process and the 0.5% XVT. The effect of xanthan gum in conserving the loss of water and solids is shown clearly by comparing the WVT with the XVT (Table 1). There were no statistically significant differences between the percent retort loss values (Table 1); however, the 1% XVT and the 3S treatment had similar but significantly lower total loss (blanch+retort) values than the other treatments. The XVT mushrooms were also whiter and had a less tough texture than the 3S or control samples.

Table 1. Shrinkage, colour and shear press values for day 0 mushrooms canned using xanthan gum treatments in comparison with the 3S process and water treated controls

	Pre-blanching treatment*						
Factor/test	Control	72 hr 3S process	Vac. soak water	Vac. soak 0.5% xanthan gum	Vac. soak 1% xanthan gum	*F*-test	s.e.
Weight gain (%)	0	30.1	48.6	52.6	45.2	$P < 0.001$	1.08
Blanch loss (%)†	25.9	10.4	22.8	14.3	8.2	$P < 0.001$	0.34
Retort loss (%)	12.5	12.0	12.2	12.7	13.2	NS	0.54
Total loss (%)‡	35.2	21.2	32.2	25.3	20.3	$P < 0.001$	0.32
Whiteness (L)	54	67	73	72	70	$P < 0.001$	0.44
Shear press value (kN)	0.81	0.80	0.76	0.68	0.65	$P < 0.001$	0.01

*See materials and methods section.

†Based on fresh weight.

‡Comprises weight loss during blanching and retorting.

Experiment 2: Effect of chill storage/xanthan treatment

The weight losses in the chill storage (2–4°C) of mushrooms in 1.36 kg chips covered with polythene were 0, 2.3, 3.3, and 4.3% on days 0, 1, 2 and 3 respectively. This result was statistically significant ($p < 0.001$; s.e. = 0.09).

The data (Table 2) for the uptake of water/solution during the soak treatments show that there was a highly significant ($P < 0.001$) interaction between days of chill storage and the subsequent soak treatments. There was a progressive increase in water percent/solution taken up by the mushrooms following chill storage from 0 to 3 days for the WVT and the XVT samples while there was a decline in the amount of water soaked up in the 3S process with increasing chill storage time post-harvest. Chill storing mushrooms for up to 3 days prior to vacuum soaking is, therefore, advantageous in terms of the weight gained by the mushrooms during vacuum soaking in water or xanthan gum solution.

Blanching loss decreased (Table 2) with increasing time of chill storage of the mushrooms prior to blanching. The effect was most dramatic for the 1% XVT where a net gain in weight after blanching was recorded for mushrooms chill stored for 2 or 3 days prior to blanching; the blanching loss/gain was based on the mushroom weight post-chill storage and not on the inflated weight after vacuum soaking. Retort loss was

Table 2. Weight gain/loss for mushrooms held for 0–3 days in chill storage (2–4°C) prior to canning

Factor	Days stored in chill prior to processing*	Soak treatment*			
		Vac. soak water	Vac. soak 1% xanthan	3S soak	Mean
Water/solution	0	46.5	48.0	33.5	42.6
uptake (%)	1	49.0	53.5	29.0	43.8
during	2	51.0	56.0	25.5	44.2
soaking	3	56.0	57.5	21.0	44.8
	Mean	50.6	53.8	27.3	
F-test: Days (D), NS; Soaks (S), $P < 0.001$; D×S, $P < 0.001$; s.e. 1.03					
Blanch	0	25.0	15.0	12.5	17.5
loss (%)†	1	18.0	4.2	11.5	11.2
	2	16.5	+1.1	7.2	7.5
	3	12.8	+3.6	7.8	5.7
	Mean	18.1	3.6	9.7	
F-test: D, $P < 0.001$; S, $P < 0.001$; D×S, $P < 0.001$; s.e. 0.75					
Retort	0	10.4	11.5	13.0	11.6
loss (%)	1	12.8	14.5	11.4	12.9
	2	12.7	16.3	14.5	14.5
	3	13.8	16.4	12.8	14.3
	Mean	12.4	14.6	12.9	
F-test: D, $P < 0.001$; S, $P < 0.001$; D×S, $P < 0.01$; s.e. 0.45					
Total	0	33.0	25.0	23.5	27.2
loss (%)‡	1	29.5	20.0	23.0	24.2
	2	29.5	18.5	23.5	23.8
	3	28.5	17.0	23.0	22.8
	Mean	30.1	20.1	23.3	
F-test: D, $P < 0.001$; S, $P < 0.001$; D×S, $P < 0.001$; s.e. 0.44					

*See Materials and methods.

†Based on weight after chill storage.

‡Comprises weight loss during chill storage, blanching and retorting.

greatest for the 1% XVT in mushrooms chilled for 1–3 days (Table 2); chilling had a smaller effect on retort loss in mushrooms from the WVT and the 72 hr 3S treatment. Total weight loss comprises weight loss during chill storage, blanching and retorting. The data (Table 2) show that the 1% XVT gave the lowest total weight loss in mushrooms chill stored for 1–3 days but not in day 0 samples; the effect was largest in mushrooms chilled for 3 days prior to processing i.e., the 1% XVT had a total weight loss value 6% lower than the 72 hr 3S process and 11.5% lower than WVT samples. These data show the very pronounced effect of xanthan gum in reducing total weight loss in canned mushrooms.

Table 3. Whiteness and shear press values for canned mushrooms which were held for 0–3 days in chill storage (2–4°C) prior to canning

Factor	Days stored in chill prior to processing*	Soak treatment* Vac. soak water	Vac. soak 1% xanthan	3S soak	Mean
Whiteness (Hunter L)	0	72	71	67	70
	1	71	72	67	70
	2	70	72	64	68
	3	69	70	61	67
	Mean	70	71	65	
F-test: Days (D), $P < 0.001$; Soaks (S), $P < 0.001$; D×S, $P < 0.01$; s.e. 0.50					
Shear press value (kN)	0	0.75	0.68	0.84	0.76
	1	0.83	0.75	0.87	0.81
	2	0.89	0.83	0.94	0.88
	3	0.90	0.81	0.96	0.89
	Mean	0.84	0.76	0.90	
F-test: D, $P < 0.001$; S, $P < 0.001$; D×S, NS; s.e. 0.02					

*See Materials and methods.

The total weight loss data must be viewed together with the whiteness results (Table 3). Chill storage prior to processing had no adverse effect on the whiteness of the WVT or XVT samples but there was a progressive decrease in whiteness of the 72 hr 3S canned samples as the length of the chill storage period prior to processing increased from 1 to 3 days. A Hunter L reading of 70 or above indicates a canned product with an excellent bright colour (white/yellow). The data (Table 3) show that the 1% XVT canned samples had an excellent colour in addition to their favourable total weight loss values. There was no significant day×soak treatment interaction in the case of mushroom shear values (Table 3). Chill storage for 0–2 days prior to processing increased shear values of canned mushrooms from the three soak treatments; however, chill storage for an extra day i.e., 3 days had no further effect on texture. The 1% XVT samples had the lowest shear values.

Experiment 3: Long term colour and shrinkage test

Mushroom shrinkage in-can was lower ($P < 0.001$) at the higher SMBS levels (Table 4) but this may be a function of blanching losses as well as SMBS level since blanching and retort losses are inversely related. Time of testing also influenced ($P < 0.01$) in-can shrinkage but the effect was inconsistent, being highest at the first and third dates and lowest at the second and fourth (Table 4).

The 0.10% SMBS level gave the best coloured mushrooms (Table 4); 0.25% SMBS gave equally white mushrooms early in the trial but in the later stages it was responsible for in-can de-tinning which discoloured the mushrooms (see significant interaction, Table 4); mean Hunter L values for the mushrooms 'ex-can' from this treatment fell from 73 on 18 May (1984) to 66 on 15 February (1985). The extent of the de-tinning is evident from in-can Hunter L reflectance values which ranged from 24 in the zero SMBS treatment to 9 in the 0.25% SMBS treatment. Some de-tinning also took place at the other SMBS levels (Table 4), suggesting that lacquered cans are needed when using even low levels of SMBS.

Table 4. Long term quality tests on canned mushrooms, treated with 1% xanthan gum solution containing different levels of sodium metabisulphite

	Sodium metabisulphite (%)*					
Factor	0.00	0.05	0.10	0.25	*F*-test	s.e.
Shrinkage (%) in-can	11.6	12.9	10.7	10.6	$P < 0.001$	0.32
Mushroom whiteness (L)	58	64	71	71	$P < 0.001$	0.45
Shear value (kN)	0.80	0.76	0.74	0.66	$P < 0.001$	0.02
SO_2 in mushrooms (mg/kg)	0.00	1.00	2.92	39.30	$P < 0.001$	0.67
pH of can liquor	5.4	5.3	5.3	5.4	$P < 0.001$	0.02
Can reflectance (L)	24	22	18	9	$P < 0.001$	0.30
	Testing date†					
	18 May	7 July	30 Nov	15 Feb		
Shrinkage (%) in-can	12.1	10.8	12.0	11.0	$P < 0.01$	0.32
Mushroom whiteness (L)	65	65	68	65	$P < 0.001$	0.45
Shear value (kN)	0.75	0.74	0.73	0.74	NS	0.02
SO_2 in mushrooms (mg/kg)	12.42	10.50	10.08	10.25	NS	0.67
pH of can liquor	5.3	5.3	5.4	5.4	$P < 0.001$	0.02
Can reflectance (L)	—	—	19	18	$P < 0.01$	0.21

Significant interactions: test date × sodium metabisulphite
Mushroom whiteness, $P < 0.001$
SO_2 in mushrooms, $P < 0.001$
Can reflectance, $P < 0.05$

*Means over four testing dates.
†Means over four SMBS levels.

The use of increasing amounts of SMBS resulted in a softening in mushroom texture (Table 4) and the effect was greatest between the 0.1 and 0.25% levels. However, no changes in texture took place between the first and last testing dates (Table 4). The amount of SMBS used significantly ($P < 0.001$) affected the pH of the can liquor, as also did ($P < 0.001$) time of testing (Table 4), but in practical terms the differences were very small and were no greater than 0.1 of a pH unit. Levels of SO_2 in the canned mushrooms were either zero or small for the 0.05 and 0.1% SMBS treatments; however mushroom SO_2 content was about 39 mg/kg for the 0.25% SMBS level (interaction, $P < 0.001$) with values of 46, 42, 37 and 32 mm/kg of drained mushrooms on the four testing dates respectively.

Discussion

The total loss value (blanch loss plus retort loss) of about 20% in the 1% XVT canned mushrooms corresponds to an increase of 23% in weight retention compared with the control and is similar to the value found by Singh *et al.* (1982) using 1.5% carboxymethylcellulose rather than xanthan gum. The 1% xanthan treatment compared favourably with the 72 hr 3S process in terms of weight retention in the mushrooms. The XVT mushrooms were whiter and had a more acceptable texture than 3S treated mushrooms and the time for vacuum treating with xanthan gum (0.5 hr) is much shorter than the long soaking and chill storage times required in the 3S process. The advantage of xanthan treatment is even more striking in view of the fact that the 3S process gives relatively low total loss canning values in comparison with other mushroom canning procedures (Gormley & Walshe, 1982; Beelman *et al.*, 1973; Parrish *et al.*, 1974; Beelman & McArdle, 1975). While the results showed that the 1% XVT gave a considerably lower total loss value in the canned product than 0.5% XVT, cost considerations may also be a factor in deciding the concentration of the xanthan gum solution to be used. A 1% xanthan gum solution is also quite viscous and could present problems during soaking and blanching of mushrooms on an industrial scale. The softening effect of the XVT on canned mushroom texture is a desirable feature as canned mushrooms often have a 'leathery' texture; a similar effect on texture was found in xanthan treated frozen mushrooms (Gormley, 1984). The ability of xanthan gum to reduce shrinkage in canned mushrooms is due, presumably, to its hydrocolloid nature. Some of the xanthan gum may also be bound by the mushroom protein, thereby preventing expulsion during blanching and retorting; Magsam (1977) has shown that xanthan gum can become bound to dairy proteins under certain conditions.

The XVT had an even more marked effect on weight retention in the canning of mushrooms which were chill stored prior to processing (experiment 2). The water uptake data post-chilling suggest that the mechanism by which water is soaked up in the 3S process (water binding by protein has been suggested by Eby, McArdle & Beelman, 1977) changes during the chill storage of the mushrooms; the same held true for the vacuum soak treatments except the effect was the opposite, i.e., chill storage enhanced the uptake. The increased water/solution uptake by WVT or XVT mushrooms with time of post-harvest storage was not 'making up' for water loss during chill storage as the increased uptake was larger than the water loss during chill storage. The net gain in weight after blanching 1% XVT mushrooms (blanching data based on mushroom fresh weight; not on weight after vacuum treatment) agrees with previous data from this laboratory on blanching/xanthan treatment (Gormley, 1984) and is also similar to the findings of Ferguson & Malick (1983) who reported (in a U.S. patent) blanching losses/gains in the range 2% loss to 5% gain for freshly harvested mushrooms subjected to a modified atmosphere and infused with a suspension containing a particulate microscopic heat stable material. Increasing lengths of chilling prior to blanching also reduced blanching losses in WVT and 3S process mushrooms but to a lesser extent. These reductions in blanching loss with increasing time of chilling pre-processing were paralleled by increased retort losses in most cases. The 1% XVT treated mushrooms had the highest retort loss and the lowest blanching loss. However, the overall beneficial effect of the 1% XVT was retained as shown by the total weight loss (during chill storage, blanching, retorting) values; these decreased with increasing time of chill storage and the effect was larger than that found by Beelman & McArdle (1975) for mushrooms stored for 1–3 days at 2°C and canned without any pre-soak treatment.

The poor whiteness values for pre-chilled 3S processed canned mushrooms relative to the XVT treated samples was expected in view of the long time involved before blanching in the 3S process; for example if the mushrooms are chill stored for 3 days prior to the commencement of the 3S process this results in a 6 day interval between harvesting and blanching i.e., 3 days for the chill storage and 3 days for the soak–chill–soak treatments of the 72 hr 3S process. Inevitably this will cause a loss in product whiteness despite the presence of 0.25% SMBS in the soak waters.

The data from experiment 3 indicate the desirability of using SMBS to maintain a good appearance in canned mushrooms. A level of 0.1% SMBS, or below, in the pre-blanch soak water/solution is desirable. Sodium metabisulphite had a softening effect on canned mushroom testure; this effect was also observed in frozen mushrooms treated, pre-blanching, with SMBS (Gormley, 1984). There were no consistent trends in mushroom shrinkage (in-can) during storage at ambient temperatures over a 9 month period; it was felt that the weight conserving effect of the xanthan gum in the canned mushrooms might change over time resulting in an increase in the loss of water and in the leaching of solids in the mushrooms. However, this did not occur.

Acknowledgments

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Effect of drying on the quality of milled rice

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Summary

The effect of drying on the quality of milled rice was investigated. Variables studied were drying temperature, air velocity and air relative humidity. The degree of breakage during milling increased as temperature of drying increased (from 40 to 70°C) and relative humidity decreased. Varying the air velocity between 0.26 and 2.12 m/sec had no effect on breakage. The 'activation energy' of grain breakage was much higher than of drying.

Introduction

It is known that the quality of milled rice is affected by variables such as drying and milling conditions, storage and moisture content of the grains at harvesting. The influence of grain moisture content at harvesting has been studied by Angladette (1969), Wasserman & Caldepwood (1972) and Topolanski (1975). According to these authors moisture contents ranging from 19% to 26%, dry basis at harvest cause minimum breakage.

The relation between cracks and breakage has been recognized by Rhind (1962). Breakage of grains during milling is greater for samples which show a greater proportion of cracks. Development of fissures during harvesting and drying operations, as well as in post-drying, have been studied by Autrey *et al.* (1955): Kunze & Prassad (1978) and Sharma & Kunze (1982), among others. The effect of moisture gradients during adsorption or desorption has been recognized by Kunze & Prassad (1978). They suggested that fissures caused by moisture adsorption develop during adsorption but those caused by rapid moisture desorption develop primarily after drying ceases. In either case, it was indicated that a grain with a fissure is likely to break during milling (Stahel, 1935; Rhind, 1962). Among criteria for evaluating the quality of milled rice (U.S. Department of Agriculture, 1972) the term 'broken kernals' is defined as those kernels which are less than three-fourths of the whole kernels. This term will be used in the present work, as a measure of grain breakage caused by milling.

Materials and methods

An Argentine variety of rice (Itape, medium grain) was used in the present work. The grains were freshly harvested with a moisture content of 20–26%, dry basis, and stored in a refrigerator at 4°C before use. The grains were previously cleaned from foreign matter and screened to obtain samples of more uniform size (mesh 2.8–3.3 mm).

The through circulation laboratory dryer has been described in detail by Suárez, Viollaz & Chirife (1980). For runs at high relative humidity, steam was added to air through a steam line connected at the inlet of the heated chamber. Wet and dry bulb

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temperatures were measured (±0.1°C) and relative humidity calculated from these values. The air velocity used for each run was measured with a flow meter.

After drying the grains were sealed in plastic bags and stored in an environmental chamber at 20°C for a week, prior to milling. The need for a storage period has been recognized by Sharma & Kunze (1982) for total development of the internal stresses generated during drying.

Moisture content

Moisture content of grains was determined gravimetrically using a vacuum oven at 70°C for 72 hr with magnessium perchlorate as dessicant.

Milling equipment

Rough rice samples were milled in a Kepler-Weber disc sheller laboratory mill consisting of hull and bran removal sections (Fig. 1). The sheller section has two horizontal metal discs (18 cm in diameter). The lower, emery faced disc revolves on a vertical axis at 1440 rpm and the upper disc, covered with rubber, is stationary. The distance between discs was adjusted for minimum efficiency of hull removal (90%). The bran removal section consists essentially of a metal truncated cone, also emery faced, which rotates in a larger cavity of similar shape.

The mill was fed with samples of 100 g of rough rice and the grains milled to a 'well-milled' degree (U.S. Department of Agriculture, 1972). The milling yield (U.S. Department of Agriculture, 1972) for all tests was between 66.4% ad 67.0% of the initial weight of rough rice.

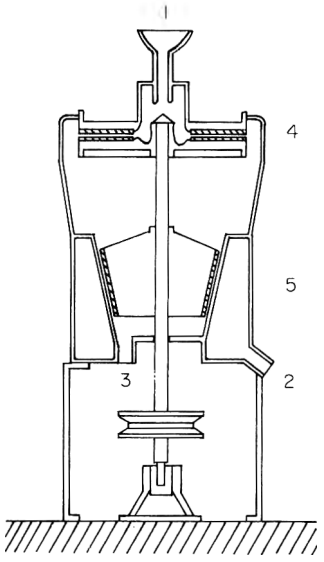

Figure 1. Cross section of the laboratory mill.

After milling the rice kernels were separated in two fractions 'whole' and 'broken', by manual inspection. The results of the milling experiments are reported in terms of whole kernels fraction W.

$$W\% = (M/M_0)\times 100 \quad (1)$$

where M is the weight of whole kernels and M_0 the initial weight of rough rice.

Results and discussion

Samples of rough rice were dried to a moisture content of 14% (dry basis) at 40, 50, 60 and 70°C with an air velocity of 5 m/sec. The effects of subsequent milling are shown in Table 1 where it can be seen that increase of temperature produces increased grain breakage (decreased W).

Table 1. Effect of drying temperatue on the whole kernels fraction

Drying temperature (°C)	W(%)
40	39.6
50	31.6
60	16.4
70	3.2

To investigate whether the causes of breakage are due to moisture and/or temperature changes, closed metal cups, full of grains, were heated at 40 and 60°C for different periods. (No significant variation of moisture content was observed). Table 2, shows that the degree of breakage was not affected by the time or temperature of treatment suggesting that changes in temperature alone do not influence the degree of breakage. Similar conclusions were reported by Kunze & Hall (1967), who found that thermal gradients do not cause fissures in rice as long as the grains remain at a constant moisture content.

Table 2. Whole kernels fraction *versus* heating time without moisture losses

Time (min)	W(%)	
	40°C	60°C
0	18.6	18.6
30	18.5	18.7
60	18.7	18.5
90	18.4	18.3
120	18.5	18.7

The effect of the air relative humidity on breakage was investigated by drying grains from 10 to 14% (dry basis) at 52°C with varying air humidity. As shown in Fig. 2, the fraction of whole grains increased when drying was performed with air of high relative humididy.

The effects of varying the air velocity are reported in Table 3 (the drying temperature 55°C, relative humidity 6.5%). The unbroken fraction remained practically

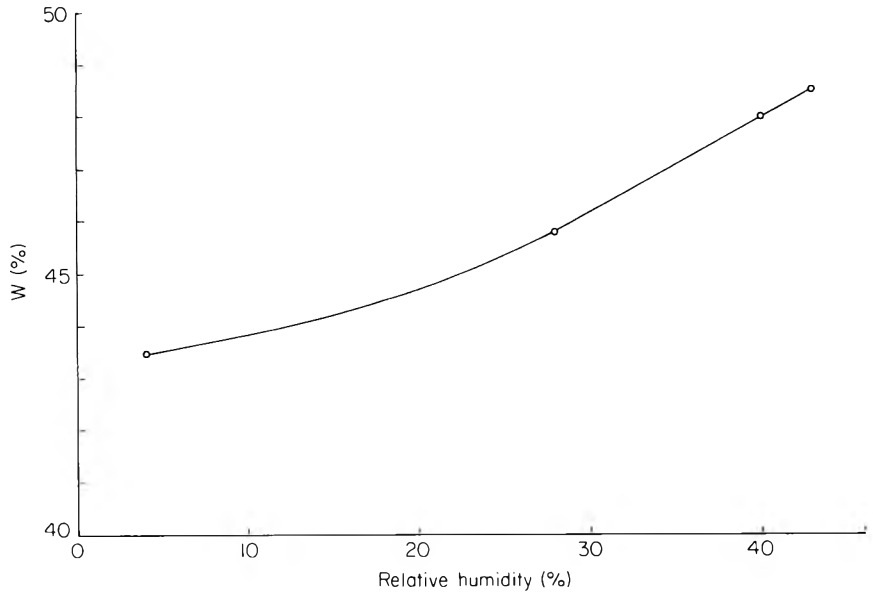

Figure 2. Influence of the air relative humidity on grain breakage.

constant for the different drying conditions, suggesting that the drying rate was not modified by the air velocity in the range investigated. To interpret this result, the mass transfer Biot number corresponding to the lower velocity was calculated. Its value was 102, which indicates that the drying process was controlled by moisture migration into the kernel for the whole range of velocities investigated (Vaccarezza, Lombardi & Chirife, 1974).

Table 3. Effect of air drying velocity on whole kernels fraction

Air velocity (m/sec)	W(%)
0.26	44.2
0.65	44.0
1.06	44.2
2.12	44.3

The relation between breaking of grains and drying time was also studied at 40, 50 and 65°C. Initial moisture content of the grains was 20% (dry basis), air velocity in all cases was 5 m/sec and relative humidity of the air was 24, 14 and 6% respectively. After drying the grains were stored for a week and milled. As shown in Fig. 3, W decreases with increase of drying temperature and, for a given temperature, with the drying time. At 40°C, the values of W show little effect on drying time as described by Sharma & Kunze (1982) in their work at 39°C.

Assuming a first order kinetic between W and time, the following relationship was postulated:

$$\frac{dW}{d\theta} = -kW \tag{2}$$

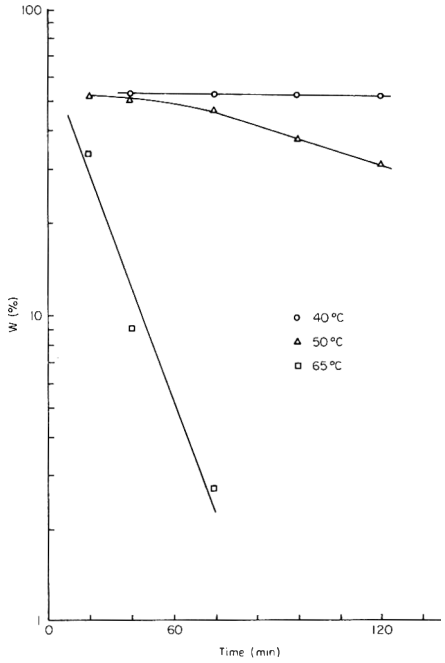

Figure 3. Effect of drying temperature on grain breakage.

where θ is the drying time and k a constant. The values of k calculated by means of equation 2 were plotted as a function of the inverse of the absolute temperature in Fig. 4 and seem to indicate that k varies exponentially with temperature, approximating to an Arrhenius type equation:

$$k = k_0 \exp(-E_a/RT). \tag{3}$$

From this equation the value of E_a corresponding to the breakage process was 39.8 kcal/mol.

In an earlier paper Aguerre, Suárez & Viollaz (1982) reported the activation energy for drying of rough rice as 9.9 kcal/mol. Expressing the effect of temperature on the specific reaction velocity in terms of the parameter Q_{10} (the ratio of the velocities at $T+10$ and T) and the relation between Q_{10} and the activation energy as:

$$\log Q_{10} = \frac{2.289\, E_a}{T(T+10)}, \tag{4}$$

gives values of Q_{10} for drying and grain breakage processes as 1.63 and 7.27, respectively. The much higher value of Q_{10} is a measure of the increased breakage and reduced quality of milled rice as drying temperature increases.

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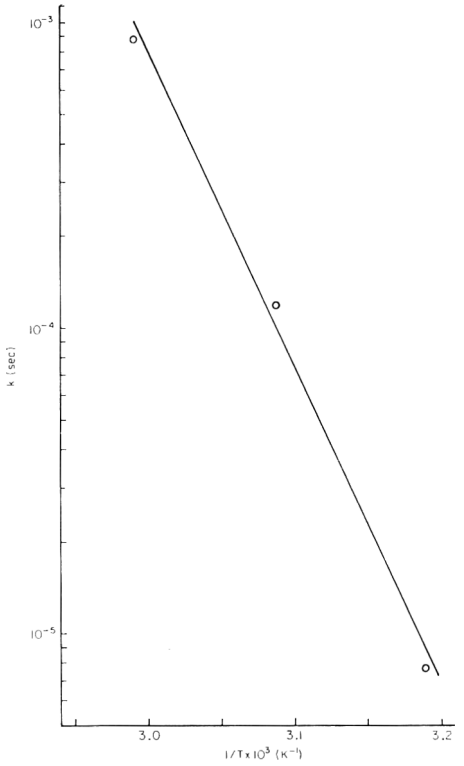

Figure 4. k Dependence on the inverse of the absolute temperature.

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Rapid *in vitro* enzymic predictive model for the *in vivo* digestibility of food proteins

A. I. IHEKORONYE

Summary

The absorbance of the deep orange colour produced by incubating protein hydrolysates with trinitrobenzene sulphonic acid was used to follow the liberation of free amino groups. Five animal and five plant proteins were hydrolysed with pepsin-pancreatin and papain-pronase E and aliquots were taken every 4 hr. To facilitate proteolysis the proteins were presolubilized in 0.05 M HCl at 70°C for 2 hr with 1.0 ml 0.8% mercapto-ethanol added to reduce disulphide bonds. An increase in absorbance occurred in all enzymatic digests between 8–20 hr incubation. No further hydrolysis took place after 20–28 hr incubation. The extent of the hydrolysis *in vitro* correlated with *in vivo* measurements of digestibility and so may serve as a more rapid predictor of digestibility.

Introduction

In recent years, the role that protein digestibility with its concomitant amino acid release may play in determining the nutritional quality of foods has been increasingly studied. Perhaps a more important consideration is the extent and proportion of digestible peptides released, for it is the amount of each amino acid absorbed that determines protein utilization in the body. Digestibility data alone can provide estimates of the nutritional quality of a protein (Buchmann, 1979; Furuya, Sakamoto & Takahashi, 1979), or they can be used in combination with data on the essential amino acid content of the protein to yield such estimates (Buchanan, 1969; Hsu *et al.*, 1977).

Current *in vitro* assays for estimating protein digestibility have tried to simulate rat digestibility data since the essential goal has been to use the *in vitro* digestibility data to help in the eventual prediction of *in vivo* performance. Satterlee, Hendrick & Miller (1977) went a step further by correlating pH changes during proteolysis with *in vivo* digestibility for a number of proteins. The method was rapid but applicable only to reactions in which a few peptide bonds are split and the pH shift is so small that it can be assumed that the buffering capacity of the newly formed peptides remains unchanged. Also protein may coat the pH electrodes and cause unreliable performance. These limitations necessitate continued interest in finding rapid procedures for evaluating the nutritional quality of foods.

The objective of this study was to develop a simple, rapid and reliable colorimetric procedure for evaluating the extent of hydrolysis of food protein after sequential treatment with enzymes.

Materials and methods

The proteins examined are listed in Table 1. This contains the proteins used by Saterlee *et al.* (1977) in their rat assay. They were obtained from the University of Nebraska,

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Table 1. Food products used to evaluate course of activity of papain-pronase enzyme system*

Food or food ingredient	% Protein	Kjeldahl factor
Whole cooked egg†	83.30	6.25
Cooked chicken muscle	83.75	6.25
ANRC casein	90.55	6.38
Ribonuclease‡	95.50	6.25
Egg white	77.60	6.25
Shredded wheat	9.69	5.70
Textured soy protein	48.15	5.70
Soy flour	48.15	5.71
Corn meal	7.20	5.70
Breakfast bars	18.69	6.25
Fish meal§	57.36	6.25

*Protein samples supplied by L.D. Satterlee, University of Nebraska.

†Eggs boiled for 15 min, freeze-dried, homogenized and defatted with chloroform methanol (2 : 1).

‡Sigma Chemical Type 1-A, St. Louis, MO.

§From Experimental Chemical Laboratories, UMC.

Lincoln, together with data on protein content and results of *in vivo* digestibility studies. The enzymes used (pepsin, EC 3.4.23.1, papain, EC 3.4.22.1, pancreatin, and pronase E, EC 3.4.4) were of commercial quality and purchased from Sigma Chemical Company, St Louis, Missouri. Prior to use, they were dialysed at 5°C for 24 hr against 0.01 M phosphate buffer, pH 7 using seamless cellulose dialyser tubing (Fisher Scientific Company) with an approximate molecular weight cut off of 12000 Daltons.

Sample preparation

Fifty mg of the test sample was solubilized in 10 ml 0.05 M HCl, pH 2.0 by stirring at 70°C. To reduce disulphide bonds 1.0 ml of 0.8% mercaptoethanol was added and the mixture held at 37°C. After 1 hr, the pH was adjusted to 7.5 with 1.0 M NaOH and the total volume brought to 20 ml with 0.01 M phosphate buffer, pH 7.5. One ml of the solution now contained 2.5 mg sample. A 15 ml aliquot corresponding to 37.5 mg sample was transferred to 50 ml KIMAX culture tube, stoppered and, following addition of enzyme, placed in a 37°C shaking water bath.

In vitro *enzymic hydrolytic procedures*

Two incubation mixtures, one containing papain-pronase E and the other containing pepsin-pancreatin were used (Fig. 1) for enzymic hydrolysis. With reference to the pepsin-pancreatin incubation mixture, hydrolysis was started with the addition of 1 ml pepsin containing 1.0 mg/ml enzyme at pH 2.0. After 8 hr, the pH of the hydrolysate was adjusted to 8 with 1.0 M NaOH and a 1.0 ml solution containing 1.0 mg of pancreatin was added and the incubation continued, with shaking, for further 16 hr.

At the end of this period, all the culture tubes were removed from the water bath and immediately transferred to a beaker of boiling water for 5 min to inactivate the protease. At this point, the concentration of the sample protein in the hydrolysate was 2.18 mg per ml.

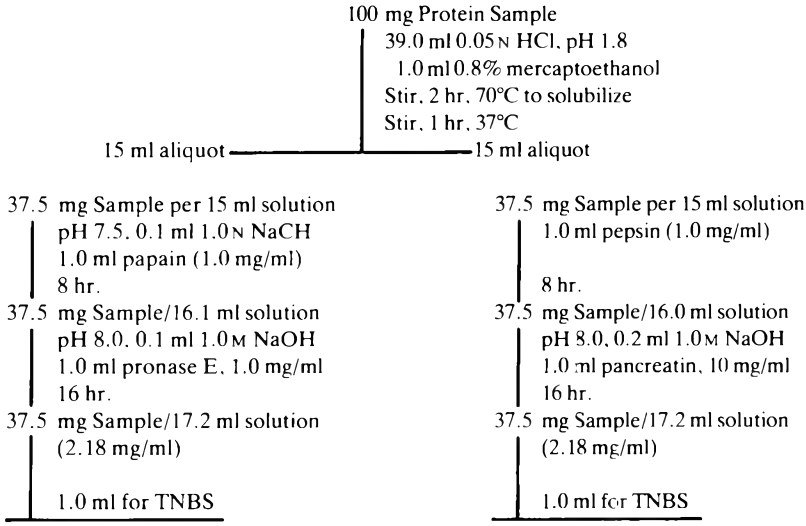

Figure 1. Sampling procedure for measuring enzymic hydrolysis.

A 5 ml aliquot (representing 10.90 mg sample) was transferred to a 30 ml Corex centrifuge tube containing 20 ml of a saturated solution of picric acid. To remove the undigested residue, the tubes were centrifuged for 30 min at 54000 g and the supernatant used for colorimetric reaction with trinitrobenzene sulphonic acid (TNBS).

Colorimetric determination of digestibility and degree of hydrolysis

Trinitrobenzene sulphonic acid reacts stoichiometrically with the amino groups of proteins, peptides and amino acids (except proline), in a basic solution to give trinitrophenyl derivatives with absorbance maxima at 345 and 420 nm. The various procedures described in the literature (Satake *et al.*, 1960; Fields, 1971; Soley & Alemany, 1980), seem to have been developed for soluble synthetic peptides, hence had to be modified for application to the present work. The modifications included: the use of 0.01 M phosphate buffer of pH 8.2, rather than bicarbonate solution since this was observed to produce carbon dioxide bubbles during the acidification step which interfered with the absorbance readings; a reaction time of one hour at 60°C was found to be optimal for the development of the orange colour and allowing completion of the analysis within a reasonable period.

The exact procedure for TNBS analysis was as follows: the hydrolysate was heated at 100°C for 3 min and centrifuged to deproteinize the sample. A 1 ml aliquot diluted with 0.01 M phosphate buffer, pH 8.2, to contain 0.100 mg sample was added to a test tube and mixed with 1.0 ml 0.01 M phosphate buffer, pH 8.2, and 1.0 ml 0.01% TNBS was added. The contents were mixed for 30 sec and incubated at 60°C. Tubes containing 1 ml distilled water instead of sample, and leucine standard solutions (0.005–0.10 mg/ml) were incubated simultaneously. During incubation, all the tubes were covered

with aluminium foil. After 1 hr, 1 ml 1.0 M HCl was added to the resulting orange coloured solution, to stop the reaction, and the tubes cooled to room temperature.

The absorbance of the acidified yellow solutions was measured at 420 nm using a Bausch and Lomb spectronic 20 spectrophotometer. Digestibility (D) was then calculated as:

$$\frac{\text{Absorbance at 420 nm of 1 ml pepsin-pancreatin hydrolysate}}{\text{Absorbance at 420 nm of 1 ml papain-pronase E hydrolysate}} \times 100.$$

The procedure for measuring the course of enzyme hydrolysis involved hydrolysing a 15 ml aliquot containing a 37.5 mg sample with papain-pronase E enzyme systems as described previously. Aliquots were taken every 4 hr, for a total period of 40 hr, and analysed by reaction with TNBS. From a plot of relative absorbance at 420 nm of each hydrolysate against time, the course and extent of enzymic hydrolysis was determined.

Results

Colorimetric determination of the course and extent of enzyme hydrolysis of the food proteins

An increase in absorbance occurred in all enzymic digests between 8 and 20 hr (Table 2). After 20 hr the increase in absorbance was very small for all proteins indicating the limit of hydrolysis. All samples prior to analysis appeared to contain appreciable amounts of amino groups as indicated by absorbance measurements. However all the plant proteins, except the soy products had lower absorbance values than the animal proteins. This observation may either reflect the protein content of these samples before enzymic hydrolysis or the content of lysine and arginine in the proteins, since TNBS would react with these groups. Considerable time was required to solubilize completely all the plant protein in 0.05 M HCl, but the animal proteins went into solution readily. Thus, for example, there was a 4.7% degree of hydrolysis for corn meal between 0 and 4 hr compared to 10.1% hydrolysis for cooked chicken muscle.

There was a greater number of digestion products as shown by the extent of hydrolysis at any given time (Table 2) from the time pronase E was added to the system

Table 2. Course and extent of hydrolysis of proteins by papain-pronase*

	Hours									
Food proteins	4	8	12	16	20	24	28	32	36	40
Egg white	4.8	14.5	38.7	54.8	100.0	100.0	95.2	95.2	87.1	87.1
Ribonuclease	7.8	14.1	68.1	81.3	100.0	100.0	98.4	96.9	96.9	89.1
Whole cooked egg	17.7	33.9	80.6	82.3	100.0	100.0	100.0	100.0	100.0	100.0
Cooked chicken muscle	10.1	36.4	45.5	55.5	81.8	100.0	100.0	100.0	90.9	90.9
Fish meal	7.2	9.1	16.4	72.7	100.0	100.0	100.0	98.2	98.2	98.2
ANRC casein	26.7	33.7	73.3	86.0	94.8	100.0	100.0	100.0	100.0	98.8
Breakfast bars	29.4	58.8	88.2	94.1	97.1	100.0	99.4	99.4	94.1	94.1
Shredded wheat	4.3	8.7	17.4	34.8	95.7	100.0	100.0	95.7	95.7	87.0
Textured soy protein	4.2	10.5	35.8	54.7	98.9	100.0	97.9	98.9	94.7	90.5
Corn meal	4.7	5.8	11.8	58.8	82.4	100.0	100.0	82.3	100.0	76.5
Soy flour	27.5	38.1	42.4	46.6	69.9	100.0	100.0	99.6	91.1	91.1

*Percent of maximum hydrolysis at a given time, $t = \frac{\text{Absorbance at time, } t - \text{Initial absorbance}}{\text{Absorbance maximum} - \text{Initial absorbance}} \times 100.$

(8–20 hr) than the period (0–4 hr) when papain was the only enzyme in the hydrolysate. These differences confirm the earlier findings of Nomoto & Narahashi (1959) who hydrolysed casein, ovalbumin and wheat gluten for 72 hr with *Streptomyces griseus* protease and by pepsin, trypsin, and chymotrypsin and concluded, after analysis of amino nitrogen of the digests by formol titration, that the proteolytic activity of *Streptomyces griseus* protease (pronase E) was far stronger than that of any other proteases. The data presented here suggest that with pre-solubilization, a period of 24–28 hr was effective for hydrolysing proteins by papain-pronase E. Continuing the hydrolysis beyond this period was marked by a decrease in absorbance. In this case there was a decrease in alpha amino nitrogen content, perhaps due to inhibition of the enzymic action by the increasing accumulation of proteolytic products or resynthesis of peptides from the free amino acids: peptidyl transferases present in the system would be efficient catalysts in these circumstances.

In vitro *enzymic digestibility measurements with pepsin-pancreatin and papain-pronase E*

Table 3 lists *in vitro* digestibilities of a diverse number of proteins as determined by the pepsin-pancreatin and papain-pronase E enzyme systems. Also presented are *in*

Table 3. Enzymic hydrolysis and *in vivo* digestibility of food proteins

	Pepsin-pancreatin	Papain-pronase E	Hydrolysis	
Type of food	Absorbance	420 nm*	*In vitro*†	*In vivo*‡
Breakfast bars	0.220	0.242	91.70	84.20
Cottonseed meal	0.578	0.712	81.11	85.10
Beef noodle	0.305	0.352	86.65	81.00
Lean beef	1.219	1.332	91.50	91.70
Sausage analogue	0.595	0.712	83.57	81.80
HRW wheat flour	0.170	0.186	91.40	81.90
Clam chowder	0.918	0.085	84.60	—
Fish fillets	0.495	0.572	86.54	—
Macaroni and cheese	0.505	0.564	89.54	87.50
Chicken dumpling	0.335	0.384	87.24	83.00
Soy flour 70 PDI	0.865	1.081	80.02	78.92
Turkey pot pie	0.278	0.311	89.40	89.50
Whole cooked egg	1.290	1.305	98.80	91.10
ANRC	1.290	1.418	90.97	90.60
Textured vegetable protein	0.630	0.753	83.70	83.30
Life cereal	0.450	0.545	82.30	83.30
Durum wheat flour	0.310	0.355	87.32	86.10
Cooked chicken	0.865	0.985	87.82	89.30

*Average of two independent determinations; *B*-amino nitrogen determined by TNBS.

†Hydrolysis $= \frac{\text{Absorbance, 420 nm of pepsin-pancreatin hydrolysate}}{\text{Absorbance, 420 nm of papain-pronase E hydrolysate}} \times 100$.

‡Digestibility determined by rat studies, data supplied by Dr L.D. Satterlee, University of Nebraska, Lincoln.

vivo rat based protein digestibilities provided by Dr Satterlee. Correlation between *in vitro* hydrolysis and *in vivo* rat based digestibilities were also calculated for all the food proteins with the exception of clam chowder and fish fillets.

The results indicate a correlation of ($r = 0.77$) between the papain-pronase E procedure and the *in vivo* rat based procedure. The papain-pronase E *in vitro* method, however, overestimated the digestibilities of breakfast bars, wheat flour, beef noodle and whole cooked egg, perhaps due to their proximate composition and higher content of free amino groups in these samples as indicated by absorbance measurements prior to enzymic hydrolysis. The high protein digestibilities reported for wheat products agree with values published by Saunders & Kohler (1972) who used pronase E to assess protein digestibility in wheat millfeeds. Under the conditions employed by the above authors, digestion effected by pronase E went to completion in about 12 hr; agreeing with the period of 16 hr between the addition of pronase E and termination of hydrolysis found in this study. It must be mentioned however, that the extent of digestion and not merely rate of digestion was being measured.

Discussion

Although the amino acid profile is important in evaluating the nutritional quality of a protein, the digestibility of that protein is the primary determinant of the availability of its amino acids, for it is the amount of each amino acid that is absorbed that determines protein utilization in the body. Numerous *in vitro* procedures have been proposed for predicting protein digestibilities but none has gained universal acceptance.

The relative resistance to proteolysis is generally explained by the composite tertiary structure of the protein which protects bonds. On the other hand, when proteins are solubilized, some peptide bonds are exposed and available for enzymic cleavage. With the addition of papain, the polypeptides will be cleaved to smaller peptides plus a few amino acids. While this partial hydrolysis of the protein molecule proceeds, an increasing number of peptide bonds become available. The non-specific protease, pronase E, would have more free carboxyl and amino end groups to attack and so its contribution to the hydrolytic process is enhanced. This process continues until amino acids become the predominant end product of the hydrolytic process. In essence, enzymic hydrolysis by this combination of enzymes involves sequential action, where the endopeptidase papain initiates the attack and the new chain ends that are generated are then hydrolysed by the non-specific protease, pronase E.

Conclusion

Two main conclusions may be drawn. Presolubilization of proteins is necessary in order to achieve maximum enzymic hydrolysis, and hydrolysis of proteins using papain-pronase E is complete 20–28 hr after presolubilization. An *in vitro* procedure utilizing the reaction of TNBS with the alpha amino groups released by enzymic hydrolysis using pepsin-pancreatin and papain-pronase E has been developed and can be used to predict *in vivo* digestibility.

Acknowledgments

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Studies on production of a ghee-like product from vegetable fats

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Summary

A typical ghee flavour was obtained by heating vegetable fats with fermented milk or fermented whey at 125°C for 2 min. The resulting product had an acceptability preference and better storage stability than the untreated vegetable fat. It is suggested that whey proteins and fermentation products are the major contributors to development of ghee flavour in vegetable fats. The increase in storage stability could be due to the formation of reactive sulphydryl groups from whey proteins during the heating process.

Introduction

Ghee has long been used in Egypt and other Middle Eastern and Asian countries for culinary purposes. In recent years, because of the price increase of anhydrous milk fat, and the association between heart disease and saturated fatty acids, there has been an increase in the consumption of vegetable fats (FAO, 1978; UNIDO, 1984). However ghee has a characteristic flavour, referred to as a cooked or curdy flavour (Ganguli & Jain, 1973), traditionally accepted by consumers for cooking or making confectionery. This flavour is thought to be brought about by a heat induced interaction between the milk fat and solid not fat (SNF) during the manufacturing process (Gaba, Bindal & Jain, 1975).

Wadhwa, Bindal & Jain (1977) reported that butter oil, which has a bland flavour, could be converted to ghee by heating in the presence of small amounts of SNF. Patel & Gupta (1983) succeeded in simulating ghee flavour in vegetable fats by heating in the presence of cultured cream. They concluded that the presence of a certain amount of butter fat as well as SNF is essential for the developing of ghee like flavour in vegetable fats.

In a previous study (Iskander, Bayoumi & Shalabi, 1985) we showed the differences in composition and storages stability between ghee, butter oil and vegetable fat. In this investigation an attempt has been made to produce a product analogous to ghee by heating vegetable fat with more abundant dairy product i.e., fermented milk (Zabadi), skim milk and whey. The physico-chemical characteristics and storage stability of the resulting product were examined.

Materials and methods

Vegetable fats: Hydrogenated sunflower oil Nefertiti brand (The Nile Cotton Ginning Co., Product, Minia, Egypt) was used throughout. Milk solids not fat sources were fresh skim milk (0.16% acidity, 0.2% fat, 3.5% protein), soft cheese whey (0.13%

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acidity, 0.3% fat, 0.68% protein) and Zabadi (0.8% acidity, 5.0% fat, 3.7% protein) obtained by fermenting milk at 40°C for 6 hr using 1.0% mixed starter of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*.

Preparation of the samples

Ganguli & Jain (1973) reported that the intensity of ghee flavour depended on the temperature used and ghee usually prepared at temperatures above 110°C had a strong flavour. The vegetable fat was first mixed with the solids not fat source at 10 or 20% w/w level in a 2 l capacity conical flask (total weight of the sample was 1 kg). The mixture was then heated up to 125°C (coming up time was 15 min) in a thermostatically controlled glycerol bath (Grant HE, Grant Instruments, Cambridge, U.K.) and held for 2 min. This resulted in the formation of a brown residue. The mixture was filtered through cheese cloth to remove the residue and stored in brown glass jars at 35°C for 6 months.

Analytical methods

Colour was determined by the colour Wesson method using Lovibond glasses and calibrated according to Cocks & Van Rede (1966). Refractive index and melting point were determined as described in AOAC (1975).

Moisture content, free fatty acids (FFA), peroxide value (PV), thiobarbituric acid value (TBA), iodine value and saponification value: these were determined as described in AOCS (1974).

Organoleptic test

The treated vegetable fat was judged for its flavour by the departmental members of staff using a 10 point rating scale, with genuine ghee (10 points) as references. A second test was done by a group of fifty people to give a representative sample of consumers, in which the samples were arranged in order according to their preferences.

Results and discussion

Ghee flavour development

Results in Table 1 confirm earlier findings that ghee flavour could be simulated in vegetable fats by heating in the presence of SNF. However, flavour intensity depended to a great extent on the source of SNF used. Heating with fresh skim milk at 10 or 20% level resulted in an appreciable induction of ghee like flavour. When whey was used at 10% level the flavour intensity increased and increased more by increasing the level of addition to 20%. Treatment with Zabadi at 10 or 20% level produced a typical ghee flavour. The resulting product had nearly the same flavour impression as genuine ghee.

The high flavour scores with whey and fermented milk suggested that whey proteins and fermentation products are the key factors in ghee flavour development. This is supported by the observation that ghee made from lactic butter has a stronger flavour than that from sweet butter (Ganguli & Jain, 1973). Confirmation was made by heating vegetable fat with whey fermented in the same way as Zabadi. The flavour scores of the resulting product were closer to that obtained with Zabadi (Table 1). High temperature treatment forms reactive sulphydryl groups from whey proteins which are believed to be responsible for the cooked flavour in heated milk (Patrick & Swaisgood, 1976; Hanson & Melo, 1977). Therefore ghee flavour could be the result of interactions between SH groups and fermentation products and the fat. The presence of milk fat

Table 1. Average flavour scores of vegetable fat heated with different sources of milk solid not fat

Vegetable fats were subjected to the following treatments then heated at 125°C for 2 min	Flavour* (10 point scale)
10% fresh skim milk	5.5
20% fresh skim milk	6.0
10% whey	6.5
20% whey	7.0
10% zabadi	8.2
20% zabadi	8.7
10% fermented whey	8.0
20% fermented whey	8.5

*No ghee flavour (untreated fat 1 point) genuine ghee (10 points).

may improve the flavour because of the phospholipid content, but it is not a major flavour contribution as Patel & Gupta (1983) suggested.

Induction of ghee flavour greatly improved the acceptability of the vegetable fat. All treated samples showed an acceptability preference by testers over the untreated fat (Table 2). This demonstrates that SNF could be successfully used as a flavour modifying agent in the processing of vegetable fats associated with flavour problems e.g., cotton seeds oil and soybean oil.

Table 2. Acceptability preferences of vegetable fat and flavour modified fat

Vegetable fat + zabadi
Vegetable fat + fermented whey
Vegetable fat + whey
Vegetable fat + skim milk
Vegetable fat (untreated, control)

Physico-chemical characteristics

Data in Table 3 demonstrate that heating vegetable fat with different sources of SNF had no practical effect on the physico-chemical characteristics of the fat. There were slight increases in the moisture content, peroxide value and TBA value but within the permissible limits. The most significant effect was on red colour intensity. The red colour value decreased from 4.1 to 3.6, 2.5, 3.2 and 2.0 on heating with skim milk, whey, Zabadi and fermented whey respectively, possibly due to the precipitation of some of the colour pigments with SNF during heating process.

Storage stability

Free fatty acids development (Table 4) confirm the previous findings in this laboratory (Iskander *et al.*, 1985) that lipolytic rancidity is not a major problem when the fat is

Table 3. Effect of heating vegetable fat with different source of milk solid not fat on the physico-chemical characteristics

Analysis	Vegetable fat	Vegetable fat heated with:			
		Skim milk	Whey	Zabadi	Fermented whey
Colour, lovibond R/Y 5.25 in cell	4.1/35	3.2/35	2.5/35	3.2/35	2.0/35
Refractive index (40°C)	1.462	1.462	1.462	1.462	1.462
Melting point (°C)	37.8	38.0	38.0	38.0	38.0
Moisture (%)	0.22	0.37	0.38	0.36	0.32
FFA % (oleic acid)	0.22	0.22	0.25	0.25	0.24
Peroxide value	4.3	4.9	5.2	4.5	4.8
TBA value	0.40	0.51	0.53	0.47	0.45
Iodine value	78.6	79.4	79.1	79.1	79.1
Saponification value	186.0	186.5	186.2	188.0	186.2

Table 4. Lipolytic rancidity development in vegetable fat and flavour modified fat after storage for 6 months at 35°C

Storage period (months)	Free fatty acids as % oleic acid				
	Vegetable fat (control)	Flavour modified fat with:			
		Skim milk	Whey	Zabadi	Fermented whey
0	0.22	0.22	0.25	0.25	0.24
1	0.23	0.24	0.26	0.27	0.28
2	0.25	0.25	0.30	0.31	0.30
3	0.26	0.27	0.30	0.32	0.32
4	0.27	0.28	0.31	0.34	0.34
5	0.29	0.30	0.31	0.34	0.35
6	0.30	0.31	0.34	0.35	0.36

Table 5. Oxidative rancidity development in vegetable fat and flavour modified fat after 6 months of storage at 35°C

Storage period (months)	Vegetable fat (control)		Flavour modified fat with:							
			Skim milk		Whey		Zabadi		Fermented whey	
	PV*	TBA†	PV	TBA	PV	TBA	PV	TBA	PV	TBA
0	4.3	0.40	4.9	0.51	5.2	0.53	4.5	0.47	4.8	0.45
1	10.2	0.92	9.3	0.90	10.3	1.10	9.1	0.88	8.5	0.81
2	16.1	1.36	15.2	1.25	13.5	1.36	11.9	1.20	12.7	1.15
3	23.2	1.54	17.7	1.38	17.0	1.51	14.0	1.31	14.7	1.26
4	31.6	1.77	21.0	1.54	19.1	1.73	18.3	1.40	18.5	1.30
5	36.5	2.20	28.0	1.65	28.0	1.80	23.9	1.44	21.7	1.47
6	40.7	2.47	33.1	1.93	30.4	1.92	27.9	1.56	24.1	1.52

*PV, Peroxide value as mEq peroxide/kg sample.

†TBA, Thiobarbituric acid as mg malonaldehyde/kg sample.

stored under tropical conditions. Furthermore, the results show that heat treatment with different source of SNF had no practical effect on lipolytic rancidity development. In all cases the FFA content after 6 months of storage at 35°C were within the permissible limit of 0.3%.

However, oxidative rancidity is the principal problem. Data in Table 5 show remarkable increases in the peroxide and TBA values as the storage time proceeded. It is of interest to note that the rate of increase was slower in the samples that had been heated with SNF. Heating with zabadi or fermented whey resulted in a greater reduction in the rate of increase in PV and TBA values (Table 5).

The present findings reveal that SNF caused an inhibition of oxidative rancidity development. This conclusion is substantiated by the findings of Taylor & Richardson (1980) who found that skim milk had an antioxidant activity in linoleate emulsion. It has been suggested that formation of reactive sulphydryl groups could be responsible for the antioxidant activity of SNF (Parks, 1972; Taylor & Richardson, 1980). The greater effect of zabadi and fermented whey indicates that fermentation products may have an additional effect.

Conclusions

The results of this investigation show that vegetable fats can be simply converted to a product analogous to ghee by heating with fermented milk or fermented whey. This treatment also inhibited the oxidative rancidity development in the fat. As flavour is the critical factor that determines the limit of use of any food product, the induction of ghee flavour in vegetable fat would favour consumers who are accustomed to ghee flavour. Also, it may extend the use of vegetable fat in products in which vegetable fat as such would be less desirable.

The present results suggest that production of ghee flavour and inhibition of oxidative rancidity development in vegetable fat, as a result of heating with SNF, could be due in part to formation of reactive sulphydryl groups. Fermentation products may also be involved. Further study is needed to clarify the actual effect of fermentation products and perhaps the type of fermentation on ghee flavour development and antioxidant activity.

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Sorption properties of raw hake muscle

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Summary

Adsorption and desorption isotherms of raw hake (*Merlucius hubbsi*) muscle were determined at 5, 20 and 30°C. From the temperature dependence of the isotherms, isosteric heats of sorption were evaluated by means of the Clausius-Clapeyron equation. Monolayer moisture contents are calculated by means of the B.E.T. analysis. The magnitude of the hysteresis between desorption and adsorption isotherms was quantified. Results are compared with those reported in the literature for other fish species.

Introduction

Preservation of foods by dehydration, although an ancient technique, is still widely used. Critical consideration of the effects of water–solid equilibria is of fundamental importance in determining the ease and extent of drying, in specifying drying conditions and in producing foods with desirable attributes.

The properties of water in relation to biological systems has been, within limits, successfully described using a thermodynamic approach involving the determination of the amount of water in the food in equilibrium with its surroundings, at different relative humidities and temperatures. This relationship between total moisture content and the corresponding water activity (a_w) at a constant temperature yields, when graphically expressed, the moisture sorption isotherm.

Several moisture sorption isotherms have been published for raw cod muscle following either the adsorption path: Cutting, Reay & Shewan (1956) (10°C), Cooper & Noel (1966) (20°C), Doe *et al.* (1982) (25°C), or the desorptive path: Jason (1958) (30°C), Taylor (1961) (18/21°C). Wolf, Spiess and Jung (1973) determined the moisture adsorption and desorption isotherms of raw and cooked trout muscle at 5, 45 and 60°C.

The equilibrium moisture content of a sample depends on sorption mode, equilibrium temperature and composition. With reference to composition, the sorption characteristics of fish muscle may be modified by the relative protein and fat contents. Proteins absorb much more water at low a_w's than do fatty materials (Labuza, 1968) and consequently the presence of fat may depress the sorption ability of a protein sample (Hermansson, 1977). Accordingly, the sorption isotherms published for various fish species usually present differences. The sorption behaviour of tropical fish, dried and salted under semi-commercial conditions, was found to be similar to that of dried cod (Curran & Poulter, 1983). The authors plotted moisture content on a fat free, salt free, dry basis against a_w as suggested by Doe *et al.* (1982).

The purpose of the present study was to determine the adsorption and desorption isotherms of raw hake muscle at different temperatures. From these the monolayer moisture contents, the isosteric heats of sorption and the magnitude of hysteresis were calculated. When possible, results were compared with previously reported isotherms.

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Materials and methods

Adsorption and desorption isotherms were determined by a static method. Hake (*Merluccius hubbsi*) fillets were ground in a solids mill. Samples of 2–3 g of this homogenate, in weighed dishes, were placed inside hermetically sealed glass containers and allowed to reach equilibrium over sulphuric acid solutions with a_w values in the range of 0.125–0.85. Determinations were performed at 5, 20 and 30°C. Constant temperatures were maintained by placing the glass containers in thermostatically controlled chambers. Samples for the adsorption test were freeze dried before they were allowed to reach equilibrium. Samples that reached equilibrium by adsorption presented mould growth at a_w's of 0.85 and 30°C. To avoid spoilage on samples that reached equilibrium at high a_w by desorption, the samples were previously freeze dried to an a_w of about 0.85.

The final moisture content of the sample, once equilibrium had been reached, was determined gravimetrically by the weight lost after 24 hr in an oven at 102 ± 2°C. The a_w of the solution was determined by titration against NaOH solutions. Reported results are the average of three determinations which varied by less than 3%.

Results and discussion

Temperature dependence of sorption isotherms

Desorption and adsorption isotherms for the three temperatures analysed are shown in Figs 1 and 2 respectively. All exhibit the characteristic sigmoidal curves typical

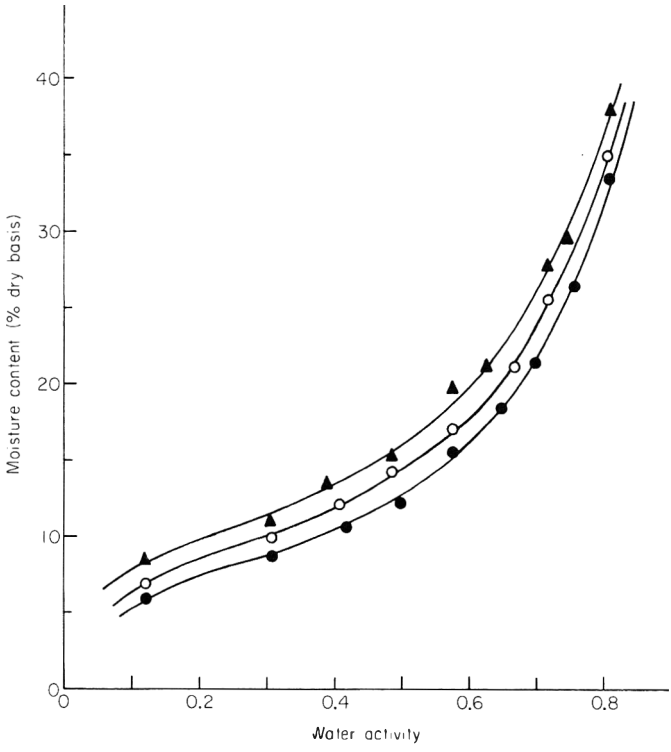

Figure 1. Moisture desorption isotherms of raw hake muscle as a function of temperature: (▲) 5°C, (O) 20°C and (●) 30°C.

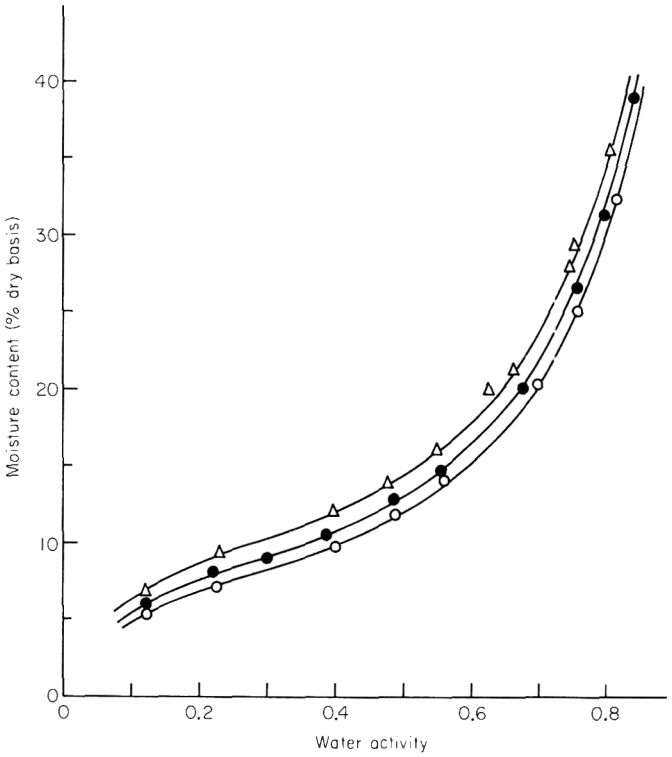

Figure 2. Moisture adsorption isotherms of raw hake muscle as a function of temperature: (△) 5°C, (●) 20°C and (O) 30°C.

of high-protein foods (Wolf *et al.*, 1972). The effect of increasing temperature is to lower the equilibrium moisture content over the whole range of a_w studied. Consequently, in an atmosphere of constant relative humidity, the sample adsorbs more moisture at lower temperatures than at higher temperatures.

The sorption data obtained at different temperatures were used to calculate the isosteric heat of sorption by application of the Clausius-Clapeyron equation:

$$\left[\frac{d \ln a_w}{d (1/T)}\right]_m = -\frac{Q_{sn}}{R} \qquad (1)$$

where:

$Q_{sn} = Q_s - \Delta H_{vap}$ = net isosteric heat of sorption,
Q_s = isosteric heat of sorption,
ΔH_{vap} = latent heat of vaporization of pure water,
T = absolute temperature,
m = equilibrium moisture content,
R = molar gas constant (1.987 cal/°K gmol).

The net isosteric heats of sorption were obtained by plotting ln a_w *versus* 1/T, the slope then being equal to $-Q_{sn}/R$. Calculated heats of adsorption and desorption are plotted in Fig. 3 against moisture content. Desorption heats are higher than adsorption heats; the difference is greater at low moisture content. Similar results were found for a wide variety of foods by Iglesias & Chirife (1976a).

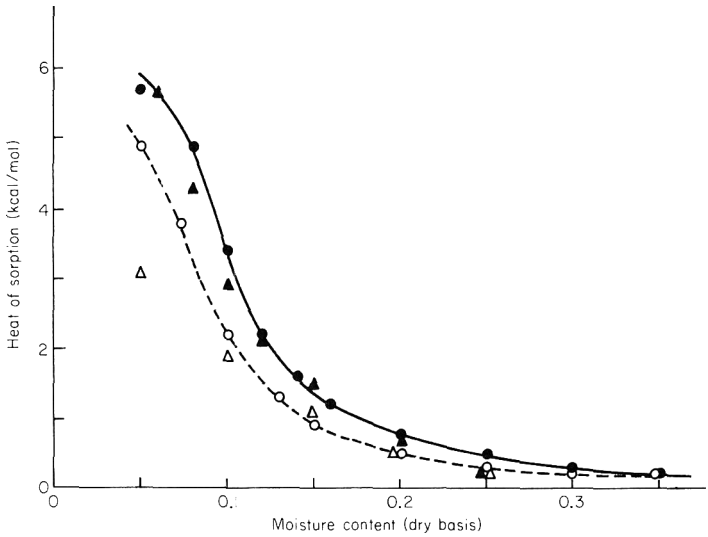

Figure 3. Isosteric heat curves for water sorption on raw hake, (—●— desorption; - -○- - adsorption) and on raw trout (▲ adsorption; △ desorption).

The isosteric sorption heats of raw trout calculated from the data of Wolf, Spiess & Jung (1973) are also plotted on Fig. 3. Desorption heats match very closely those calculated for hake. On the other hand, adsorption heats are somewhat smaller for trout than for hake at low moisture contents.

Application of B.E.T. (Brunauer, Emmett, Teller) equation

Table 1 shows the monolayer moisture content calculated by applying the classical B.E.T. analysis to the hake sorption data. It can be seen that the monolayer moisture content decreases with increasing temperature. This has been attributed to a reduction in the number of active polar sites for water binding as a result of physical and/or chemical changes induced by temperature (Iglesias & Chirife, 1976b).

Table 1. Application of B.E.T. equation to sorption isotherms for raw fish

Species	Sorption mode	T (C)	m_M (g water/g dry solid)	Reference	Reference* code
Hake	Adsorption	5	0.075		
Hake	Adsorption	20	0.067		
Hake	Adsorption	30	0.065		
Hake	Desorption	5	0.082	This work	
Hake	Desorption	20	0.074		
Hake	Desorption	30	0.066		
Cod	Adsorption	10	0.073	Cutting *et al.* (1956)	1
Cod	Adsorption	20	0.070	Cooper & Noel (1966)	2
Cod	Adsorption	25	0.076	Doe *et al.* (1982)	3
Cod	Desorption	18–21	0.077	Taylor (1961)	4
Cod	Desorption	30	0.078	Jason (1958)	5
Trout	Adsorption	5	0.079	Wolf *et al.* (1973)	6
Trout	Desorption	5	0.088	Wolf *et al.* (1973)	7

*See Table 2.

The sorption mode also affects the monolayer values as hysteresis persists down to low a_w values. Consequently, the monolayer values calculated on the desorption branches are higher than the corresponding ones on adsorption.

Monolayer moisture contents for other fish species, evaluated from literature sorption data, are also reported in Table 1. Although the calculated value for the other species are generally higher than the corresponding monolayer moisture contents found for hake, the differences are, in most cases, within the limits of experimental error.

Hysteresis

Figure 4 shows the adsorption and desorption isotherms for hake muscle at 20°C. Hysteresis is evident. The total hysteresis is moderate and it is fairly evenly distributed along the isotherms, beginning at about $a_w = 0.85$ and extending to the monomolecular region.

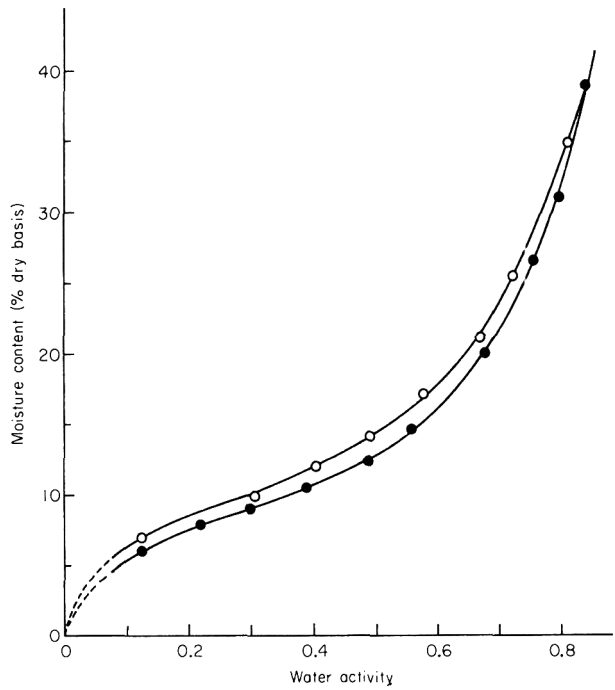

Figure 4. Hysteresis effect of sorption isotherms of raw hake muscle at 20°C: (O) desorption, (●) adsorption.

As hysteresis is a manifestation of irreversibility during the sorption process, Rizvi & Benado (1984) suggested it could be quantified in terms of the uncompensated heat, or irreversible entropy production. The net work, W, over a closed cycle (recalling that work is a path and not a point function) is given by:

$$W = -RT \oint \ln a_w \, dN \qquad (2)$$

where:

R = molar gas constant (1.987 cal/°K gmole),

N = m/M_r = number of moles of water sorbed,

T = absolute temperature,
m = equilibrium moisture content,
M_r = relative molecular mass of water.

Since $dE = TdS - dW$, and by the law of conservation of energy for a closed system $dE = 0$, then $TdS = dW$ and

$$\oint dS = \Delta S_{irr} = \frac{R}{M_r} \oint \ln a_w \, dm, \tag{3}$$

where:
S = entropy and E = internal energy.

In this way, the work lost irreversibly is the area enclosed by the plot of $\ln a_w$ against moisture content. The irreversible entropy production calculated by numerical integration of equation 3 was 0.00335, 0.00322 and 0.00123 cal/(°K g dry solid) for the hysteresis loops at 5, 20 and 30°C respectively.

Thus the effect of increasing temperature was to decrease the total hysteresis and to limit the span of the loop along the isotherms. These results are consistent with those reported in the literature by Wolf *et al.* (1972) who found that rice, apple and pork the total hysteresis decreased with increasing temperature. For raw trout hysteresis at 5°C, calculated from the data of Wolf *et al.* (1973), was 0.00865 cal/(°K g dry solid), that is 2.5 times greater than is found for hake at the same temperature. For trout hysteresis was not detected at 60°C.

Comparison between isotherms

The determination of the adsorption and desorption isotherms at different temperatures allows comparison to be made between sorption values found for hake and those reported in the literature for other species. At those temperatures for which there were no experimental sorption data for hake (10 and 25°C), the corresponding isotherms were calculated from the integrated Clausius-Clapeyron equation:

$$a_w(T) = a_w(T_o) \exp(-Q_{sn} (1/T - 1/T_o)/R). \tag{4}$$

In this way, from the relationship between Q_{sn} and water content (Fig. 3), the a_w at different temperatures may be calculated.

To make the comparison, an absolute value of the mean percentage difference was defined as

$$|D|\% = \frac{100}{n} \sum \frac{|m_h - m_o|}{m_h},$$

where m_h is the equilibrium water content in hake and m_o the equilibrium water content in other species, taken at n equally spaced a_w values over the isotherm.

Results and intervals over which the comparisons were made are shown in Table 2. The greatest percentage differences were found in samples that had not been freeze dried before they were allowed to reach equilibrium. This fact would indicate that freeze drying modifies the sorptional capacity of muscle by altering its structure.

At the same temperature and sorption mode, calculated |D|% values for cod are less than 5%. These results indicate that the sorptional characteristics of hake and cod, both species with high protein and low fat contents, are similar.

Table 2. Comparison between sorption isotherms of fish muscle

Reference code	Sorption mode	|D|%	Range of comparison (a_w)
1	Adsorption	2.0*	0.2–0.7
2	Adsorption	10.5	0.2–0.8
3	Adsorption	4.2*	0.2–0.8
4	Desorption	4.5	0.1–0.6
5	Desorption	4.5	0.2–0.8
6	Adsorption	15.0	0.2–0.8
7	Desorption	7.0	0.2–0.8

*Freeze dried prior to equilibration.

Conclusions

The sorption isotherms for raw hake muscle were determined. The heat involved in the sorption process was calculated from the temperature dependence of the sorption isotherms. Hysteresis was evaluated from the adsorption and desorption isotherms. The results on raw hake muscle were very similar to reported data on the sorptional characteristics of cod. However, the sorptional properties of fish muscle will be affected by the composition which depends on, among other factors, fish size and feeding conditions. Further research is being carried out in this area to cover all aspects of the sorptional characteristics of raw hake muscle.

Acknowledgments

This research was supported in part by Consejo Nacional de Investigaciones Científicas y Técnicas of Argentina.

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Technical note: The calculation of the heat of water sorption in foods on the basis of B.E.T. theory

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Introduction

It is believed that the C term in the B.E.T. isotherm equation can be used to estimate the heat of water sorption in foods. This has been done to estimate the heat of sorption for a wide variety of foods and food products (Masuzawa & Sterling, 1968; Bettelheim & Volman, 1957; Bushuk & Winkler, 1957; Román, Urbicain & Rotstein, 1982). However, some authors notably Iglesias & Chirife (1976a); Chirife, Iglesias & Boquet (1978) showed that B.E.T. heats, for a large number of foods, are always much less than the isosteric or calorimetric ones, and consequently of little practical interest.

Recently, Suárez, Aguerre & Viollaz (1983) and Aguerre, Suárez & Viollaz (1984) used a more general expression for the C parameter in the B.E.T. theory to calculate the heat of sorption for seven different foods. With this procedure they found that the values were substantially higher and showed good agreement with isosteric heats (at the monolayer coverage). In this paper we report the results of an extensive investigation on the evaluation of the heat of water sorption using this technique to confirm/extend the results of Suárez (1983) and Aguerre *et al.* (1984).

Results and discussion

According to B.E.T. theory the C term in the B.E.T. isotherm equation:

$$\frac{a_w}{a_w(1-a_w)} = \frac{1}{X_m C} + \frac{C-1}{X_m C}, \tag{1}$$

where a_w and X_m are the water activity and monolayer coverage respectively, is related to the net heat of sorption, Q_n, as:

$$Q_n = RT \ln C - \ln \frac{b_1 a_2}{a_1 b_2}. \tag{2}$$

For the purposes of calculation it is usually assumed that the ratio $b_1 a_2 / b_2 a_1 = K = 1$, so:

$$Q_n = R T \ln C, \tag{3}$$

which is the simplified equation widely used to estimate the heat of water sorption in foods.

If values of C are available over a certain temperature range (i.e., if the isotherms at different temperatures are known), it is not necessary to assume *a priori* that $K = 1$ as equation 2 may be rewritten:

$$\ln C = \ln K + \frac{Q_n}{RT}. \tag{4}$$

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Thus a plot of ln C *versus* 1/T should be a straight line from which the parameters Q_n and K may be calculated. Suárez *et al.* (1983) and Aguerre *et al.* (1984) found that the temperature dependence of the desorption data for seven different items (namely: rice, thyme, aniseed, sweet marjoram, fennel, wheat kernel and sugar beet root) followed this behaviour. They found that in the seven cases studied the K values obtained differed markedly from unity and thus the Q_n values obtained from equation 3 (single isotherm method) were lower than those calculated from equation 4. We performed a similar analysis for thirty-one different food products for which the adsorption or desorption isotherms at three or four temperatures are available. The data were obtained from the compilations reported by Iglesias & Chirife (1976b) and Iglesias & Chirife (1982). We attempted to calculate the B.E.T. heat of sorption in each case through equation 4 by plotting ln C against 1/T. Analysis of the results for the thirty-one different foods were as follows. For some food products we confirmed that C decreases with increasing temperature closely following the behaviour indicated by equation 4; in agreement with the results previously reported by Suárez *et al.* (1983) and Aguerre, *et al.* (1984). However, many of the foods examined did not follow this behaviour. In some cases C increased rather than decreased with increasing temperature, while for others it remained approximately constant or varied rather 'randomly' with temperature. Figure 1 illustrates the temperature dependence of C for some selected foods and Table 1 summarizes the results. It can be seen that about 26% of the thirty-one food products examined obey, to some extent, equation 4, but the rest (≃ 74%) do not. For the foods which obeyed equation 4, the B.E.T. heat of sorption was calculated by least-squares analysis of ln C *versus* 1/T. These are given in Table 2 and compared with the B.E.T. heats obtained from the simplified equation 3. As reported by Suárez *et al.* (1983) and Aguerre *et al.* (1984), we also found that in all cases the net heats of sorption (ad or desorption) calculated from the simplified equation 3 were much lower than those obtained from equation 4 i.e., K is less than unity.

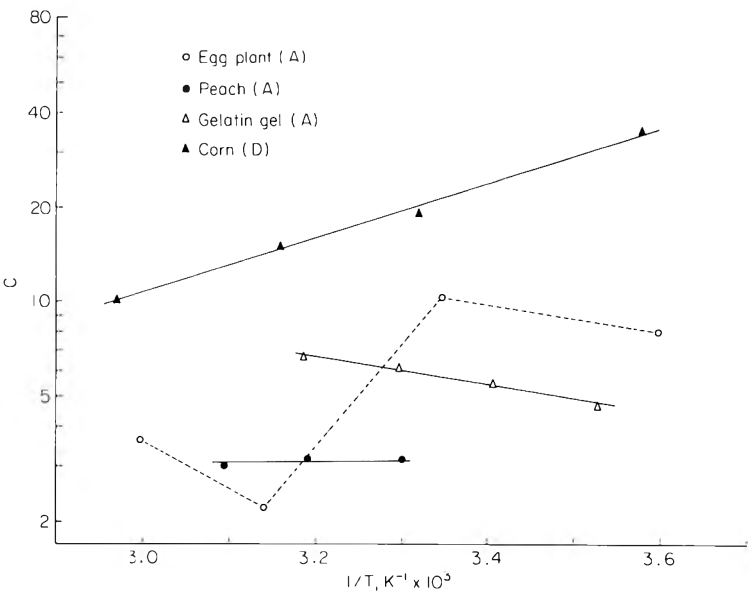

Figure 1. Temperature dependence of the parameter C for some selected foods. (A), Adsorption; (D), Desorption.

Table 1. Temperature dependence of C for different food products

	Temperature interval (°C)
(A) Foods for which C was found to increase with increasing the temperature	
1 Chicken, cooked (Ads.)	5–60
2 Chicken, raw (Ads.)	5–60
3 Trout, cooked (Ads.)	5–60
4 Banana (Ads.)	25–60
5 Pineapple (Ads.)	25–60
6 Thyme (Ads.)	5–60
7 Celery (Ads.)	5–60
8 Gelatin gel (Ads.)	10–40
(B) Foods for which C was found to remain approximately constant with temperature	
1 Dextrin (Ads.)	10.7–39.7
2 Peach (Ads.)	30–50
3 Radish (Ads.)	5–60
(C) Foods for which C was found to vary 'randomly with temperature	
1 Avocado (Ads.)	25–60
2 Egg plant (Ads.)	5–60
3 Cloves (Ads.)	5–60
4 Ginger (Ads.)	5–45
5 Winter savory (Ads.) (Des.)	5–60; 5–45
6 Lentil (Ads.)	5–45
7 Yeast (Des.)	16–44
8 Pekanut (Ads.) (Des.)	25–60; 5–60
9 Peppermint (Des.)	5–45
10 Mushrooms (Ads.)	25–60
11 Cardamon (Ads.)	5–60
12 Chamomille tea (Ads.)	25–60
(D) Foods for which C was found to decrease with increasing temperature	
1 Carrots (Ads.)	10–60
2 Corn (Des.)	10–68.3
3 Potatoes (S)	28–70
4 Starch gel (Ads.)	0–50
5 Wheat flour (Ads.)	20.2–50.2
6 Onion (Ads.)	10–45
7 Fish protein concentrate (Ads.)	25–42
8 Wheat starch (Ads.)	20.2–40.8
Summary of data	
Number of foods analysed: 31	
Foods for which C follows equation 4: 25.8%	
Foods for which C does not follow equation 4: 74.2%	

(Ads.) adsorption; (Des.) desorption; (S) sorption.

It may be concluded that although the use of equation 4 may yield more realistic heats of sorption than obtained by the single isotherm method, for a few foods it is not widely applicable since for 74% of the foods examined C did not obey the temperature dependence indicated by equation 4. This may be due to the known weakness of the B.E.T. theory to describe the energetics of water sorption in food and/or to the existence of irreversible changes which may occur in foods subjected to increased

Table 2. Comparison of B.E.T. heats of sorption calculated from equations 3 and 4.

Materal	Heat of sorption (kcal/mol)		Correlation* coefficient
	Equation 4	Equation 3‡	
Carrots (S)	7.1	1.1	0.988
Corn (D)	4.0	1.8	0.996
Potatoes (S)	6.5	2.0	0.998
Starch gel (A)	2.6	1.6	0.892
Wheat starch (A)	4.0	1.7	0.976
Wheat flour (A)	11.6	2.6	0.975
Onion (A)	9.7	1.8	0.989
Fish protein concentrate (A)	3.2	1.8	0.981

*For plot of ln C *versus* 1/T.
‡Average for the temperature interval studied.
S. Sorption; A. adsorption; D. desorption.

temperatures (Bandyopadhyay, Weisser & Loncin, 1980; Iglesias & Chirife, 1984). Such changes include changes in the crystallinity of polymers, cross-linking and denaturation of proteins and non-enzymatic browning reactions. The nature and magnitude of these changes depends not only on the food composition and the time and temperature of treatment but also on the moisture content since it is well known that, in low-moisture and intermediate moisture foods, water plays an important role in governing the rate of many physical and chemical changes. A further complication arises from the fact that the equilibrium time (at any particular a_w and temperature), is not identical for all situations since it is determined by the physical characteristics of the food and the experimental device used to determine the isotherm.

It is apparent that these effects make any theoretical analysis of the effect of temperature on the sorption of foods very difficult.

Acknowledgments

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Book Reviews

Plant Proteins for Human Food. Ed. by C.E. Bodwell and L. Petit.
The Hague: Martinus Nijhoff/Dr W. Junk, 1983. Pp. xi+267. ISBN 90 247 2856 8. $54.50.

This book contains the proceedings of a European Conference held in Nantes, France 5–7 October 1981, organized by the Insitut National de la Recherche Agronomique (INRA). It is the second time that these proceedings have been published. Indeed, the book is a direct reprint from the first publication of the *Conference in Qualitas Plantarum: Plant Foods for Human Nutrition*, Vol. 32, Nos 3/4. In fact, little concession has been made to the book form. Even the original journal page numbers remain with an extra set of page numbers in brackets for use with the table of contents of the book.

The book consists of fifteen papers divided into five parts as follows: (i) The importance of plant proteins; (ii) the composition of raw materials; (iii) extraction processes and end product characteristics; (iv) functional properties; and (v) nutritional aspects. In all there are 22 contributors from six European Countries.

While some chapters deal with more speculative sources of plant protein, such as 'The protein from leaves' by R. Fiorentini and C. Galoppini or 'Protein extraction from tobacco leaves' by P. Fantozzy and A. Sensidori, others deal with more pragmatic aspects, such as 'Extraction processes and the effect on protein functionality', by P.J. Lillford and 'Texturization of vegetable proteins and various technological processes used' by C. Giddey. The two papers in Part 5 on 'Nutritional aspects', namely 'Influence of processing on the nutritional value of protein' by P.A. Finot and 'Natural anti-nutritional factors present in European plant proteins', by R. Ferrando, contain material published in several reviews published in other books, but their inclusion here is important to the integrity of the subject.

Each chapter is well presented and referenced, with clear diagrams and tables. The main drawback to the book is the lack of an index. The intended readership includes students, food scientists, nutritionists, agronomists and anyone seeking information on plant protein sources, processing or utilization. The scope is broad, with sufficient detail to make it a useful book for a wide range of readers.

Ann F. Walker

Statistical Methods in Food and Consumer Research. By Maximo C. Gacula and Jagbir Singh. Orlando: Academic Press, 1984. Pp. xiii+505. ISBN 0 12 272050 4. £61.00.

This text assumes the reader has a working knowledge of the basics of statistical inference. There are three chapters on 'Sensory testing', 'Sensory difference tests' and 'Paired comparison methods'. However the bulk of the book is concerned with the analysis of data from designed experiments. It is intended for researchers, instructors and applied statisticians, and according to the authors is suitable for a first course in

applications of statistical methods which appears to presuppose a course in basic statistics.

In the preface the authors refer to the growth of data analysis methodology attributing this to the availability of computers, but neither 'computer' nor 'package' appears in the index, and evidence of their influence is not obvious in the text. Computational details for hand calculation abound. Along with solid algebra they produce a text likely to daunt most food scientists despite the very limited use of calculus. This is not a basic book.

The applied statistician's training may well have given him more sophisticated ideas than are presented here on topics like modelling and testing the goodness-of-fit of a model, but he will be able to cope with the algebra easily enough. He will find a large selection of results presented and may be quite happy to use this book as a reference on specific technical matters. He will not find much novel statistical material. More than 90% of the hundreds of references were 10 or more years old when the book was published. If he is engaged as a consultant to food scientists he probably has access to computing facilities that would make some rather different 'cookbook' help relevant. Reasonably modern statistical packages will handle designs much less stringently constrained to balance form than those discussed herein. Blocks of unequal sizes and missing values, are examples of practical sizes not elucidated in an up-to-date way.

There is a concentration on details of analysis and an organization in terms of analysis methods that seems to this reviewer undesirable and narrow. The supposed readership of non-specialists, as far as statistics is concerned, need to look at analysis in the context of other phases of the data generating processes of experimentation and get inadequate help to do so.

How should the structure of experimental units, e.g., in blocks, be determined? How should the treatments applied to the units be structured for efficiency? These are questions of design rather than analysis and the book's emphases make it unhelpful on this side. Even before that the laboratory scientist needs to decide exactly what measurements he is going to make, and there are decisions here that have implications for analysis.

When he goes to the trouble of designing an experiment in the formal way assumed in the classical statistical analyses, the food scientist is unlikely to restrict himself to one observation on each unit. Multiple responses such as repeated measures through time, or a profile comprising numerous characteristics, are almost inevitable if the experimenter is, sensibly, squeezing as much as he can from his material. It is an unfortunate feature of the generality of statistical texts that they have little to say about what one does to analyse multiple responses and this is a rather derivative text following that tradition.

Full-scale formal multivariate analyses are not the answer except for the expert; they are stultifyingly complex. But it is highly likely that the structure of the multiple measurements is more important to the experimenter than the other artificial block and treatment structure imposed on the experimental material. It is a great pity the book does not encourage commonsense efforts to utilise a number of elementary analyses to build up perspectives on the measurement set. All in all this text leaves plenty scope for other books offering food scientists help with their specific problems in using statistical methods.

I. Wilson

Immigrant Foods. By S.P. Tan, R.W. Wenlock and D.H. Buss.
London: HMSO, 1985. Pp. vi+74. ISBN 0 11 242717 0. £4.50

This second supplement to McCance and Widdowson's *The Composition of Foods* (edited by A.A. Paul, D.A.T. Southgate 1978, London: HMSO) has been produced in response to increasing demand for information on immigrant foods by dietitians and nutritionists working with immigrant communities in the U.K. The 357 foods presented have been selected in consultation with dietitians, nutritionists, the food industry and restaurateurs. Foods eaten by the most numerous immigrant groups from the Indian sub-continent, the West Indies and Hong Kong are emphasized. Some items have already been covered by the 4th edition, and are included in the supplement for completeness.

The supplement has been prepared following the general principles of the 4th edition, using a combination of direct analysis and selection of values from literature sources. However, the tables are not as extensive or as elaborate as the 4th edition. The entry columns for proximates are simplified, seven minerals quoted in place of ten in the 4th edition, with nine columns for vitamins in place of fifteen in the 4th edition. The tables are presented in the same way throughout the food groups which makes them easy to use and allows all the data to be accommodated on two facing pages. Nevertheless, the simplification is at the expense of some detailed information given in the 4th edition, which makes the supplement less valuable, particularly for research workers.

The appendices contain recipes for cooked dishes and systematic names for fish and plant foods. The index allows for easy location of most foods. Although the book is useful in providing a single source of reference for immigrant foods, the lack of a considerable amount of data is disappointing. In particular, for many foods, no information is available on dietary fibre, as number of minerals, especially zinc, and certain vitamins. In my view it would have been preferable to have delayed publication until more data were available.

Ann F. Walker

Modern Chromatographic Analysis of the Vitamins. Ed. by André P. De Leenheer, Willy E. Lambert and Marcel G.M. de Ruyter.
(Chromatographic Science Series, Vol. 30).
New York: Marcel Dekker, 1985. Pp. ix+556. ISBN 0 8247 7221 0. US$102.00, SFr.255.

This volume is divided into twelve sections, each devoted to one vitamin, written by different teams of authors from Europe, the United States and Japan. The vitamins covered are A, D, E, K, C, folates, niacin, B1, B2, B6, B12 and biotin. Each section has an introduction summarizing the chemical properties of the vitamin, its biochemical significance and in some cases an historical perspective of its discovery and analysis. The remainder of each chapter reviews the application of chromatographic techniques to the separation and determination of the different forms of the vitamin, its provitamins and metabolites in biological fluids, pharmaceuticals and food.

The editors state the book is intended primarily for those with an acquaintance with chromatographic techniques, whilst providing some useful information for a beginner

in the field. They aim to provide a detailed overview of the techniques, particularly the newer ones, applicable to each vitamin group in different matrices.

The authors of each section are clearly expert in their fields and give a detailed account of their specialities, involving such techniques as GC-MS of vitamin D metabolites, multidimensional HPLC of Vitamin K1 and HPLC/RIA of cobalamin. All the sections give thorough accounts of the different forms of each vitamin and devote much space to their separation. The problems and pitfalls of the extraction of each vitamin are covered although not always in the separate sub-section which they deserve.

The references range in number from 68 on flavins to 225 on vitamin A. The majority are from the years 1970 to 1981, the most recent being from 1984. The quality of the printing is very high: well laid out, legible and virtually free from typographical errors, and there is a comprehensive index.

The different authors sub-divide their subject matter in different ways, some taking analytical technique as a major sub-heading, others taking the vitamin form or the matrix. Most of the chapters are very well provided with clear chemical diagrams, tables and example chromatograms, an exception being that on folates which is mostly text. Some of the authors provide useful tables summarizing the main characteristics of the methods reviewed others however bury much of the information in page-sized paragraphs of turgid prose.

There is a strong bias towards clinical applications in many of the chapters at the expense of food and pharmaceuticals. For example the chapter on thiamine contains little more than half a page on each food and pharmaceuticals but ten pages on clinical samples.

With the current interest in the nutritional labelling of foodstuffs and the associated increase in demand for vitamin analysis of foods, it is a pity that this subject is not given more prominence. Moreover a separate section on multivitamin methods would have been useful.

In conclusion, this volume provides in itself a wealth of up-to-date information on the chromatography of vitamins and comprehensive references to the literature.

J.P. Wootten

Biotechnology Biotransformations. (Vol. 6A. Ed. by K. Kieslich. Weinheim: Verlag Chemie, 1984. Pp. Xii+473. IBN 3 527 25768 3. DM 495.

This is the latest addition to an excellent but very expensive series of books. It contains ten chapters most of which deal with the biological transformations of a specific chemical group. Apart from an introduction and a short chapter on general methodology the one chapter which diverges from this pattern is on antibiotics. I feel this was a very sensible move by the editors since the structurally diverse antibiotics could easily have been lost in small sub-sections in the other chapters.

While the whole of this volume should be of interest to food scientists and technologists three chapters deserve particular mention: those concerned with the biotransformations of terpenoids, amino acids and peptides and carbohydrates. The various biological transformations of amino acids, peptides and carbohydrates are of course well known and of increasing importance to the food industry. The more important of these processes are described here in detail. For example, the chapter on carbohydrates

considers fructose production by glucose isomerase; bioconversion in L-ascorbate acid synthesis; bioconversions of gluconic acid, kojic acid, dihydroxyacetone and isomaltose, the conversion of raffinose by α galactoside-galactohydrolase, of lactase by β-lactase and sucrose by invertase are also discussed. The coverage of each of these topics is admirable, ranging from the technical aspects of microbiology and chemical engineering to its economic viability.

The biological transformations of terpenoids outlined in the book were new to me. However, their potential importance in synthesizing these important flavour agents is effectively brought over. This chapter in particular contains many examples which illustrate how the remarkable stereospecificity of enzymes can be utilized in a commercial process.

Each chapter is complete in itself containing an introduction, outline of methods and a summary of the more important biological transformations of that particular class of compound. Due to the vast range of information in each chapter the authors cannot always go into the details their subject requires, but each chapter contains a well balanced list of references. This volume holds together admirably and each chapter is so well written and organized that I find it hard to criticize any aspect of the book's content. All the contributors should be congratulated on the thoroughness with which they have obviously searched the patent and scientific literature.

The book has a adequate index, is attractively presented and I found no typographical errors. Undoubtedly the purchase of this series is a major financial undertaking for most scientific collections. However, if the rest of the series is of the same high standard as this volume, then it will be well worth the money.

F.F. Morpeth

Microbial Survival in the Environment: Bacteria and Rickettsiae Important in Human and Animal Health. By Eilhard Mitscherlich and Elmer H. Marth. Berlin: Springer Verlag, 1984. Pp.x+802. ISBN 3 540 13726 2. DM 390.

This book is stated by the publishers to be "a collection of data on the tenacity in the environment of bacteria and some rickettsiae important in medicine and veterinary medicine", and is expected to be useful by providing "data of fundamental importance to physicians, veterinarians, epidemiologists and public health officials confronted with epidemics of contagious diseases or outbreaks of foodborne illnesses".

The first 560 pages consist of tables listing the bacterial and rickettsial species in alphabetical order. For each species there is first a short sentence or paragraph on the organism's "significance and habitats", although this section is often extremely brief, e.g., the entry for "*Vibrio* non-cholera" reads simply: "non-cholera vibrios (Heiberg group I, II and V) are incriminated in incidents of foodborne illness". Then follows information on survival data. These derive from published papers, and details are given for each substrate followed by the reference. Using as an example the entry for "*Vibrio* non-cholera" once again, survival data are given separately for buffers, casserole-type foods, eggs, insects, meat and shellfish, although five of the six entries derive from the same paper by Diane Roberts and Richard Gilbert.

Section II, of 146 pages, starts with an 11 page table listing the temperature and pH limits for the growth of the organisms. Then follow a further 327 tables listing data for

the survival of a range of microorganisms in a given environment. The environment may be a foodstuff (e.g. honey, milk), or a natural habitat (e.g., seawater, soil), or a surface which may have been contaminated (e.g. metal, paper, plastic, rubber), or the contaminating agency (e.g., air), but some tables arranged alphabetically with all the rest relate to an environmental component or parameter (e.g. salt, water activity). I feel that closer attention should have been paid to the organization of this section: perhaps it would have been more helpful to the reader to separate these different categories.

Section III (36 pages) provides very short commentaries on the effect of environment or environmental parameters. This Section is bound to be of very limited value, e.g., the discussion of the effects of low temperature takes up only about two-thirds of a page.

The book is completed by a list of around 2000 references, and indices of microorganisms and environments. The compilers of the book point out that some data are included which derive from papers published around the turn of the century where the data can be considered reliable (for example in the case of *Salmonella typhi* and *Vibrio cholerae*). However, microbiologists involved with newer food processes and products and with the use of newer food preservatives should note that few references derive from the 1980s.

I doubt that this book will prove particularly useful to the target audience. Those concerned with investigations of outbreaks of diseases such as food poisonings will often need to consider quite complex interactions between many aspects of the food history during production, distribution and consumption, and of the patient status such as age of consumers, state of health etc. Such workers would get more data of fundamental importance in relation to food borne diseases by consulting the 2 volume work *Microbial Ecology of Foods* produced by the International Commission for Microbiological Specifications for Foods and published by Academic Press. The present work by Mitscherlich and Marth is much more likely to prove useful to research workers seeking information on previous work carried out on survival of particular organisms or of organisms in particular environments, and thus it would be a useful addition to university and research institute libraries unless they already have access to computer-generated databases for information retrieval.

W.F. Harrigan

Books received

Developments in Soft Drinks Technology, Vol. 3. Ed. by H.W. Houghton. Barking, Essex: Elsevier Applied Science, 1984. Pp. xiii+246. ISBN 0 85334 278 4. £26.00.

The contributions in the latest volume in this occasional series are: Modern design and treatment of bottles used for carbonated drinks (H. Ono); Modern bottle washers (D. Bosi); High fructose corn syrups (B.H. Landis & K.E. Beery); The comminuted citrus base (H.W. Houghton); Further microbiology of soft drinks (V.J. Batchelor); Powdered soft drink mixes (R.P. Verrall).

The Farinograph Handbook, 3rd ed. by Bert L. D'Appolonia and Wallace H. Kunerth.
St. Paul, Minnesota: American Association of Cereal Chemists, 1984. Pp. vii+64. ISBN 0 913250 37 6. US$ 33.50 (softback).

There are chapters covering: a discussion of the farinograph; theoretical aspects; types of farinograph curves and the factors affecting them; physical factors affecting farinograms; interpretation of the curves; special uses (including evaluation of cereal grain quality, gluten properties, carbohydrate properties, enzyme activity, baking quality, and composite flours); dough rheology; modifications and developments; and precautions to be followed during use. Appendices discuss an AACC collaborative study, and AACC, ICC and RACI procedures.

Ethylene and Plant Development. Ed. by J.A. Roberts & G.A. Tucker.
London: Butterworths, 1985. Pp. ix+416. ISBN 0 407 009230 5. £55.00.

This volume comprises the Proceedings of the 39th University of Nottingham Easter School in Agricultural Science held in 1984. There are thirty-three contributions, some being research papers and some being reviews. The subject areas covered include the practical methods and advantages of either applying ethylene to, or removing from, various commercial products; the more fundamental aspects of ethylene synthesis and action during the developmental process of growth, ripening, abscission and senescence; and the effects of ethylene on gene expression and cell development.

Advances in Cereal Science and Technology, Vol. VII.
St. Paul, Minnesota: American Association of Cereal Chemists, 1985. Pp. ix+359. ISBN 0 913250 39 2. US$ 60.00.

The six chapters in this volume are concerned with: Seed storage proteins of economically important cereals; Phospholipases of cereals; Changes in rice during parboiling, and properties of parboiled rice; Dietary fibre in cereals; sprouted grain; and Starch damage.

Leitfaden der Milchkunde und Milchhygiene. By Gerhard Kielwein.
Berlin: Paul Parey, 1985. Pp. 156. ISBN 3 489 684168. DM 34.–. (softback, in German).

Insect Management for Food Storage and Processing. Ed. by Fred J. Baur.
St. Paul, Minnesota: American Association of Cereal Chemists, 1985. Pp. xiv+384. ISBN 0 913250 38 4. US$ 65.00.

Nutritional Bioavailability of Calcium. Ed. by Constance Kies. (ACS Symposium Series 275). Washington, D.C.: American Chemical Society, 1985. Pp. vii+200. ISBN 0 8412 0907 3. US$ 45.95.

Food Microbiology and Hygiene. By P.R. Hayes.
Barking: Elsevier Applied Science, 1985. Pp. xvi+403. ISBN 0 85334 355 1. £48.00.

Diet-Related Diseases: The Modern Epidemic. By Stephen Seeley, David L.J. Freed, Gerald A. Silverstone & Vicky Rippere.
Beckenham: Croom Helm, 1985. Pp. xi+272. ISBN 0 7099 3365 7. £9.95 (softback).

Starch Conversion Technology. Ed. by G.M.A. Van Beynum & J.A. Roels.
New York: Marcel Dekker, 1985. Pp. xi+362. ISBN 0 8247 7194 X. US$ 78.00.

Food Allergy. Ed. by Patricia Scowen, Gisela Medhurst & Jill Leslie.
London: Edsall, 1985. Pp. 96. ISBN 0 90263 45 1. £3.90 (softback).

Quality Control in the Food Industry, Vol. 1. 2nd ed. Ed. by S.M. Herschdoerfer.
London: Academic Press, 1984. Pp. xiii+469. ISBN 0 12 343001 1. £45.00.

Principles of Plant Biotechnology: An Introduction to Genetic Engineering in Plants. By S.H. Mantell, J.A. Matthews and R.A. McKee.
Oxford: Blackwell, 1985. Pp. iii+269. ISBN 0 632 07215 3. £10.80 (softcover).

Lipid Protein Vegetable Sources. By H.O.A. Osman, Y.G. Moharram, R.A. Bakr, and revised by A.A. Abdel-Bury.
Alexandria: University of Alexandria, 1985. Pp. vii+294 (In Arabic with 45 pages of tables in English).

Evaporation, Membrane Filtration and Spraydrying in Milk Powder and Cheese Production. Ed. by Robert Hansen.
Vanløse: North European Dairy Journal, 1985. Pp. xviii+420. ISBN 87 7477 000 4.

Processing Aquatic Food Products. By Frederick W. Wheaton and Thomas B. Lawson.
New York: John Wiley. 1985. Pp. xvi+518. ISBN 0 471 09736 5. £77.60.

Toxigenic Fungi—Their Toxins and Health Hazard. (Developments in Food Science, 7). Ed. by H. Kurata and Y. Ueno.
Amsterdam: Elsevier, 1984. Pp. xix+363. ISBN 0 444 99630 3. US$ 86.50.

JOURNAL OF FOOD TECHNOLOGY: NOTICE TO CONTRIBUTORS

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Arrangement. Papers should normally be divided into: (a) Summary, brief, self-contained and embodying the main conclusions; (b) Introduction; (c) Materials and methods; (d) Results, as concise as possible (both tables and figures illustrating the same data will rarely be permitted); (e) Discussion and conclusions; (f) Acknowledgments; (g) References. Authors should consult the current issue in order to ensure that their manuscript conforms to the Journal conventions on such things as subheadings, layout of tables, etc.

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Steiner, E. H. (1966). Sequential procedures for triangular and paired comparison tasting tests. *Journal of Food Technology*, **1**, 41–53.

Reference to books and monographs should include (a) name(s) and initials of author(s) or editor(s); year of publication in parentheses; (b) title, underlined; (c) edition; (d) page referred to; (e) place of publication and publisher—e.g.

Lawie, R. A. (1979). *Meat Science*. 3rd edition. Oxford: Pergamon Press.

In the case of edited multi-author monographs, the editor(s) should be indicated in parentheses after the book title—e.g.

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

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

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

SI UNITS

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

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

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

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

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

Offprints. Fifty offprints will be issued free with each paper but additional copies may be purchased if ordered on the printed card which will be sent to the senior author with the proofs.

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