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The detection and estimation of soya protein in food products by isoelectric focusing

J. W. LLEWELLYN AND B. FLAHERTY

Summary

The analysis of non-meat protein in meat products by the technique of isoelectric focusing in polyacrylamide gels is described. When laboratory prepared pork sausages containing known amounts of added soya protein isolate were analysed, 1% (of fresh sausage weight) added isolate could be detected. At low levels of added isolate (<5% fresh sausage weight) the technique could only be considered semiquantitative, but at higher levels of added isolate (>5% fresh sausage weight) better quantitation was possible. The technique cannot be applied to the analysis of products that have undergone extensive heat treatment during manufacture, however, due to denaturation of the soya protein.

Introduction

The identification and estimation of soya protein in meat products by gel electrophoresis has been widely reported. Polyacrylamide as a support medium has been favoured by most workers, although starch gels (Olsman, Houtepen & van Leeuwen, 1969) and cellulose acetate membranes (Gils & Hidskes, 1973) have also been used. Both disc (Fischer & Belitz, 1971; Spell, 1972; Guy, Jayaram & Willcox, 1973; Lee *et al.*, 1975) and slab (Freimuth & Krause, 1970; Hofmann & Penny, 1971; Parsons and Lawrie, 1972; Mattey, 1972; Hofmann, 1973) techniques have been investigated together with a variety of extractants and buffer systems. A preference has been shown for those based on urea alone (Freimuth & Krause, 1970; Spell, 1972; Parsons & Lawrie, 1972; Gils & Hidskes, 1972), urea and 2-mercaptoethanol (Olsman *et al.*, 1969; Guy *et al.*, 1973) and also sodium dodecyl sulphate (Hofmann & Penny, 1971; Mattey, 1972; Hofmann, 1973; Lee *et al.*, 1975). Use of a trishydroxymethylmethylamine and glycine system as both extracting and running buffer has also been reported (Fischer & Belitz, 1971).

None of these analytical procedures completely resolves soya protein from meat and corrections for a 'meat background' are necessary. Quantitative analysis of soya protein in products which have not undergone extensive heat treatment during preparation, for example, raw sausages, has been possible but with cooked products,

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such as pies and luncheon meats, denaturation of the proteins on severe heating renders the electrophoretic band patterns diffuse, making the method insufficiently accurate for quantitative analysis (Lawrie *et al.*, 1972; Guy *et al.*, 1973).

The technique of isoelectric focusing in polyacrylamide gels (Wrigley, 1968a, b) offers the advantages of higher resolution and superior reproducibility over conventional electrophoresis. The proteins are separated on a gel which has an internal pH gradient maintained by a system of amphoteric buffers (ampholytes). The proteins migrate under the influence of a strong electric field into regions of the gel in which the pH is equal to their respective isoelectric points (pI), where, since the overall charge on each protein is zero, they remain. Separation is thus based on isoelectric point and not on molecular weight. The proteins are resolved into extremely narrow and highly reproducible bands, at equilibrium positions in the gel. The theory and practice of isoelectric focusing in gels has recently been comprehensively reviewed (Righetti & Drysdale, 1974).

Isoelectric focusing of soya protein in milk whey–soya protein blends on polyacrylamide gels containing urea have been reported (Badui & Josephson, 1974), but the technique has not been applied to meat products. It was thought that the more distinct band pattern and better reproducibility obtained by isoelectric focusing might readily afford a simpler and more reliable indentification of soya and other non-meat proteins than was hitherto possible. Earlier qualitative investigations carried out in this laboratory have been described (Flaherty, 1975) and we report below a quantitative procedure developed from this.

Materials and methods

Preparation of sausage meats

Sausage meats were prepared to the following recipe.

Lean pork	$33 - x\%$ where $x = 0, 1, 2{\cdot}5, 5, 7{\cdot}5, 10, 15$
Soya protein isolate (Promine D)	$x\%$
Fat	33%
Finely ground rusk	12%
Water	20%
Salt	2%

The ingredients were blended into a smooth paste which was then lyophilized, defatted by continuous extraction with diethyl ether and dried in air at room temperature. Samples of commercial sausages, and canned and frozen beefburgers were first freeze-dried and then defatted as above.

Extraction of proteins

Freeze dried and defatted products were suspended in water in sealed Universal

bottles and heated in a boiling water bath for 15 min. On cooling, urea, to make the solutions 6 M and 2-mercaptoethanol (2%) were added and the suspensions shaken for 30 min at room temperature. They were centrifuged at 17 000 rev/min (34 000 ***g***) at 20°C and the clear supernatants retained. Three separate extractions of each sample were taken.

Standard solutions of soya protein isolate (Promine D) were similarly prepared.

Preparation of gels

Polyacrylamide slab gels (1·5 mm thick) 4·5% (3% cross linked, final ampholine conc. 1·8%), pH 3·5–10 containing 6 M urea were prepared as follows. Acrylamide (2·7 g), N,N′-methylenebisacrylamide (0·083 g), urea (21·6 g), LKB ampholines pH 3·5–10 (2·4 ml), pH 4–6 (0·15 ml) and pH 5–7 (0·15 ml) were dissolved in deionised water (42 ml) and riboflavin solution (0·2 mg/ml × 0·4 ml) added. The solution was deaerated under high vacuum, the gel cell filled taking care to avoid trapping bubbles, and the mixture polymerized by u.v. irradiation for 2 hr.

Isoelectric focusing

Two aliquots (20 μl) of the Promine D standard and of each of the three sample extracts were dispensed from a micrometer syringe on to 5 × 15 mm filter paper strips placed at the anode side of the gel. Focusing was performed on an LKB 2117 Multiphor at a constant power of 20 W for 3 hr. (Initial voltage 200 V, 50 mA, gradually increased to 400 V, 50 mA after 20 min, then power kept constant. Final voltage 800 V, 25 mA). The anode buffer was 1% aqueous orthophosphoric acid (pH 1·7) and cathode buffer 1% aqueous diaminoethane (pH 11·2). Two gels were normally run simultaneously on separate Multiphors.

After focusing, the gels were stained by immersion in a solution of 0·2% bromophenol blue in ethanol:acetic acid:water, 5:1:4 for 2 hr (Awdeh, 1969), then destained by immersion in a solution of ethanol:aetic acid:water, 6:1:13 for 24 hr, with one change of solution during this period. The proteins appeared as opaque dark green bands on a clear pale yellow background.

Densitometric scanning

Gels (which remained attached to a glass backing plate throughout the experiment) were immersed in a tray of water, covered with a second glass plate, taking care to eliminate trapped air bubbles, to form a sandwich which was then scanned on a Joyce Loebl Chromoscan 200 with 201 thin layer attachment, using 620 nm optical filters and narrowest slit width. For quantitative measurement, a 3·5 cm length of each set of bands was scanned to avoid irregularities due to streaking from the sample application area at the anode end. Corrections for gel background absorption were made by scanning a corresponding length of adjacent blank gel. Each set of bands (and corresponding background) was scanned four times and an average value of the integral

taken. Results were calculated for each of the three sample extracts on each gel (total of six values) and the average quoted.

pH gradient measurement

The pH of the gel was measured at 1 cm intervals across the gel with an antimony microelectrode. Readings were taken at each end of the gel and at two intermediate positions and the average taken. The pH profile was then plotted.

Results

Results of analysis of laboratory-prepared pork sausages containing Promine D are summarized in Fig. 1. The 10 and 15% levels of Promine D represent replacements of the minimum meat content much greater than those recommended in the report of the Food Standards Committee on Novel Proteins in Food Products (1975), hence only one analysis at each level was performed. Analysis of eight separate sausage preparations each containing 5% (of fresh sausage weight) Promine D were carried

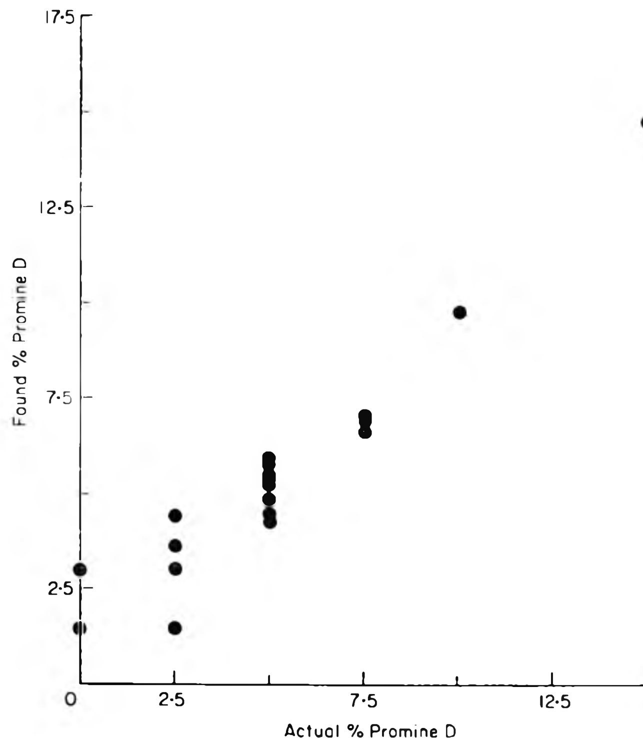

Fig. 1. Results of analyses of laboratory prepared pork sausages containing added Promine D (weight Promine D expressed as percentage of total weight of sausage meat).

out to examine the reproducibility of the method at this level which approximates to the 30% (maximum) recommended replacement of the minimum meat content of meat products by hydrated TVP. Analyses were carried out on one commercially available canned brand and two frozen brands of beefburgers, which were known to contain soya protein. None could be detected in the canned product but the frozen products both gave soya protein contents of 7·2 ± 1·4% (of fresh weight of products), expressed as Promine D.

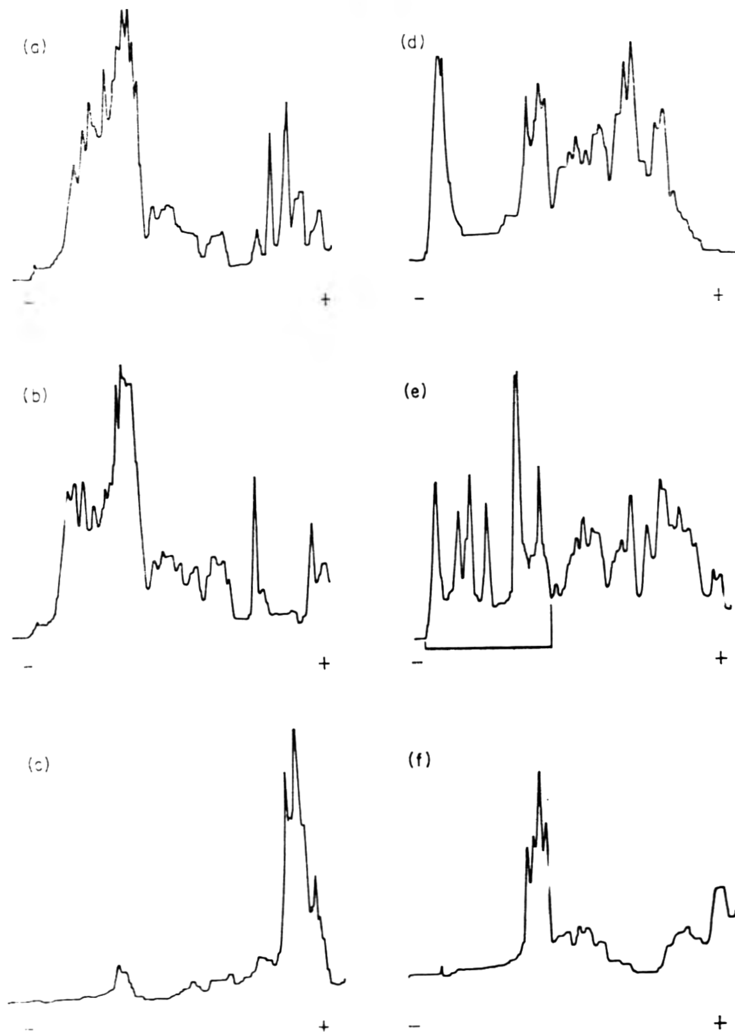

FIG. 2. Microdensitometric traces of band patterns. (a) Pork; (b) chicken; (c) spray dried milk powder; (d) field bean protein; (e) soya protein isolate (Promine D); (f) dried egg powder. Underlined section of 2e indicates 3·5 cm length used to quantify Promine D; anode right, cathode left.

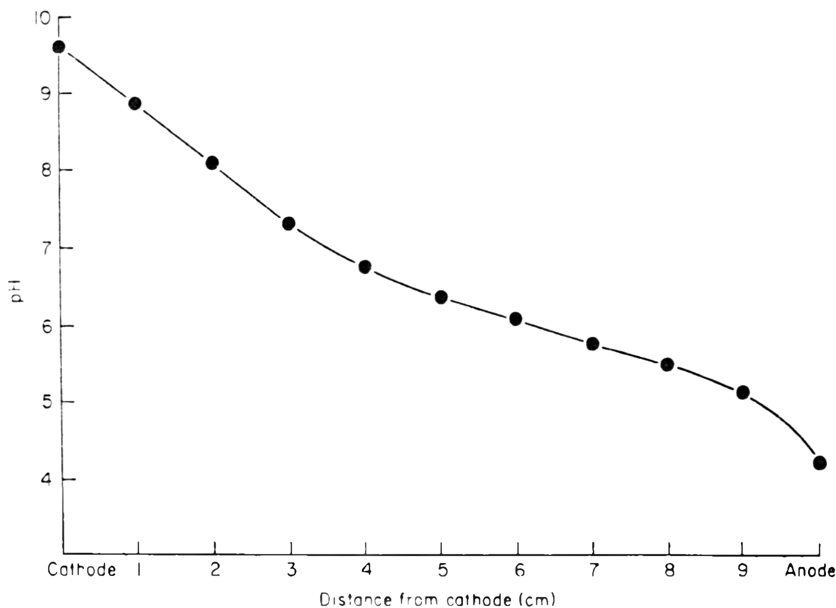

FIG. 3. pH gradient of polyacrylamide (4·5% pH 3·5–10) gel containing 6 M urea. ● Average of four readings.

Isoelectric band patterns of urea-mercaptoethanol extracts of other possible meat extenders, namely field bean protein, dried egg powder and spray dried milk powder were qualitatively compared with the patterns obtained from soya protein, pork, and chicken and are shown in Plate 1. The corresponding densitometric traces are displayed in Figs 2a–f. Individual bands were not assigned to specific proteins. The pH profile (Fig. 3) is not linear but was reproducible and, apart from the extreme ends, variation along the length of the gel was minimal. The pH decreased from 9·6 adjacent to the cathode buffer strip to 4·2 adjacent to the anode buffer strip.

Discussion

The isoelectric patterns of some soya isolates, concentrates and flours, various meats and soya based cheese substitutes have already been reported (Flaherty, 1975). The characteristic patterns (and corresponding microdensitometric traces) of egg powder, dried milk powder, soya and field bean, (Plate 1 and Figs 2a–f) protein sources which may be used either to replace or extend meat, or to increase the protein content of meat products, are easily distinguishable from those of meats, thereby affording a simple qualitative identification of 'added protein'. However, as the band patterns obtained by isoelectric focusing are more complex than those obtained by conventional electrophoresis, patterns obtained from a mixture of protein sources exhibit a multitude of

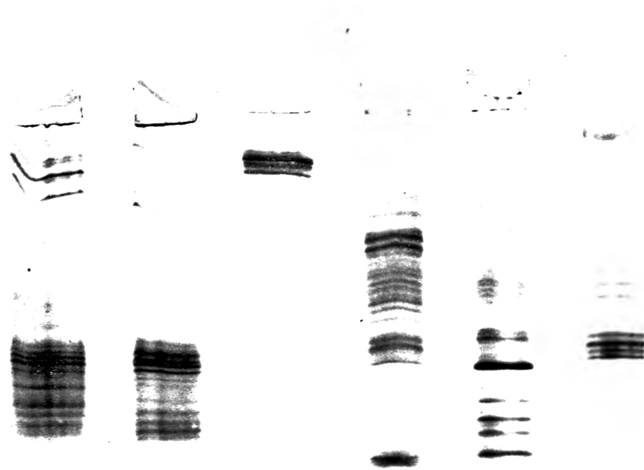

PLATE 1. Isoelectric band patterns on polyacrylamide (4·5% pH 3·5–10) gel containing 6 M urea. Left to right: pork; chicken; spray dried milk powder; field bean protein; soya protein isolate (Promine D); dried egg powder. Samples at anode—anode at top of photograph).

(*Facing p.* 560)

lines from which it is difficult to identify the individual components. The complex patterns can be simplified, however, when one component is a meat. Limited heating of meat and non-meat protein mixtures renders the meat essentially insoluble in urea-mercaptoethanol whereas the non-meat protein remains soluble, thus only its band pattern is observed. Meat products which contain appreciable quantities of two added proteins, for example, soya and field bean, would, however, still afford the complex combined pattern of the two added species.

Selective extraction of added protein by the limited heating technique enabled the quantitative analysis of soya protein isolate in uncooked sausage meat, the results of which are shown in Fig. 1. Isolate added at the 1% (of total fresh sausage weight) level (i.e. 5% replacement) could just be detected but not quantified, but 5% added isolate (i.e. 25% replacement) could be determined with a relative error of ±20% (i.e. 5 ± 1%) as confirmed by the eight individual analyses at this level. At levels below this results were considerably less accurate. The replacement levels were calculated as replacement of statutory minimum meat content of pork sausages by *hydrated* soya protein where hydrated weight soya protein = 3 × weight of dry isolate (i.e. hydration ratio of 2 : 1) without taking account of any added fat. If an added fat content is taken into account replacement levels are higher.

The principal sources of error were, variation in extraction efficiency, a residual meat effect, scanning, and variation in gel background due to uneven staining. Of these the extraction variations were the most important and were minimized by taking three extractions of each sample, applying each extraction twice to each of two gels and quoting the average results. The 'residual meat' effect was due to extraction of a small quantity of meat protein which remained soluble after the heat treatment. The effect was variable, as shown by the results obtained from the pork sausage controls, and was most important with low levels of added protein (2·5% and below). The results indicated are not corrected for this effect. No effect was noted with a sample of a beef sausage when examined, however. Scanning errors were due to insufficient sensitivity of the microdensitometer to bands of low intensity and to an inherent integrator error which was minimized by taking the average of four values of each integral. Variations due to uneven staining were difficult to overcome. Blank sections of gel adjacent to sample bands were scanned to obtain a gel 'background' integral and it was assumed that there was no variation between this and the true value in the band pattern. Where variation was obvious the bands were not scanned.

Analysis of frozen beefburger samples known to contain soya protein was possible but the technique failed with their canned equivalents. The temperature involved in canning processes are sufficient to denature certain soya proteins, rendering them insoluble in 6 M urea-mercaptoethanol. This effect is paramount with basic proteins and 'cathode bands' (proteins of pI greater than 6) were not seen on IEF of canned luncheon meats or cooked pies which contained added Promine D. The acidic proteins, which appear as the 'anode bands' remain soluble, however, and are heat stable. Thus,

IEF of heat processed products affords a less complex pattern of anode bands as previously reported (Flaherty, 1975). Trial experiments using narrow range gels (pH 4–7) to examine the anode band patterns more closely were unsuccessful. The bands obtained in this pH range were weak and diffuse and the gels tended to shrink and distort on destaining, making scanning and therefore quantitation, impossible. Analysis of heat processed products containing soya protein is further complicated in that different processing conditions denature proteins to different extents and there is therefore difficulty in defining a 'standard denatured soya protein'. Thus analysis of such products may not be possible by this technique.

A wide range of soya isolates, concentrates, flours and extruded texturized soya proteins has previously been examined in this laboratory and all show the same band patterns (see Plate 1 and Fig. 2e) although the relative intensities of the bands differ. Spun soya protein products display band patterns similar to those of heat processed products thereby enabling differentiation from their extruded counterparts. In conclusion, it may be said that the limitations of the technique are those of electrophoretic methods in general, *viz.* problems of extraction, staining and microdensitometric scanning. It may only be useful for the analysis of non-meat protein in products that have not been extensively heated during manufacture, for example, sausages and beefburgers, and it is imprecise, especially at low levels of added protein. As a qualitative technique the superior resolution displayed affords better differentiation of protein species by their characteristic band patterns than is possible by conventional electrophoretic methods.

Acknowledgment

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Equilibrium moisture contents of air dried beef. Dependence on drying temperature

HÉCTOR A. IGLESIAS AND JORGE CHIRIFE

Summary

The water adsorption isotherms at 30°C of precooked beef previously dried at three different temperatures: 30°C, 55°C and 70°C, respectively, were determined. It was found that the higher the drying temperature the lower is the sorption capacity of dried beef. A B.E.T. analysis of the isotherms demonstrated that the quantity of water contained in the monolayer is affected by the temperature of drying. The adsorption and desorption isotherms at 50°C were also determined; no hysteresis was found. A multilayer adsorption equation is used to describe adequately the water sorption behaviour of cooked beef in the range of water activities studied.

Introduction

Hot air drying has been thoroughly used for the drying of pre-cooked meat minces (Sharp, 1953). However, despite many improvements in drying techniques, dried meat suffers a noticeable deterioration in quality during drying as well as when storaged in the dried state (Connell, 1958; Sharp & Rolfe, 1958). Data on the water sorption properties of dried beef may help our understanding of the nature of changes suffered by meat during drying. Besides that, information is needed for evaluating the storage characteristics of dried beef, as there is a well established relationship between water sorption isotherms and the chemical, physical and stability characteristics of dehydrated foods (Loncin, Bimbenet & Lenges, 1968; Labuza, Tannenbaum & Karel, 1970). Saravacos & Stinchfield (1965) studied the sorption isotherms of raw freeze-dried beef at various temperatures. MacKenzie & Luyet (1967) studied the dependence on initial freezing treatment of the sorption isotherms from freeze dried muscle fibres. Wolf, Walker & Kapsalis (1972) prepared sorption isotherms for myosin A and myosin B, and Palnitkar & Heldman (1971) reported the equilibrium moisture characteristics of freeze dried beef components and fractions.

The present study was intended to obtain fundamental data on the water sorption properties of air dried beef, and particularly on the effect of drying temperature on the sorption properties of dried beef. An attempt was also made to describe the various

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experimental sorption isotherms using a multilayer adsorption equation developed by Halsey (1948) and recently shown by Iglesias, Chirife & Lombardi (1975a) to describe the water sorption behaviour of several foods.

Materials and methods

Materials

Cooked beef was used in all the experiments. Most of the fat and connective tissue were removed from the sample prior to the sorption studies. Samples were prepared by cutting cubes of about 0·5 cm in size which were subjected to air drying in the conditions described below.

Drying procedure

Beef samples were dried in a laboratory cross circulation drier at an air velocity of 3 m/sec, until the moisture content reached a value less than 9% (dry basis). The samples were further dried over magnesium perchlorate in a vacuum desiccator at room temperature. Three different air drying procedures were employed characterized by three constant air dry bulb temperatures: 30°C, 55°C and 70°C, respectively.

Isotherms

The moisture adsorption isotherms were determined gravimetrically by exposing the samples to atmospheres of known relative humidities. Vacuum desiccators were prepared with saturated salt solutions (Young, 1967) or aqueous sulphuric acid solutions of known concentration (Wilson, 1921). The desiccators were placed in a constant temperature cabinet (±0·5°C). The samples were placed in the desiccators at the various relative humidities and a vacuum was pulled in order to speed up the equilibrium. The moisture content of the equilibrated samples was determined by placing the sample in a vacuum oven at 70°C over magnesium perchlorate for 48 h. The technique has not been described in detail here, because it was done in previous papers (Iglesias, Chirife & Lombardi, 1975b, c). Sorption isotherms were determined over the range 10–80% RH at 30°C and 50°C. One desorption isotherm was also established at 50°C. The desorption isotherm was obtained following an adsorption cycle and after exposing the samples to a space saturated with water vapour. Equilibrium moisture contents were reported as grams of water per 100 g dry non-fat beef. For this purpose the samples were analysed for fat content after each experiment. Fat content was determined by solvent extraction. Each point on the isotherm represents an average of two measurements made during the experimental run.

Results and discussion

The adsorption isotherms at 30°C of pre-cooked beef dried at three different air temperatures, 30°C, 55°C and 70°C respectively, are shown in Fig. 1. It is apparent that the

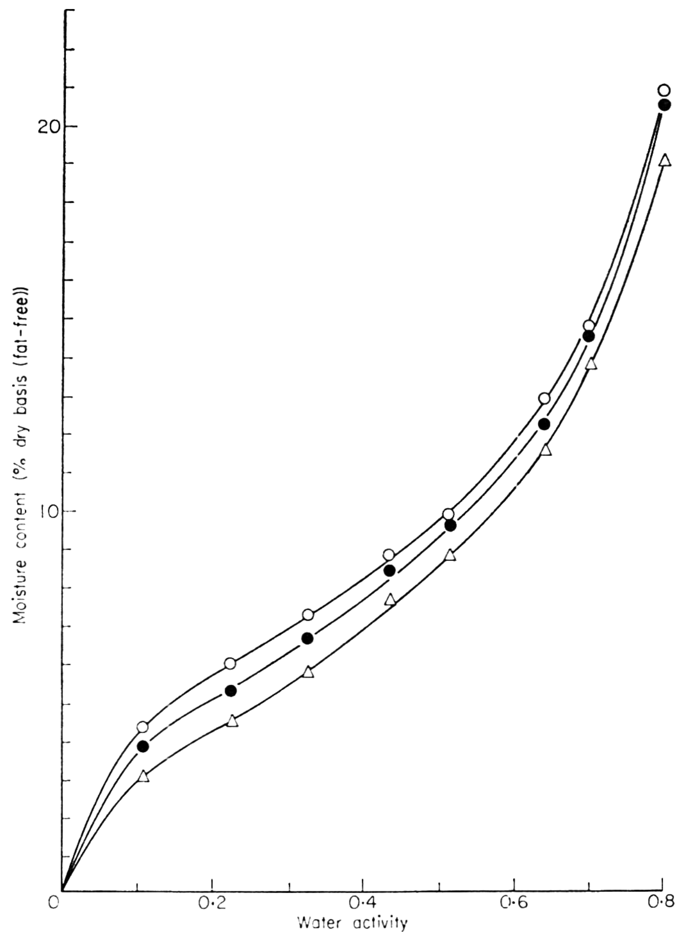

FIG. 1. Effect of drying temperature on the adsorption isotherms at 30°C of pre-cooked dried beef. ○ Dried at 30°C; ● dried at 55°C; △ dried at 70°C.

higher the drying temperature the lower the sorption capacity of beef, and that the absolute differences in moisture contents are approximately constant in the range of 0·10–0·40 water activities. The three curves have the typical sigmoid shape of type II isotherms in accordance with B.E.T. classification (Labuza, 1968). Consequently, they were subjected to analysis by the B.E.T. method (Labuza, 1968). In accordance with the usual practice we used water sorption data at water activities of about 0·10 to 0·40 for the B.E.T. analysis. Least squares analysis was used to obtain the slopes and the intercepts of the B.E.T. plots from which the monolayer was calculated. The validity and application of B.E.T. equation has been recently analysed and discussed by the authors (Iglesias & Chirife, 1976a). The results are shown in Table 1; it is

TABLE 1. Effect of drying temperature on the monolayer moisture content of dried cooked beef (from adsorption isotherms at 30°C)

Drying temperature (°C)	Monolayer value (% non-fat dry basis)
30	5·4
55	5·1
70	4·5

noteworthy that the quantity of water contained in the monolayer decreases with increasing drying temperature. As proteins are mostly responsible for the binding of water in beef, the interactions of water with proteins must be considered in order to explain the observed results. The mechanism of water sorption by proteins has been the subject of several studies. Early workers (Sponsler, Bath & Ellis, 1940; Shaw, 1944; Bull, 1944; Pauling, 1945) concluded that the amount of water sorbed depended primarily on the number and availability of two types of hydrophilic groups which are capable of binding water through hydrogen bond formation. These are the polar side chains and the carbonyl and imido groups of peptide bonds. Speakman (1944) postulated that water sorption by proteins occurred onto polar side chains at low humidities, spread to peptide linkages and then to multilayer formation at higher humidities. A point of view generally accepted (Bull, 1944; Pauling, 1945; McLaren & Rowen, 1951) suggests that there is a good correlation between the number of water molecules calculated to exist in a B.E.T. monolayer and the number of polar side chains. This suggests that each polar group initially sorbs one molecule of water followed at higher humidities by multimolecular adsorption. Accordingly, the results shown in Table 1 may indicate that the number of available polar groups in beef proteins is decreased as a result of increasing drying temperature. It is known that proteins in beef may suffer several changes during drying. Among those changes are (Karel, 1968): denaturation, crosslinking and interaction of the native or denatured proteins with lipids or carbohydrates. It may be supposed that the availability of hydrophilic sites for water binding in beef is reduced due to one or several of the above mechanisms. It is noteworthy that the observed lowering of the adsorption isotherms with increasing drying temperature from 30°C to 70°C does not necessarily indicate a reduction in the rehydration capacity of dried beef. The amount of water taken up on rehydration cannot be related solely to specific sorption sites and mostly consists in loosely bound water associated in a three dimensional hydrogen bonded network (Brooks, 1958; Leeder & Watt, 1974).

The adsorption isotherms at 30°C and 50°C of pre-cooked beef dried at 55°C are shown in Fig. 2. The desorption isotherm at 50°C is also shown in the same figure. It

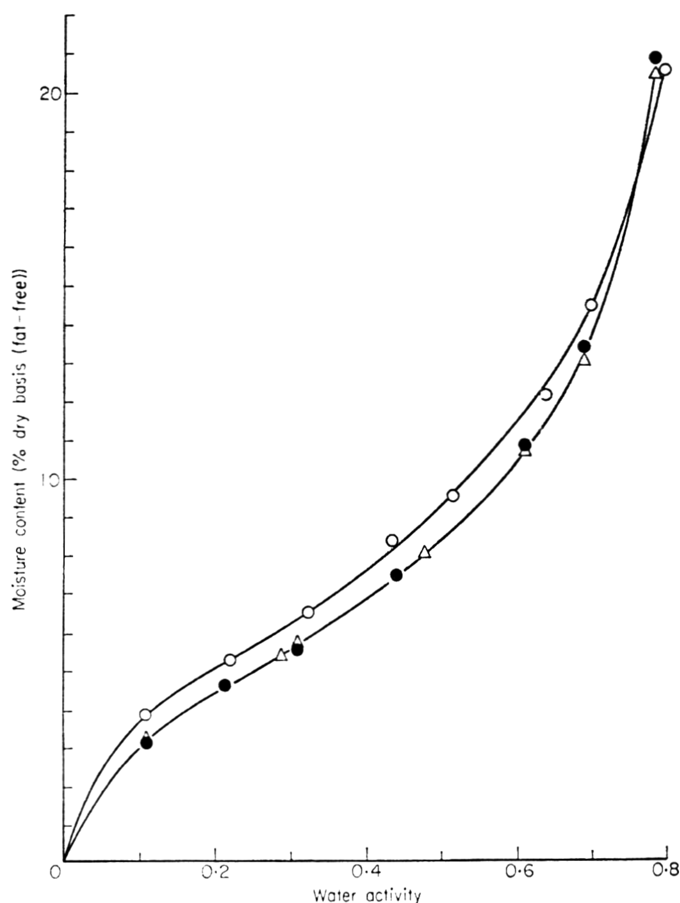

FIG. 2. Sorption isotherms at 30°C (○ adsorption) and 50°C (● adsorption and △ desorption) of beef dried at 55°C.

can be seen that temperature has the expected effect on the sorption isotherm, i.e. the quantity of sorbed water at a given relative humidity increased as the temperature was decreased. It is also seen that there is no hysteresis effect. Palnitkar & Heldman (1971) found a very small degree of hysteresis in the sorption isotherms of freeze dried beef at 21·1°C.

The isosteric heat of sorption (net heat) was calculated using the Clausius-Clapeyron equation (Iglesias & Chirife, 1976b). The isosteric net heat versus the moisture content is shown in Fig. 3. In the same figure are also plotted the heats of sorption for pre-cooked fish and chicken for the same range of moisture content, reported by Iglesias & Chirife (1976b). The curves show a similar behaviour, that is they show a continuous and steep decay with increasing moisture content. The decrease in the isosteric net heat with the amount of water sorbed can be qualitatively explained considering that

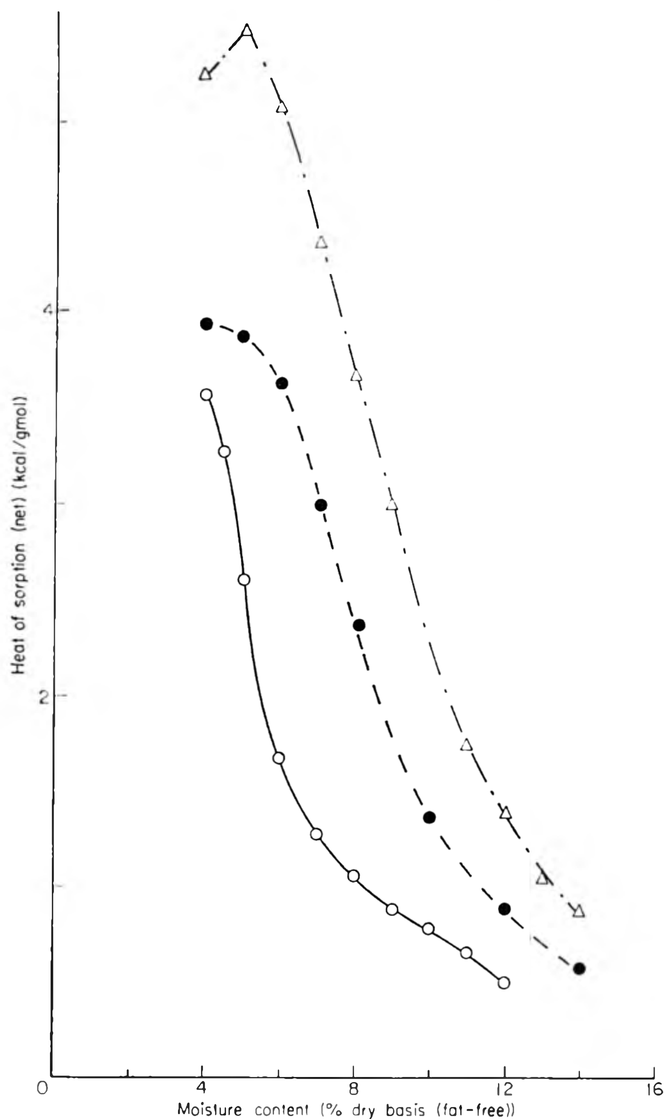

Fig. 3. Variation of net isosteric heat of sorption with moisture content. ○ Beef; ● trout muscle (Iglesias & Chirife, 1976a); △ chicken muscle (Iglesias & Chirife, 1976a).

initially, sorption occurs on the most active sites given rise to greatest interaction energy. As these sites become occupied, sorption occurs on the less active sites giving lower heats of sorption (Bushuk & Winkler, 1957; Iglesias & Chirife, 1976b).

Mathematical expressions which describe the adsorption of water on foods are of interest not only for theoretical but for practical reasons. Equations fitting water sorption isotherms are needed for the prediction of equilibrium conditions after mixing

products with different water activities (Salwin & Slawson, 1959). An analytical expression for the isotherm is also required for the quantitative analysis of storage stability (Karel, Mizrahi & Labuza, 1971; Labuza, Mizrahi & Karel, 1972). Iglesias *et al.*, 1975a) showed that a multilayer adsorption equation, originally developed by Halsey (1948) could be used to describe the water sorption behaviour of a great variety of foods. The sorption data on beef were treated according to the Halsey's equation as used by Iglesias *et al.* (1975a) and Iglesias & Chirife (1976c):

$$\ln \ln p_0/p = -r \ln X + \ln a'' \quad (1)$$

where p/p_0 = water activity; r, a'' = parameters; X = equilibrium coverage, percentage dry basis (fat-free). A plot of $\ln \ln p_0/p$ versus $\ln X$ should be a straight line from which the parameters a'' and r may be calculated. Table 2 shows the calculated values

TABLE 2. Application of Halsey's equation to sorption isotherms of pre-cooked dried beef

System	r	a''	% error (average)
Isotherm A	1·448	18·50	2·6
Isotherm B	1·392	15·08	1·8
Isotherm C	1·301	10·70	3·7
Isotherm D	1·250	9·91	2·6

A: isotherm at 30°C, beef dried at 30°C; B: isotherm at 30°C, beef dried at 55°C; C: isotherm at 30°C, beef dried at 70°C; D: isotherm at 50°C, beef dried at 55°C.

for the adsorption isotherms of beef dried at three different temperatures and for the adsorption isotherm at 50°C of beef dried at 55°C. It also shows the percentage error obtained upon application of the Halsey's equation. This error means an average of the percentage errors at several equally spaced water activities over the whole range studied of water activity (0·10–0·80). It can be seen that Halsey's equation represents adequately the sorption behaviour of dried beef for each of the situations examined. Calculated and experimental water sorption isotherm, for one of the situations examined, is plotted in Fig. 4, and show the degree of applicability of the proposed equation.

Conclusions

Water adsorption isotherms of air dried pre-cooked beef at 30°C and 50°C and the corresponding desorption isotherm one at 50°C have been described and it has been shown that the adsorption isotherms are affected by the temperature at which the drying was carried out.

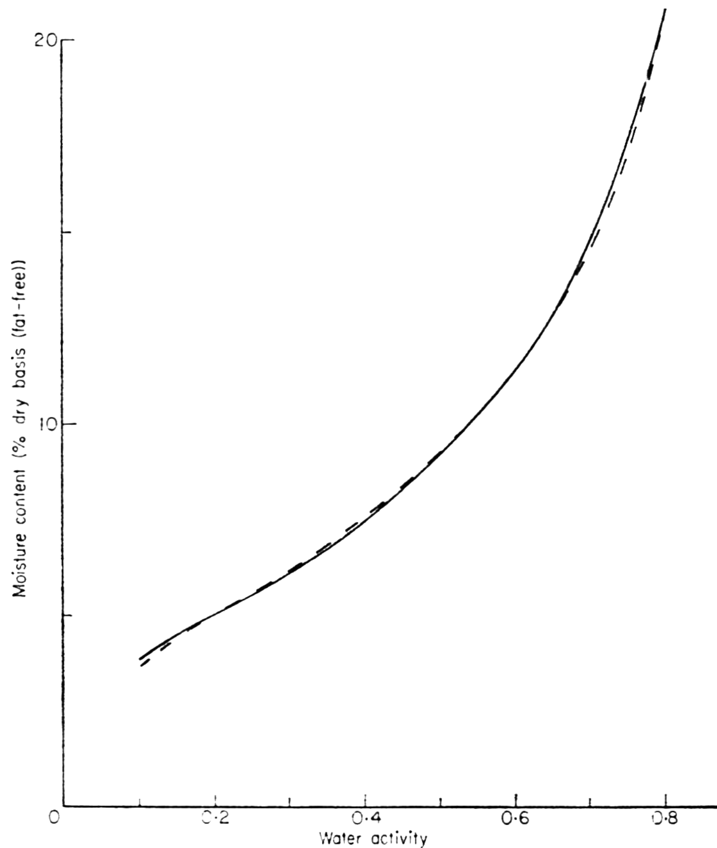

FIG. 4. Application of Halsey's equation to the adsorption isotherm at 30°C of beef dried at 55°C (——). – – – Calculated.

The net isosteric heat of sorption was also calculated and compared with reported values for other pre-cooked meats. Halsey's equation can be used to describe any of the isotherms obtained in the range of water activity studied (0·10–0·80). This study indicates that care must be taken in the selection of drying conditions for the purpose of determining the adsorption isotherms of meat products.

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The protein of intermediate moisture meat stored at tropical temperature

IV. Nutritional quality

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Summary

The effect of storage at 38°C on the nutritional value of the proteins of intermediate moisture (i.m.) meat was studied by both rat bioassay and chemical methods. Freshly prepared i.m. meat was always of similar nutritional quality to fresh meat but, if complexing reactions occurred that led to insolubilization in 3% sodium dodecyl sulphate/1% β-mercaptoethanol then a marked decrease in both net protein utilization (NPU) and protein efficiency ratio (PER) was found. As these complexing (crosslinking) reactions normally occur quite readily in i.m. meats stored at tropical temperature it is desirable, if the nutritional value of these meats is to be maintained during storage, that means be found to inhibit them.

Introduction

Previous studies have shown that the nature of the protein in intermediate moisture (i.m.) meats is, in several respects, different to that in fresh and cooked meat and with storage, further changes occur (Obanu, Ledward & Lawrie, 1975a, b, 1976). The changes appear to be due to the occurrence of both crosslinking and degradation of the proteins in the glycerol-infused i.m. meats.

Chemical studies have indicated that the complexes formed during storage are very stable (Obanu *et al.*, 1975a, b) and, from the nutritional point of view, may well make some amino acids unavailable to the body and for this reason rat bioassays have been performed to evaluate the protein nutritional quality of these foods and to correlate these results with selected chemical tests. The chemical tests used were:

(i) determination of available lysine (Carpenter, 1960) as this is often used as an index of nutritional quality; and

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(ii) determination of the solubility of the meats in solutions of sodium dodecyl sulphate (SDS) plus β-mercaptoethanol as decreases in solubility in this solvent appear to correlate with increased protein crosslinking (Obanu *et al.*, 1975a, 1976).

Materials and methods

Preparation and storage of the i.m. samples

Two feeding trials were performed. In Feeding Trial 1 the *l. dorsi* muscle of an eleven-month-old bull was used (Obanu *et al.*, 1976) and in Feeding Trial 2 the *l. dorsi* muscle of a thirteen-month-old steer. The i.m. samples were prepared and processed to a_W 0·85 as described in the earlier paper (Obanu *et al.*, 1975a). Storage was in Cryovac PVDC bags (W. R. Grace Ltd, London) at 38°C. Samples were taken at regular intervals and either pulverized with solid CO_2 prior to chemical analyses (Obanu *et al.*, 1976a) or freeze dried for use in the feeding trials. Some of the uncooked muscle was blast frozen at −20°C and stored at −10°C for use as a control; this was cooked in water at 70°C prior to use (Obanu *et al.*, 1975a).

Chemical analysis

Available lysine was determined by Carpenter's method (Carpenter, 1960). The solubility of the samples in 3% sodium dodecyl sulphate (SDS)/1% β-mercaptoethanol was determined as described previously (Obanu *et al.*, 1975a).

Feeding trials

Net protein utilization (NPU) (Miller, 1963) and an estimate of Protein Efficiency Ratio (PER) of all i.m. meat samples was made using male and female rats of the Wistar strain. These rats were bred on the premises. Intermediate moisture meat samples replaced part of the maize starch in the basal, protein-free, diet (Table 1) so that, except for the basal diet, all the diets contained 100 g protein/kg diet.

A casein diet supplemented with 2·5 g/kg DL methionine was included for comparison in both trials.

In Feeding Trial 1 the rats were all specific pathogen free males and were caged in equal weight groups of three. Three rats per cage were used in Feeding Trial 2 and these were selected to give groups of the same sex and weight. This meant that each value of NPU and PER was the mean for three rats in both Trials.

Diets were fed for ten days and at the end of this period rats were killed and carcasses either dried and ground in a bench mill (Kenwood Chef) or dissolved completely in concentrated H_2SO_4. Samples of carcass, food and faeces were estimated for nitrogen by the macro Kjeldahl technique. Net Protein Utilization (NPU) was calculated from the formula:

$$\text{NPU\%} = \frac{Bt - (Bk - Ik) \times 100}{It}$$

TABLE 1. Composition (g/kg) of basal protein-free diet

D-glucose	150
Arachis Oil	150
Salt mixture*	50
Vitamin mixture*	11
Maize starch	639

* Composition as described by Payne & Stewart (1972).

where Bt = total body nitrogen of the rats fed on meat protein; It = total intake of food nitrogen of the rats fed on meat protein; Bk = total body nitrogen of the rats fed on protein-free diet; Ik = total intake of food nitrogen of the rats fed on protein-free diet.

Protein Efficiency Ratio (PER) was calculated from the formula:

$$PER = \frac{\text{Body weight gain (g)}}{\text{Protein intake (g)}}$$

Results

As shown in the previous paper (Obanu *et al.*, 1976) the bull muscle used in Feeding Trial 1 was atypical in that although extensive proteolysis occurred there was no evi-

TABLE 2. Effect of storage at 38°C on the NPU, PER, available lysine values, and protein solubility in 3% SDS/1% β-mercaptoethanol for i.m. meat prepared from a bull *l. dorsi* muscle

Storage time (weeks)	NPU* %	PER*	Avail. lysine† g/100 g protein	SDS/β-mercaptoethanol solubility† (%)
0	97·6	2·97	9·03	80·3
	90·7	2·94		
3‡	88·6	1·95	8·95	96·3
	74·0	2·74		
6	87·3	2·50	8·15	90·6
	81·9	2·63		
9	74·0	2·59	8·55	90·2
	84·9	2·94		
Freshly cooked meat	90·7	2·83	8·02	94·8
	87·1	3·43		
Casein diet	89·4	3·05	—	—
	84·7	3·21		

* These are the mean values for three male rats caged together.

† Mean of duplicate determinations.

‡ This diet was inadvertently made up with 50 g sodium chloride instead of 50 g glucose making the diet slightly unpalatable.

dence for any crosslinking. For example, there was little change in the solubility in SDS/β-mercaptoethanol of these samples when stored at 38°C (Table 2).

Feeding Trial 1

It is seen from Table 2 that there is little change in the protein efficiency ratio (PER) and net protein utilization (NPU) during storage of these samples. Table 2 also shows that there was little change in the availability of the lysine in these samples during storage at 38°C. Apparent nitrogen digestibility of all the samples in this trial was good (> 98% at all storage times).

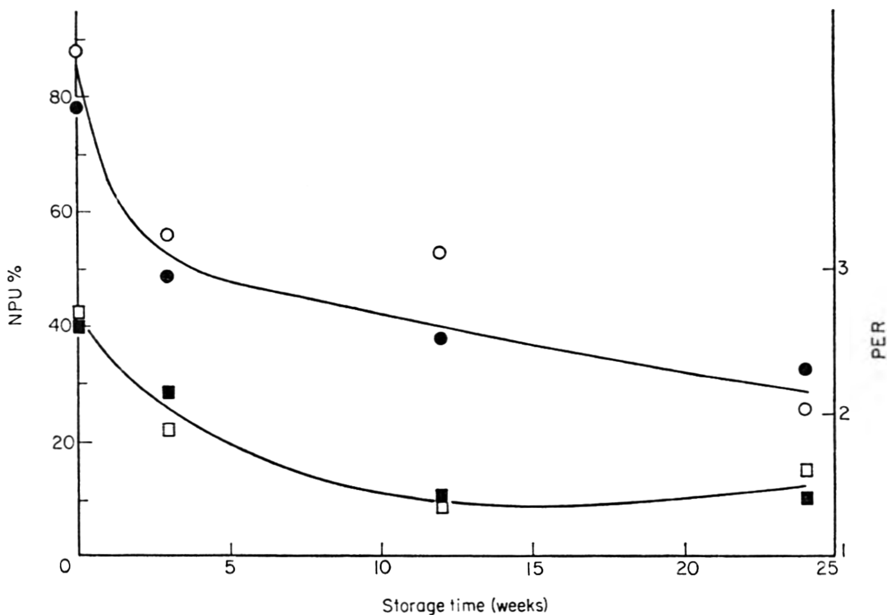

Fig. 1. Effect of storage at 38°C on the nutritional quality of the proteins of i.m. beef; ○ NPU determined on male rats, ● NPU determined on female rats, □ PER determined on male rats, ■ PER determined on female rats. For the freshly cooked meat the NPU values were 75·4 and 65·1% for the males and females respectively and the PER values were 3·2 and 2·8 for the males and females respectively.

Feeding Trial 2

The effect of storage on the PER and NPU of this steer muscle is shown in Fig. 1. It is seen that there is a marked decrease in both parameters during storage; for example, the NPU changes from a value similar to that found for fresh meat to a value typical of cereal protein (Miller & Bender, 1955).

In this feeding trial the apparent nitrogen digestibility of all meat samples was good

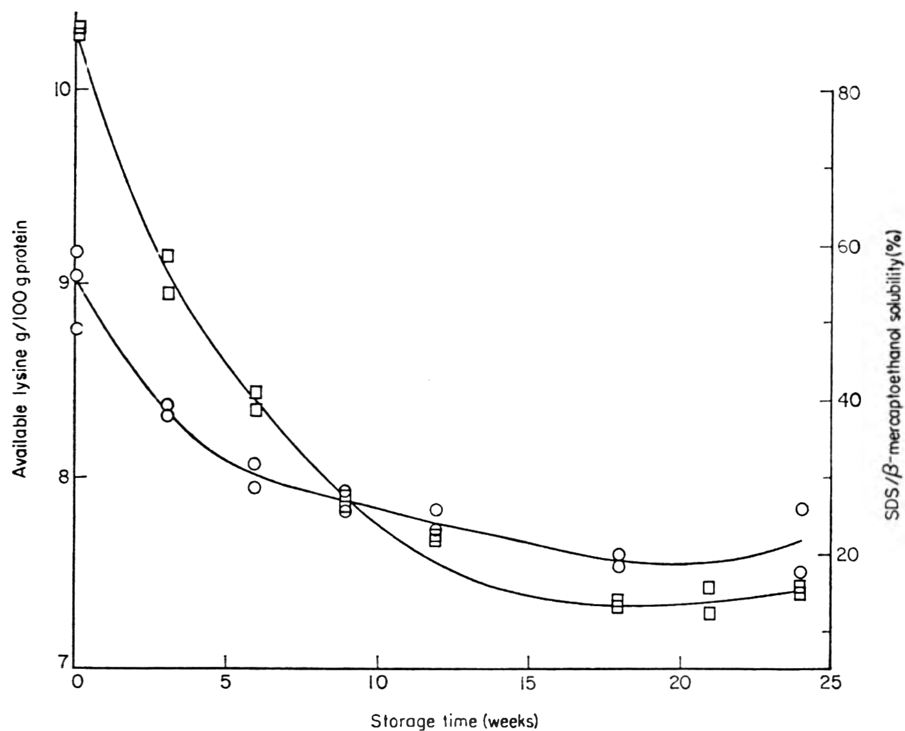

FIG. 2. Effect of storage at 38°C on the available lysine (○) and solubility in SDS/β-mercaptoethanol (□) of the proteins of i.m. beef.

(>97% at all storage times) indicating that the reduced NPU was not due to poor digestibility.

In chemical reactivity this muscle was typical of all muscles (except the bull) studied previously (e.g. Obanu *et al.*, 1975a, b, 1976) as the tests all indicated that both proteolysis and crosslinking occurred. For example, the solubility in SDS/β-mercaptoethanol decreased markedly during storage (Fig. 2) in similar manner to that found for all other i.m. meats except the bull *l. dorsi* muscle used in Trial 1 (Obanu *et al.*, 1976). The decrease in nutritional quality of the samples, and loss of SDS/β-mercaptoethanol solubility, were mirrored by a decrease in lysine availability (Fig. 2). The values for available lysine and SDS/β-mercaptoethanol solubility for the freshly processed i.m. meat were similar to the values for freshly cooked samples. The values for available lysine for cooked meat were 9·07 and 9·16 g/100 g protein and for SDS/β-mercaptoethanol solubility duplicate values were both 94·6%.

Discussion

Both feeding trials show that, immediately after processing, the nutritional quality of the i.m. meat is similar to that of ordinary cooked meat. In fact the NPU values for the

freshly prepared i.m. samples appear to be slightly higher than for fresh meat suggesting that the glycerol in the system may be improving the quality by a protein sparing effect. However, the NPU and PER values in the two trials differ markedly in their variation with storage time. The results of Trial 1 indicate that the nutritional quality of the protein in the i.m. bull samples remained very high throughout nine weeks of storage at 38°C (Table 1) whereas the protein quality in the i.m. steer sample rapidly deteriorated yielding, even after only three weeks at 38°C, a product of significantly decreased PER and NPU (Fig. 1). These apparently conflicting results in the two trials do, however, correlate with the chemical changes in the samples as in the bull samples, although proteolysis occurred during storage, there was no evidence of any crosslinking (Table 2 and Obanu *et al.*, 1976) while the steer sample was more typical in its behaviour as both proteolysis and crosslinking occurred during storage (Obanu *et al.*, 1975a, b, 1976). This crosslinking, which is shown by the decreased solubility of the samples in SDS/β-mercaptoethanol correlates well with the loss in nutritional protein quality (cf. Figs 1 and 2, $P < 0{\cdot}001$). Thus the solubility in SDS/β-mercaptoethanol may be a very good index of the nutritional quality of the proteins in these i.m. meats. The minimal changes observed in both SDS/β-mercaptoethanol solubility and nutritional quality of the protein in the Feeding Trial 1 support this suggestion. Because of the nature of the reactions that render the proteins nutritionally unavailable in these i.m. meats it is doubtful if this relationship would be applicable to other protein sources.

It is generally accepted that high glycerol intake is not hazardous to health and the results of Feeding Trial 1 confirm that, in rats, it does not affect their rate of growth. In fact the livers, spleens, kidneys and *l. dorsi* muscles of the rats in this trial were examined after slaughter and no abnormalities could be detected verifying the non-toxic nature of the additives.

It is generally recognized that in meat, a high quality protein food, lysine is not the limiting amino acid but it is seen from the present results that both PER and NPU are highly correlated with the available lysine values (cf. Figs 1 and 2). If lysine was the only amino acid involved in the crosslinking reactions, it would be anticipated that the PER and NPU values would remain relatively constant during storage, at least until the available lysine concentration was decreased to such a level that it became the limiting amino acid in the system. This is obviously not the case and it is concluded that the protein reactions leading to crosslinking are of a more general nature so that several, or even all, the amino acids become less available during storage. This observation would agree with the amino acid analyses performed earlier (Obanu *et al.*, 1975b) which showed a decrease in the concentration of all amino acids, determined after hydrolysis, in stored, compared to freshly processed, i.m. beef samples.

The crosslinking reactions, which appear to lead to decreased nutritional quality, have been found to occur in all i.m. meats studied except for the bull muscle used in Feeding Trial 1 (e.g. Obanu *et al.*, 1975a, b, 1976). Thus, if prolonged storage of i.m. meats at tropical temperatures is to be achieved, it is necessary to find means to inhibit

these reactions so that the undesirable colour (Obanu & Ledward, 1975; Obanu *et al.*, 1976), flavour (Obanu *et al.*, 1975a, b) and nutritional changes are minimized.

Acknowledgments

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Iron availability from intermediate moisture beef

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Summary

The availability of the iron present in intermediate moisture beef before and during storage at 38°C was evaluated in both normal and anaemic rats. Irrespective of the marked chemical changes that occur in the nature of the haemoproteins during storage the availability, in all samples, was as good as that in freshly cooked beef.

It is well established that cooked meat is an excellent source of iron as the haematin complexes can be readily utilized by the body (Bender, 1975) and studies using ^{59}Fe labelled meat have shown the iron to be more available, i.e. better absorbed, than iron in plant foods. Meat, therefore, as well as being an important concentrated source of protein for human nutrition, is also a most significant source of iron. Of all nutrients, requirements of this metal are the most difficult to meet. In developing countries, therefore, where iron deficiency anaemia is common (W.H.O., 1975) an increased intake of meat iron, especially in women and children, would be beneficial.

Previous studies have shown that the nature of the haematin complexes in freshly processed intermediate moisture (i.m.) beef and freshly cooked beef are spectrally similar (Obanu & Ledward, 1975). It is believed that these complexes are formed by reaction between the haematin of myoglobin and some of the denatured proteins present in cooked beef. The final products are believed to be ferric di-imadazole haematin complexes (Ledward, 1971, 1974). However, during storage at 38°C the haematin complexes in i.m. beef usually break down to yield compounds with decreased haematin–protein interactions (Obanu & Ledward, 1975); no such changes occur in cooked beef. Even though this breakdown occurs, free haematin is not released as other complexes are formed which hold the haematin even more tightly within the meat matrix (Obanu & Ledward, 1975).

In view of these marked changes in the nature of the iron complexes in i.m. meat, it was thought desirable to determine the nutritional availability of the iron. This was performed by rat bio-assay.

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Materials and methods

Preparation and storage of the i.m. beef

Two feeding trials were performed and the 0·85 a_w meats used were those described in the previous paper (Obanu *et al.*, 1976b).

Reflectance spectrophotometry

Reflectance spectra of the intact 1 cm^3 meat pieces were recorded against MgO using a Perkin-Elmer Model 124 double beam reflectance spectrophotometer. The range scanned was 700 to 340 nm at a rate of 60 nm min^{-1} (Obanu & Ledward, 1975).

Solubility in 40% pyridine

This was determined as described previously (Obanu & Ledward, 1975).

Total iron and haemoglobin concentrations

The total iron content of all diets as well as of the iron storage organs (heart, spleen and liver) were determined by reaction with either potassium thiocyanate or $\alpha\alpha$ dipyridyl (Pearson, 1970).

Blood haemoglobin concentrations were determined by formation of the cyan-methaemoglobin complex (Kampen & Zijlstra, 1961).

Rat bio-assays

Two feeding trials were performed. In Feeding Trial 1 normal rats were used and the availability of the iron assessed by the iron contents of the blood and iron storage organs. In Feeding Trial 2 iron availability was assessed by the regeneration of haemoglobin in anaemic rats.

In Feeding Trial 1 the i.m. and cooked meat diets from an eleven-month-old bull *l. dorsi* muscle were of the same composition as for the protein bio-assay described previously (Obanu *et al.*, 1976b). The casein diet, which was supplemented with finely ground $FeSO_47H_2O$ at 21·8 mg Fe/kg served as the high-iron control diet. Iron availability in this trial was determined on the same rats used in Feeding Trial 1 of the previous paper and thus all details on the rats used, their allocation to diets etc. are as described previously (Obanu *et al.*, 1976b). However, an extra group of six rats was included and used to assess the initial storage iron content of the liver, spleen and heart.

In Feeding Trial 2 an equal number of male and female Wistar rats, weaned at twenty-one days of age, were rendered anaemic by feeding on a low iron (5 mg Fe/kg) diet for twenty-eight days. The rats were split into pairs of the same sex, each pair being of similar total weight and each experimental diet was fed to a pair of male and female rats. The diets were formulated with i.m. or freshly cooked beef from the *l. dorsi* muscle of a thirteen-month-old steer (Obanu *et al.*, 1976b) to give 20 mg Fe/kg, the casein diet was also supplemented with $FeSO_47H_2O$ to this level. In both trials the rats were fed *ad lib* for ten days with free access to distilled water.

At the beginning and end of both trials the haemoglobin concentration of the blood of all rats was determined. The blood was collected from the tip of the tail of the anaesthetized rats. At the end of Feeding Trial 1 the liver, spleen, heart and *l. dorsi* muscle of each rat was dissected out and the iron content determined.

Results

Feeding Trial 1

Irrespective of diet, the haemoglobin levels in all rats were always within the normal range (Wintrobe, 1956).

It is evident from Table 1 that the iron in the i.m. bull meat was able to meet the iron requirements for haemoglobin synthesis during growth. In all except the three-week stored samples the iron in all meats was slightly better than freshly cooked beef in producing haemoglobin gain.

TABLE 1. Efficiency of conversion of the iron in i.m. (0·85 a_w) meat stored at 38°C into haemoglobin (Hb) by rapidly growing weaning rats

Storage time (weeks)	Group	Iron intake (mg/rat/ 10 days)	Iron in blood Hb† (mg/rat)		Iron in storage organs (mg/rat)		Total iron gain (mg/rat/ 10 days)	% of dietary iron in Hb + stores
			Initial	Final	Initial*	Final		
0	1	2·14	1·95	3·32	1·76	2·04	1·65	76·9
	2	1·98	1·78	3·14	1·76	1·73	1·33	66·9
3‡	1	1·37	2·03	2·69	1·76	1·50	0·41	29·8
	2	1·17	1·64	2·45	1·76	1·65	0·70	60·3
6	1	1·98	1·87	3·24	1·76	1·70	1·31	66·2
	2	1·78	1·78	3·18	1·76	1·87	1·51	85·0
9	1	1·95	1·97	3·30	1·76	1·82	1·39	71·3
	2	2·07	1·61	3·32	1·76	1·88	1·83	88·5
Freshly cooked beef	1	1·95	1·91	3·16	1·76	1·95	1·44	74·1
	2	2·03	1·77	2·96	1·76	1·68	1·11	54·6
Casein diet	1	3·1	2·13	4·13	1·76	2·10	2·34	75·0
	2	2·9	1·64	3·77	1·76	2·25	2·62	90·3
Protein and iron free diet	1	0·07	1·92	1·82	1·76	0·77	−1·09	0
	2	0·08	1·73	1·67	1·76	0·68	−1·13	0

* Initial iron content of the storage organs is the mean value for six rats. All other values are the mean values for three rats caged together.

† Iron in Hb (mg/rat) = 0·067 × body weight × 3·35 (Anderson *et al.*, 1972).

‡ This diet was inadvertently made up with 50 g of sodium chloride instead of 50 g of glucose making the diet slightly less palatable.

Table 1 also indicates that, except for the rats fed the protein and iron-free diet, iron stores were maintained at their initial levels, and in some cases increased, showing that the iron in these diets was available enough to cover endogenous losses, the demands for haemoglobin synthesis and some for storage. In addition the fresh i.m. meat was not significantly better than any of the stored samples with respect to the proportion of iron intake converted to haemoglobin and storage (Table 1) indicating that up to nine weeks' storage at 38°C had no effect on the iron availability. The reflectance spectra of these freshly processed i.m. samples were typical of ordinary cooked meat with reflectance minima at 630, 540 and 410 nm and no detectable changes occurred during nine weeks' storage at 38°C. Also the extractability, by pyridine, of the haematin from these samples was high and remained so throughout storage at 38°C (Obanu *et al.*, 1976a).

Feeding Trial 2

Irrespective of growth, the blood haemoglobin levels of the anaemic rats, on all diets except the basal control, increased slightly.

It is evident from Table 2 that at all storage times the iron in the i.m. meat was available to the rats for haemoglobin synthesis. As with the meat samples assayed in Feeding Trial 1 the iron in these i.m. beef samples compared well with $FeSO_4$ and was more available than the iron in the freshly cooked meat ($P < 0{\cdot}01$, Table 2). Also there was no significant difference in iron availability between freshly processed i.m. beef and samples stored at 38°C for up to twenty-four weeks.

The reflectance spectra of these meats were, immediately after processing, typical of cooked meat but with storage the spectra became more typical of 'free' haematin with loss of the reflectance minima at 540 and 630 nm and a shift in Soret reflectance minima from 410 nm to about 380 nm within six weeks at 38°C; no further changes occurring during subsequent storage. This behaviour is typical of most other i.m. meats studied (Obanu & Ledward, 1975; Obanu *et al.*, 1976a). Also, as found with most other samples, the extractability by pyridine of the haematin from these samples decreased during storage at 38°C (Fig. 1).

Discussion

It is apparent from the results in Tables 1 and 2 that, irrespective of whether changes in the nature of the haematin pigments occur on storage, the iron in i.m. meats is nutritionally available, at similar levels, at all times.

As there was little change in the nature of the haemoproteins during storage in Feeding Trial 1, this result is not surprising but considering the great differences which occur in the nature of the proteins (including haemoproteins) of the meat used in Trial 2, a marked effect on iron availability would not have been unexpected.

These results may be of more general applicability as they appear to demonstrate

TABLE 2. Efficiency of conversion of the iron in i.m. (0·85 a_w) meat stored at 38°C in haemoglobin (Hb) synthesis by anaemic rats

Storage time (weeks)	Group	Dietary iron intake (mg/rat/ 10 days)	Iron in blood Hb* (mg/rat)		Availability of dietary iron (%)
			Initial	Final	
0	1	2·07	2·11	3·00	42·7
	2	1·97	2·27	2·97	35·8
3	1	2·34	2·12	3·17	44·6
	2	1·69	2·37	3·01	37·8
12	1	2·17	2·39	3·29	41·9
	2	1·99	2·57	3·22	32·6
24	1	2·29	2·46	3·57	48·3
	2	2·28	2·64	3·54	39·4
Freshly cooked beef	1	2·19	2·48	3·04	25·5
	2	1·82	2·39	2·95	30·7
Casein/$FeSO_4$ diet	1	2·16	2·29	3·51	56·9
	2	2·18	2·71	3·92	55·2

All values are the means for two rats caged together; 1 = males, 2 = females.

* Iron in haemoglobin (mg/rat) = 0·067 × body weight × 3·35.

† Availability (%) = $\frac{\text{Increase in Hb iron}}{\text{Iron intake}} \times 100$.

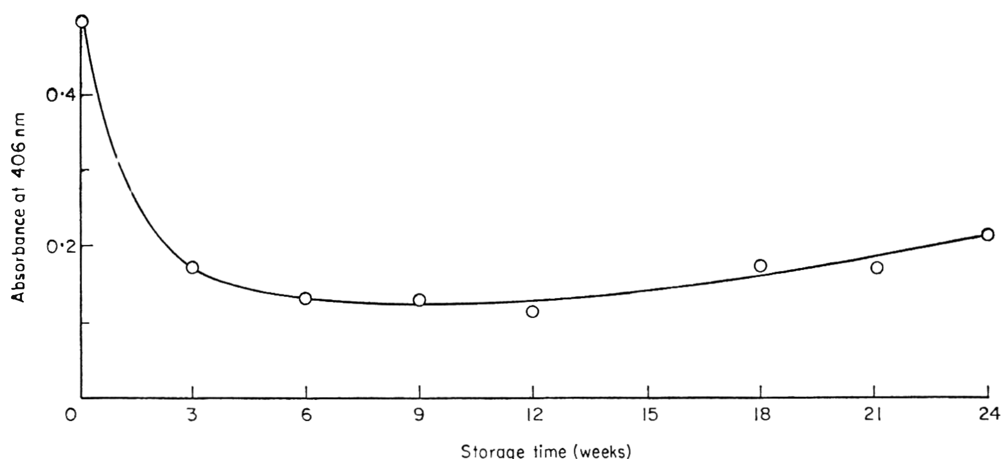

FIG. 1. Effect of storage at 38°C on the extractability of haematin by 40% pyridine in i.m. (0·85 a_w) beef.

that the availability of the iron in meat is not due to its attachment to meat proteins but rather to its presence as the haematin complex. Previous results have shown that during digestion the haematin of cooked meat haemoprotein remains attached to the two histidine residues of the protein chain even after most of the protein has been digested (Hazell, 1975). Thus these results may support the view that the good iron availability from meat is not due solely to the nature of the attached proteins but rather to the bound histidine which may serve to actively transport the iron across the mucosal membrane (Hazell, 1975). Whatever the reason for the improved iron availability from meat it is apparent that the chemical changes which occur in the i.m. meat samples on storage do not affect its availability to the body.

In fact, in both Trials, the iron availability of both freshly processed and stored i.m. meat is apparently better than in fresh meat cooked in water under the same conditions used in the preparation of the i.m. samples. This effect was most marked in Feeding Trial 2 where the availability was significantly higher in the i.m. meats. However, the iron availability for ordinary cooked beef found in this study (28·1%) was lower than found by Mahoney, van Orden & Hendricks (1974) (45%) for ground beef although their value for ferrous sulphate incorporated into a casein diet was similar to the value found in Feeding Trial 2, i.e. 51% compared with 56%.

The low iron availability found in the present study may be due to the method of cooking as the type of heat treatment may well alter the nature of the complexes formed. However, the heating regime was similar in all samples and as the effect is found in both freshly processed and stored i.m. meat the enhancement, if real, cannot be due to any of the products formed during storage. This suggests that glycerol is somehow the cause of the enhanced iron absorption and availability.

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Wiltshire curing with and without nitrate

II. Vacuum packed collar bacon and vacuum packed bacon from pigs with high ultimate pH

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Summary

The storage life of vacuum packed unsmoked bacons produced by a factory Wiltshire process from brines containing nitrite with and without nitrate has been studied at 5° and 15°C. Whilst collar bacon produced from typical bacon pigs and containing 76–129 ppm nitrite and approximately 5% salt kept slightly better when it also contained 538–568 nitrate, no consistent benefit was noted when the nitrate concentration was 196–204. Collar and back bacons with similar concentrations of nitrite and salt and produced from pigs in which the ultimate pH in the *M. longissimus dorsi* was $\geqslant 6{\cdot}0$ kept better when they also contained approximately 600 ppm nitrate. It is suggested that the benefit of nitrate in these bacons is due to the inhibition of bacterial growth by increased concentrations of nitrite produced from nitrate in relatively high pH muscles.

Introduction

The inclusion of nitrate in Wiltshire curing brines does not contribute to either quality or storage stability of typical back bacon (Taylor & Shaw, 1975). It may, however, contribute to the stability of bacon which is more susceptible to spoilage such as collar bacon, in which many of the muscles may have pH $>6{\cdot}0$, and bacon made from pigs with high ultimate pH (pH_u). The effect of nitrate on the stability of these types of bacon has therefore been studied.

Experimental

Two experiments were carried out. In the first, the effects of different levels of nitrate on storage stability of collar bacon were assessed using three separate comparisons (A, B and C). In the second experiment, collar and back bacons were used to compare the effect of curing meat from high pH_u pigs with and without nitrate.

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The levels of nitrate used in this study were designated 'high', 'intermediate' and 'nitrate-free'.

Experiment 1 (collar)
  Comparison A: high nitrate (A-1) *v.* nitrate-free (A-2);
  Comparison B: high nitrate (B-1) *v.* intermediate nitrate (B-2);
  Comparison C: intermediate nitrate (C-1) *v.* nitrate-free (C-2).
Experiment 2 (back and collar from high pH_u pigs)
  Comparison D: high nitrate (D-1) *v.* nitrate-free (D-2).

Bacon manufacture

In each comparison bacon was made from seven Grade A pigs taken from a normal factory production line. In Comparison D, the pigs were selected on the basis of pH_u, having the highest measured values. The pH_u was measured, 24 hr after slaughter, in the lumbar region of *M. longissimus dorsi* (l.d.) near the tenth rib, using a Radiometer pH Meter 29 with a GK 2321 C electrode.

TABLE 1. Composition of curing brines used in bacon comparisons

Comparison	Cure	Injection brine			Immersion brine		
		Salt (% w/v)	$NaNO_2$ (ppm)	$NaNO_3$ (ppm)	Salt (% w/v)	$NaNO_2$ (ppm)	$NaNO_3$ (ppm)
A	1	16	700	2100	26	1000	4200
	2	16	700	—	26	1000	—
B	1	16	700	2100	26	1000	4200
	2	16	700	800	26	1000	1600
C	1	16	700	800	26	1000	1600
	2	16	700	—	26	1000	—
D	1	16	700	2100	26	1000	4200
	2	16	700	—	26	1000	—

The injection and immersion brines used in each comparison are shown in Table 1 and were designed to produce bacon sides in which the back lean contained 4–5% salt, 60–100 ppm nitrite and either 500 ppm (high) or 200 ppm (intermediate) nitrate where it was included. Comparisons were always made between paired sides, the left sides receiving one treatment and the right the other.

All sides were injected by a multineedle machine (Swissvac CUR-O-MAT) to a weight gain of approximately 10% trimmed weight. Sides were then immersed for three days in freshly prepared brines followed by a further seven days' maturation at 5°C.

Slicing, packing and storage

In all comparisons a sample of collar from each side, forward from the middle of the shoulder pocket, was taken under refrigeration to the laboratory and sliced to give twenty-one slices (3 mm thickness). These slices were vacuum packed in Metathene X (Metal Box Ltd, London) pouches, three consecutive slices to a pouch to give seven packs from each side. The packs from the seven sides from each curing treatment were grouped according to a 7 × 7 Latin Square design so that, at each of the seven examination times, when seven corresponding packs were sampled from each curing treatment, each side and position in the sliced collar were represented. In all comparisons vacuum packs of collar were stored for up to twenty-one days at 5°C and fifteen days at 15°C.

In Comparison D, portions of back were also taken from each side, forward from the last rib, and twenty-eight slices cut from each. The slices were vacuum packed, four consecutive slices to a pouch, to give seven packs from each side and these were grouped in a sampling design similar to that used for the collar bacon. Back bacon samples were stored for up to thirty-five days at 5°C and nineteen days at 15°C.

Microbiological examination

All packs used in these trials were intact and vacuum-tight when examined. Packs were opened aseptically. The rind and outer fat were cut from the collar slices, and the remaining lean portions formed the sample for analysis. The eye muscle was cut from the back samples and was used as the sample for analysis. Samples were minced through a 4-mm screen. Twenty-five grams were then homogenized for 0·5 min at 6000 rev/min and 1 min at 12 000 rev/min in an Atomix Blender (M.S.E., London) in 100 ml diluent (0·85% NaCl + 0·1% peptone (Difco); pH 7·0). The total viable count and count of lactic acid bacteria were then obtained using the methods described by Taylor & Shaw (1975) with the exception that the medium used for total viable count was Plate Count Agar (P.C.A. Oxoid) + 1% NaCl.

Odour

When the packs were opened for examination the odour was assessed by a panel of four experts who noted the presence of off-odours and judged whether these would cause the bacon to be accepted or rejected by a consumer.

Chemical analysis

Chemical analysis commenced within 1 to 2 hr of opening packs and were taken from the minced bulk used for microbiological examination. One gram was homogenized with 10 ml distilled water and pH measured on either a Pye Universal pH meter or a Radiometer pH M63 Digital pH meter. Moisture content was determined and samples extracted, deproteinized and nitrate and nitrite estimated as described previously (Taylor & Shaw, 1975). Meat blanks were not estimated.

Chloride contents in the same extracts were estimated either by precipitating the chloride with excess silver nitrate and titrating the excess with potassium thiocyanate or by measurement on a Radiometer CMT 10 Chloride Titrator.

Results

Chemical analysis

Table 2 shows the pH and concentration of nitrite, nitrate and salt in the leans of bacons at the beginning of storage. The changes during storage at 5° and 15°C in the concentration of nitrite in collar bacon in comparisons A, B and C are shown in Fig. 1. The corresponding changes in back and collar bacons in comparison D are shown in Fig. 2.

TABLE 2. Analysis of bacon lean at beginning of storage

Cure	Comparison	Bacon	pH	$NaNO_2$ (ppm)	$NaNO_3$ (ppm)	NaCl (%)	NaCl (% on water)
A 1	High nitrate and	Collar	6·20	93	568	4·7	6·7
2	nitrate-free	Collar	6·20	96	26	4·8	7·1
B 1	High nitrate and	Collar	5·90	81	538	3·8	5·3
2	intermediate nitrate	Collar	5·90	76	204	4·8	6·8
C 1	Intermediate nitrate	Collar	6·40	77	196	7·5	11·1
2	and nitrate-free	Collar	6·25	129	21	5·3	7·7
D 1	High nitrate and	Collar	6·50	147	580	5·0	7·4
		Back	6·25	118	625	4·9	7·1
2	nitrate-free	Collar	6·45	120	30	5·3	7·8
		Back	6·25	111	25	4·7	6·5

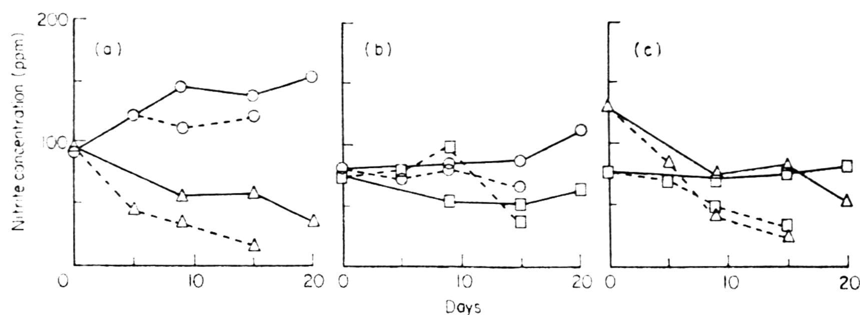

FIG. 1. Changes during storage at 5° and 15°C in the concentration of nitrite in the lean of collar bacons in comparisons A, B and C. ○——○ High nitrate at 5°C; □——□ intermediate nitrate at 5°C; △——△ nitrate-free at 5°C; ○ - - - ○ high nitrate at 15°C; □ - - - □ intermediate nitrate at 15°C; △ - - - △ nitrate-free at 15°C.

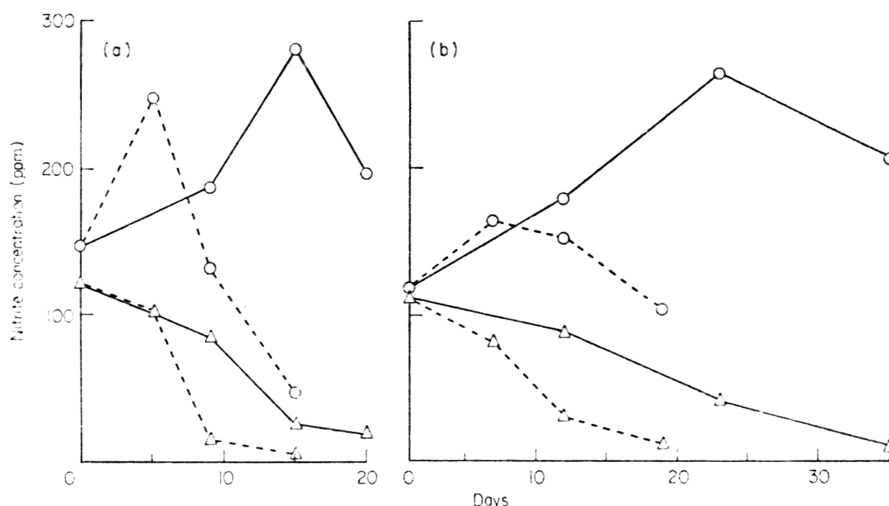

FIG. 2. Changes during storage at 5° and 15°C in the concentration of nitrite in the lean of (a) collar and (b) back bacons in comparison D. ○——○ High nitrate at 5°C; △——△ nitrate-free at 5°C; ○ - - - ○ high nitrate at 15°C; △ - - - △ nitrate-free at 15°C.

In the high nitrate bacons A-1 and B-1, nitrite remained more or less constant or increased throughout storage. In D-1 where the pH of both collar and back was higher than normal, nitrite rose to a peak during storage and then fell. In both collar and back bacon, the maximum detected concentration at 5°C was greater and came later in storage than at 15°C as was found previously by Eddy & Ingram (1962). At both temperatures, the time to reach peak nitrite concentration was longer in back than in collar. In bacons made with intermediate nitrate (B-2 and C-1) nitrite concentration remained fairly constant at 5°C but behaved erratically at 15°C. In all nitrate-free bacons, the nitrite level fell steadily at both temperatures, the rate being faster in collar than in back and faster at 15°C than at 5°C.

In all high nitrate bacons nitrate fell at a fairly steady rate, faster at 15°C than at 5°C. In general, the rate was reflected in the degree of nitrite accumulation and was greatest in bacons where the concentration of nitrite increased during storage. The two intermediate nitrate bacons had similar rates of nitrate loss, the rate again being greater at 15°C than at 5°C.

Microbiology

The total viable counts and numbers of lactic acid bacteria detected during storage of the bacon are shown in Table 4.

In Experiment 1, the nitrate-free bacon had higher total viable counts initially and during storage than the high nitrate bacon (comparison A). High nitrate bacon had higher initial counts than the intermediate nitrate bacon but after prolonged storage, counts were slightly higher on the intermediate nitrate bacon (comparison B).

The intermediate nitrate bacon had higher counts initially and during storage than the nitrate-free bacon (comparison C). Lactic acid bacteria, which may cause souring, were either not detectable or present in very low numbers on all bacons at the time of packing but growth always occurred during storage. The high nitrate bacon contained less lactic acid bacteria than the nitrate-free bacon (comparison A) and the intermediate nitrate bacon (comparison B). Lactic acid bacteria were less common in nitrate-free bacon than in the intermediate nitrate bacon (comparison C).

In Experiment 2 (high pH) the total viable count at the time of packing was higher on the bacons cured with nitrate. During storage, counts were similar on the collar bacons cured with or without nitrate. However, counts were slightly higher on the back bacon cured without nitrate during storage at both 5° and 15°C. Lactic acid bacteria were more common on both collar and back bacons cured without nitrate at the time of packing and during storage.

Odour

Table 3 shows the number of packs rejected at each sampling time.

TABLE 3. Numbers of packs, rejected on basis of odour on opening (seven packs from each cure were examined at each observation time)

Bacon	Cure	Comparison	Days stored	Storage at 5°C		Storage at 15°C	
				Cure 1	Cure 2	Cure 1	Cure 2
Collar	A 1	High nitrate	5			0	2
		and	9	0	0	0	1
	2	nitrate-free	15	0	0	2	3
			20	1	2		
Collar	B 1	High nitrate	5			1	0
		and	9	0	0	1	0
	2	intermediate nitrate	15	0	0	1	0
			20	1	0		
Collar	C 1	Intermediate nitrate	5			1	0
		and	9	1	0	2	1
	2	nitrate-free	15	1	1	6	2
			20	0	1		
Collar (high pH)	D 1	High nitrate	5			0	0
		and	9	1	0	1	1
	2	nitrate-free	15	1	1	1	3
			20	1	2		
Back (high pH)	D 1	High nitrate	7			0	0
		and	12	0	0	0	0
	2	nitrate-free	19			1	2
			23	0	1		
			35	0	2		

TABLE 4. Bacterial numbers on the lean bacons during storage at 5° and 15°C

Bacon	Cure	Comparison	Days stored	Log_{10} total viable count/g				Log_{10} count of lactic acid bacteria/g			
				5°C		15°C		5°C		15°C	
				Cure 1	Cure 2	Cure 1	Cure 2	Cure 1	Cure 2	Cure 1	Cure 2
Collar	A 1	High nitrate	0	5·6	6·1	5·6	6·1	3·2	2·5	3·2	2·5
		and	5			6·6	6·8			6·7	6·6
	2	nitrate-free	9	4·7	6·3	7·3	7·3	4·1	5·2	6·9	7·3
			15	6·0	7·0	7·1	7·8	6·0	7·0	7·2	7·8
			20	7·0	7·3			6·8	7·2		
Collar	B 1	High nitrate	0	6·2	5·5	6·2	5·5	2·1	1·4	2·1	1·4
		and	5			6·6	6·3			6·4	6·6
	2	intermediate nitrate	9	6·3	6·3	7·4	7·0	5·2	6·0	7·3	7·4
			15	6·8	7·1	7·6	7·8	6·7	7·1	7·6	7·6
			20	7·2	7·3			5·4	7·0		
Collar	C 1	Intermediate nitrate	0	5·0	4·5	5·0	4·5	< 1·4	1·4	< 1·4	1·4
		and	5			6·7	5·2			6·4	4·8
	2	nitrate-free	9	6·6	5·2	6·7	6·4	5·7	3·6	6·6	6·0
			15	7·2	5·7	7·5	5·8	6·2	5·0	6·7	5·5
			20	6·5	6·1			6·5	4·8		
Collar (high pH)	D 1	High nitrate	0	6·9	6·0	6·9	6·0	3·1	3·7	3·1	3·7
		and	5			6·6	6·6			4·8	5·8
	2	nitrate-free	9	6·6	6·2			4·9	5·6		
			15			6·8	7·1			6·5	6·9
			20	6·6	6·7			5·4	6·6		
Back (high pH)	D 1	High nitrate	0	5·7	5·4	5·7	5·4	1·9	3·9	1·9	3·9
		and	7			5·7	6·7			4·8	6·0
	2	nitrate-free	12	5·3	5·8	6·4	6·5	4·5	5·7	6·3	6·4
			19			6·3	6·6			5·8	6·8
			23	5·7	6·8			5·6	6·6		
			35	5·7	6·3			5·4	6·5		

In Experiment 1, the comparisons of collar stored at 5°C showed only marginal differences in acceptability between bacons. At 15°C, slightly fewer packs of high nitrate bacon (A-1) were rejected than the nitrate-free bacon (A-2). In comparison B, one pack of high nitrate bacon (B-1) was rejected at every sampling time but no packs of intermediate nitrate bacon (B-2) were rejected. However, in comparison C considerably more packs of intermediate nitrate bacon (C 1) were rejected than packs of nitrate free bacon (C-2).

In Experiment 2, there was no difference in the acceptability of collar bacon cured with or without nitrate during storage at 5°C. At 15°C slightly more packs of nitrate-free bacon (D-2, collar) were rejected than nitrate containing (D-1), and off-odours in the D-2 bacon were generally more noticeable at the end of storage. No packs of bacon cured with nitrate (D-1) were rejected during storage at 5°C and no off-odours were reported. However, three packs of the nitrate-free back bacon (D-2) were rejected and off-odours were detected in all but one of the packs examined after thirty-five days' storage. The difference was not so marked during storage at 15°C, although off-odours were detected in three packs of nitrate-free bacon at twelve days while none were detected in the bacon made with nitrate.

Discussion

Odour assessment and microbiological data showed that nitrate at the high concentration improved the storage stability of back bacon produced from high pH_u sides. Its effect on this bacon is therefore greater than on back bacon produced from sides with pH_u values less than 6·0, where no benefit was observed (Taylor & Shaw, 1975). This can be attributed to the higher nitrite concentrations formed during storage in the bacon from high pH_u sides, which inhibited bacterial growth. A high level of nitrite may be particularly important in high pH bacon because nitrite is less inhibitory in this pH range (Castellani & Niven, 1955). The greater production of nitrite in high pH back bacon may also explain why nitrate improved the stability of collar bacon made from sides with pH_u values less than 6·0 in the l.d., since this bacon contains muscles with high pH. The intermediate level of nitrate was not of consistent benefit to stability in collar bacon, presumably because this level is insufficient to produce effective concentrations of nitrite in the high pH muscles.

We believe that these findings are of commercial importance. High pH muscles are not only found in the collar of bacon pigs but may occur also in other cuts which are commonly vacuum packed. More importantly, 25% of pigs used for Wiltshire bacon manufacture in the UK have pH_u above 6·0 in the l.d. (Kempster & Cuthbertson, 1975) and therefore benefit from nitrate. However, in these experiments this benefit was obtained with nitrate at an initial concentration of more than 500 ppm and nitrite accumulated during storage at a level which exceeded 200 ppm. Although these figures refer to concentrations in the lean, it is possible that the whole bacon slice

would not meet current legal requirements for nitrite and nitrate. In commercial practice, therefore, lower levels of nitrate would have to be used and this would almost certainly lessen its benefit.

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Supplementation of bread with soybean and chickpea flours

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Summary

Flours from defatted soybean, raw and parboiled chickpea seeds were obtained on a laboratory scale and used as a supplement to wheat flour. The effect of supplementation level on water absorption, dough rheology, loaf quality and major chemical constituents of breads were investigated. Water absorption was slightly affected by soy and parboiled chickpea flours but was reduced by raw chickpea flour. Dough mixing time, stability and valorimetric values increased but the mixing tolerance index decreased by adding soy and parboiled chickpea flours to wheat flour. Loaves were slightly smaller in volume than the control at high levels of soy and chickpea flours. Moreover, bread score and panel evaluation showed deterioration of bread characteristics above 6% soy flour and 15% chickpea level of supplementation. The chemical analysis of the supplemented bread showed a positive trend of increasing protein and ash contents by increasing the levels of chickpea and soy flours.

Introduction

Supplementation of wheat flour with high protein and vegetable sources to increase protein content and to improve essential amino acid balance of the resultant baked products has been of great interest to nutritional scientists.

In the USA Bird (1975) estimated that 600 million pounds of soy flour and grits (50–60% protein), 60 million pounds of soy concentrate (above 70% protein) and 40 million pounds of soy isolates (above 90% protein) were utilized in food products in 1973–74. It was projected that by 1980 about 2146 million pounds of meat and other animal proteins will be replaced by plant protein. US standards of identity for enriched white bread allows the use of up to 3% soy flour as optional ingredients (Cotton, 1974). Two soy fortified wheat flours 6% and 12% soy flours are being produced for use in school lunch programmes, institutional feeding and other commodity distribution

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programmes in order to achieve nutritional improvement (Hoover, 1974). Tsen (1972) showed that bread made with 12% soy flour is acceptable by the consumer and contains more protein of a higher nutritional quality than common wheat flour breads. The protein efficiency ratio of bread increased from 0·7 to 1·9 when fortified with 12% soy flour. Moreover, lysine content increased to more than double and the protein content increased by 30% (Marnett, Tenney & Barry, 1973). Ranhotra, Loewe & Lehman (1974) studied the characteristics of breads fortified with fifteen commercial soy protein products. They found that most soy flours especially full fat and high fat products, permitted fortification at the 15–20% level and produced breads of acceptable volume, flavour and overall quality, with resultant substantial increases in protein content and greatly improved amino acid balance.

Studies on the supplementation of arabic bread with protein sources and amino acids are few. Hallab, Khatchadorin & Jabr (1974) supplemented arabic bread with chickpea and soy flours. They reported that the organoleptic properties of the supplemented bread compared favourably with the unsupplemented bread up to a level of 20% chickpea and 10% soy flour.

This investigation describes the effect of blending wheat flour with soy and chickpea flours on water absorption, physical properties of dough, bread characteristics and chemical constituents of the supplemented breads.

Materials and methods

Seeds of wheat (Giza 155), chickpea (Giza 1) and soybean (Clark) were obtained from the Seed Production Department, Ministry of Agriculture, Cairo, Egypt.

Wheat was conditioned to 14% moisture, milled on a Willey mill and sifted in an automatic plansifter. The flour, sifted through an 8 *xx* sieve, was used in this study. Chickpeas were decorticated, milled and sifted to pass through an 8 *xx* sieve. Parboiled chickpea flour was obtained by parboiling the chickpea seeds for a period of 30 min; it was then autoclaved for 20 min, dried at 65°C for 24 hr and ground to pass through an 8 *xx* sieve. Soybeans were dehulled, flaked, defatted, autoclaved for 20 min, dried at 65°C for 24 hr and ground to pass through an 8 *xx* sieve.

Physical dough properties of the fortified wheat flour with chickpea and soybean flours were determined by the farinograph method as outlined according to A.A.C.C. (1962). Physical dough characteristics such as mixing time, stability, mixing tolerance index and valorimeter values were computed from the curve obtained.

The baking test was carried out with a straight dough method employing a lean formula. The basic formula included 300 g flour, 2% yeast and 1·5% salt and water based on the farinograph optimum absorption. The dough was fermented at 30°C for 3 hr, divided, moulded, panned and proved for 50 min at 30°C. The bread was baked for 25 min at 218°C. The loaf weight and volume were determined after cooling

(2 hr). Bread quality score based on appearance and internal characteristics were assessed. Flavour and organoleptic evaluation was also made by a panel of testers.

Supplemented breads were dried and ground. Moisture, protein, fat, ash and crude fibre contents were determined according to A.A.C.C. (1962). Total carbohydrate (N.F.E.) was obtained by difference.

Results and discussion

Water absorption and dough rheology

The effect of fortifying wheat flour with chickpea and scy flour on water absorption and dough rheology as measured by the farinograph is shown in Tables 1, 2 and 3. Substituting wheat flour with 5% chickpea flour reduced water absorption by 1%. Subsequent substitutions reduced water absorption 1% on the average. A reduction of water absorption was less in the case of fortification with parboiled chickpea flour. It was only 2% at 25% of parboiled chickpea level. On the other hand, soy flour at low levels had no effect on water absorption but increasing the level up to 15% reduced the water absorption by 1·2% less than the control. Water absorption is generally related to the hydration capacity of protein. Gluten has the strongest imbibition power

TABLE 1. Effect of chickpea supplementation on water absorption, mixing characteristics and bread scores

	Supplementation level (%)					
	0	5	10	15	20	25
Water absorption (%)	55	54	53	52	52	50
Farinograph values						
Mixing time (min)	1·5	1·5	1·5	1·5	1·2	1·2
Stability (min)	1·0	1·0	1·5	1·5	1·2	1·0
Mixing tolerance (BU)	120	80	80	90	90	80
Valorimeter value	32	31	25	28	26	26
Bread properties						
Loaf weight (g)	129	130	134	135	137	139
Loaf volume (cc)	220	230	250	240	210	200
Appearance (10)	7	8	9	8	7	4
Crust colour* (10)	7	8	8	9	7	6
Crumb colour (10)	9	9	8	7	5	3
Grain (10)	7	7	7	6	5	3
Texture (10)	8	7	7	6	6	3
Flavour (10)	8	8	8	6	5	2

* Brown and very dark brown crust colours have a lower score.

compared to protein from other sources. Substitution of wheat flour resulted in decreased water absorption despite the elevated protein content. Low moisture content of soy flour and parboiled chickpea flours may explain their water absorption results. Damaged starch will absorb water and this will be diluted as the protein content is elevated. Mixing time was slightly affected by soybean flour substitution. However, the stability increased and mixing tolerance decreased with supplementation of soy flour or parboiled chickpeas. In the case of raw chickpea supplementation, dough stability and mixing tolerance were only slightly affected. The general appearances of

TABLE 2. Effect of parboiled chickpea supplementation on water absorption, mixing characteristics and bread score

	Supplementation level (%)					
	0	5	10	15	20	25
Water absorption (%)	55	55	54·5	54	53·5	53
Farinograph values						
Mixing time (min)	1·5	1·5	1·5	1·5	1·5	1·5
Stability (min)	1·0	1·5	1·5	2·0	2·5	3·0
Mixing tolerance (BU)	120	100	80	60	40	30
Valorimeter value	32	34	40	46	52	58
Bread properties						
Loaf weight (g)	127	129	130	130	131	132
Loaf volume (cc)	220	230	240	230	210	200
Appearance (10)	7	8	9	8	7	5
Crust colour* (10)	7	8	9	9	7	6
Crumb colour (10)	9	9	9	9	6	3
Grain (10)	7	7	6	6	6	3
Texture (10)	8	7	7	7	6	3
Flavour (10)	8	9	9	7	6	3

* Brown and very dark brown crust colours have a lower score.

the farinograms can be shown by the valorimeter. This value takes into consideration mixing time and dough stability. The valorimeter value increased with supplementation of soy and parboiled chickpea flours whereas it decreased with supplementation of raw chickpea flour. The inactivation of any proteolytic enzyme in the chickpeas during parboiling would be the only explanation for the differences in mixing characteristics.

Tsen & Hoover (1973) reported that fortification with more than 12% full fat soy flour adversely affected both rheological properties and baking quality of wheat flour. Jakubczyk & Haberowa (1974) studied the physical properties of dough containing

different levels of soy products as measured by means of the farinograph. They found that addition of soy flour improved dough rheological properties. On the other hand, they noticed a marked decrease in physical properties as measured by means of the extensograph during the fermentation process.

Bread quality

The effect of different levels of soy and chickpeas on the loaf weight, volume and other properties are presented in Tables 1, 2 and 3. The loaf volume increased with

TABLE 3. Effect of defatted soy flour supplementation on water absorption, mixing characteristics and bread score

	Supplementation level (%)					
	0	3	6	9	12	15
Water absorption (%)	55	55	54·5	54·2	54	53·8
Farinograph values						
Mixing time (min)	1·5	1·5	1·5	1·5	1·5	1·5
Stability (min)	1·0	1·5	2·0	2·5	3·0	4·0
Mixing tolerance (BU)	120	100	80	70	60	50
Valorimeter value	32	32	40	41	42	43
Bread properties						
Loaf weight (g)	124	125	127	130	132	134
Loaf volume (cc)	210	220	240	230	210	200
Appearance (10)	7	8	8	7	6	5
Crust colour* (10)	7	8	8	8	7	7
Crumb colour (10)	9	9	8	8	6	4
Grain (10)	7	8	8	6	6	2
Texture (10)	8	7	7	6	5	2
Flavour (10)	8	8	8	7	5	3

* Brown and dark brown crust colours have a lower score.

supplementation up to 10% of chickpea flour and 6% soy flour. This increase in volume is accompanied with slight increase in loaf weight. Soy or chickpea fortified dough became soft and less elastic during fermentation and may not have been able to hold as much of the CO_2 produced, resulting in smaller volume. Both appearance and internal characteristics of the fortified breads compared favourably with the control loaves up to a level of 15% chickpea and 9% soy flour. Beyond these levels, the total score decreased considerably. The crumb colour became distinctly yellow and the texture of crumb was dense at 25 and 15% levels of chickpea and soy flour respectively.

The results also indicated that up to 6% soy flour supplementation, the flavour

rating was the same as that of the control. More than 15% of chickpea flour adversely affected the taste and general properties. Parboiling the chickpea before grinding alleviated the adverse acceptability of the supplemented bread. Increasing the level of supplementation of chickpea flour increased the bread crust colour. Chickpea contains approximately 8% of low molecular weight carbohydrate with sucrose predominant and this value is greater than that normally found in wheat flour. Thus one might anticipate increased browning in backed products containing these chickpea flours. Lineback & Ke (1975) reported that wheat flour should probably not be supplemented with more than 20% of legume flours, in that case flatulence and increased browning would be minimized. Jakubczyk & Haberowa (1974) noticed that the flour of poor baking quality responded better to the supplementation of soy products and produced better bread than the good quality flour.

TABLE 4. Proximate analysis of wheat, chickpea and soy flours and supplemented breads

	Level used (%)	Chemical constituent*						% protein increases
		Moisture	Prot.†	Fat	Fibre	Ash	NFE‡	
Wheat flour		8·5	9·65	1·06	0·48	0·40	88·41	
Chickpea flour		8·6	21·91	5·02	2·18	3·00	67·89	
Parboiled chickpea		7·4	22·16	3·62	2·18	3·00	69·04	
Soy flour		6·7	53·22	1·92	3·01	7·00	34·84	
Wheat bread		13·3	11·04	1·00	1·03	0·60	86·33	
Chickpea bread	5	13·8	12·37	1·13	1·13	1·00	84·37	12·1
	10	14·0	13·10	1·24	1·20	1·41	85·05	18·7
	15	13·2	14·44	1·36	1·28	1·82	81·10	30·1
	20	13·5	14·84	1·50	1·35	2·26	80·05	34·4
	25	13·5	15·22	1·68	1·43	2·60	79·07	37·9
Parboiled chickpea bread	5	13·5	13·69	1·10	1·10	1·00	83·11	24·0
	10	13·6	14·20	1·21	1·17	1·50	81·92	28·6
	15	13·6	14·78	1·28	1·25	2·01	80·68	33·4
	20	13·5	15·42	1·36	1·32	2·50	81·40	39·6
	25	13·5	15·77	1·43	1·36	3·02	78·43	42·8
Soybean bread	3	13·4	13·10	1·11	1·16	1·20	83·43	18·7
	6	13·6	14·09	1·18	1·30	1·81	71·62	27·6
	9	13·8	15·24	1·26	1·46	2·38	79·66	38·0
	12	14·0	16·39	1·33	1·58	3·21	77·49	48·5
	15	14·2	17·16	1·40	1·70	3·89	75·95	55·4

* Dry weight basis.

† Protein content is $N \times 6{\cdot}25$, except $N \times 5{\cdot}7$ for wheat flour.

‡ NFE, nitrogen free extract (total carbohydrate).

§ Protein increases of supplemented bread: percentage of protein increase relative to unsupplemented bread.

Chemical analysis

The chemical composition of the supplemented breads are shown in Table 4. The crude fat and fibre of the enriched breads increased with each additional level of chickpea and soy flours. The ash content reached over 3·0% at high level of parboiled chickpea and soybean enriched bread. The most favourable change of the chemical constituents of bread is the increased protein content. Breads made with different levels of soy or chickpea flour contained more protein than the control bread. Protein increased by 30·1, 33·4 and 38·0% with supplementation of 15% raw chickpea and parboiled chickpea and 6% soy flour respectively. Moreover, Tsen (1972) reported an increase of the protein efficiency ratio of soy fortified bread which indicates high nutritional quality bread and improved amino acid balance.

In conclusion, supplementation of wheat flour with 6% soy flour or 15% chickpea flour produced bread of acceptable overall quality and contained more protein than common wheat flour bread. The organoleptic properties of the enriched bread were similar to those of unsupplemented bread. Appreciable deterioration of bread characteristics occured above 6% soy flour and 15% chickpea level of supplementation.

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A method for measuring tomato fruit firmness using a modified shear press

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Summary

Tomato fruit firmness was measured with a modified shear press by compressing individual fruit by 5 mm between two flat surfaces. The system was calibrated with a spring balance and the relationship between applied force and proving ring deformation was linear over the range tested.

The correlation coefficient between tomato fruit firmness as measured by a finger feel panel and the shear press was 0·988. Minimum shear press firmness values for acceptability of fruit for sale at retail level and use in the home were obtained by matching the panel and shear press data.

Tests showed that from fifteen to twenty-five fruit should be tested per 12-lb box to get a good estimate of the average firmness of the lot. The spread of firmness values on either side of the mean was considerable in the nineteen lots tested and it is important to quantify this and quote it, in addition to the mean firmness value, to give a complete picture of tomato fruit firmness.

The system described is useful for carrying out firmness tests on tomato fruit cultivars at time of picking and during storage in order to obtain an estimate of potential shelf-life during wholesaling and retailing.

Introduction

The production costs of tomatoes grown under glass are continuing to rise with consequent higher retail prices. Consumers are, therefore, demanding a higher level of quality. Growers are looking for higher yielding cultivars and modified methods of production in an effort to cut costs, and in such a situation there is a danger that quality may be impaired. There is increasing emphasis at both wholesale and retail level on the importance of tomato fruit firmness, especially in produce that is to be, or has been, exported, and it is important that simple practical methods are available to measure fruit firmness.

Voisey (1971) has reviewed the modernization of texture instrumentation, including various systems for carrying out compression tests. Holt (1970) described a technique

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of punch testing tomatoes to measure the firmness of the different anatomical parts. Hamson (1952) measured the amount of tomato fruit compression for a fixed load, while Shafsak & Winsor (1964) carried out a similar test on the fruit along an axis from the calyx to the stylar scar. Other firmness meters for tomatoes, the Firm-o-meter and the Ansco meter were described by Garrett *et al.* (1960). Diener, Sobotka & Watada (1971) measured tomato fruit firmness with a modification of the original F–L meter. The readings compared favourably with those obtained with the Instron Universal Testing machine. Voisey, Buckley & Crete (1974) described a system to record food deformation automatically between two levels of applied force. Philouze (1975) used a penetrometer technique to measure the firmness of thirty-eight tomato fruit cultivars. Stenvers, Rudolphij & Bruinsma (1973) designed a non-destructive, automated, fast and portable softness measuring device which measures tomato fruit firmness at the stylar scar.

The present study reports a simple modification of the shear press, using the strain gauge system described by Keppel & Gormley (1975) coupled to a high gain instrumentation amplifier. Each tomato fruit was compressed through 5 mm at a constant rate between a plunger attached to the proving ring and a flat metal plate on top of a laboratory jack. The force to achieve this compression was measured and the correlation between the instrument and a 'finger feel' panel was very high. Minimum firmness levels for acceptability of tomatoes at retail level or for use in the home are given.

Materials and methods

Modification and operation of the shear press

A shear press with a 58-kg proving ring with attached strain gauges as outlined by Keppel & Gormley (1975) was modified for use in these compression tests. The operational amplifier used with the system described by the authors above proved inadequate due to the limitations of gain versus input impedance. A Burr Brown 3660K instrumentation amplifier was used to overcome this. A schematic diagram of the strain gauge bridge and amplifier is shown in Fig. 1. The amplifier has adjustable voltage gain set by resistor R_G.

$$\text{Gain equation } G = \frac{10^5}{R_G} \text{V/V (adjustable between 1 and 1000)}$$

$$\text{(Output voltage) } E_0 = GE_i \text{ (Differential Input Voltage)}$$

$$E_0 = \frac{10^5\, E_i}{R_G}$$

An optional offset adjustment using a 100 kohm potentiometer between terminals 7, 6 and the negative supply rail was used to provide any desired offset. A range control was provided on the output by means of a 1 kΩ potentiometer in series with a

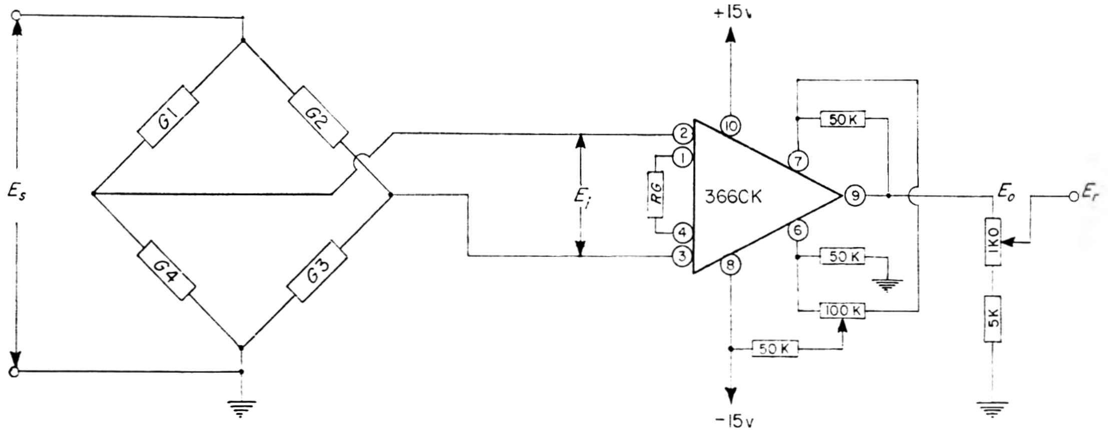

Fig. 1. Schematic diagram of strain gauge bridge and amplifier.

5 kΩ resistor. The complete system is mains operated from a stabilized power supply which provides ± 15 V for the amplifier and + 12 V E_s to power the bridge circuit. The amplifier was connected to a Philips PM8100 flatbed recorder.

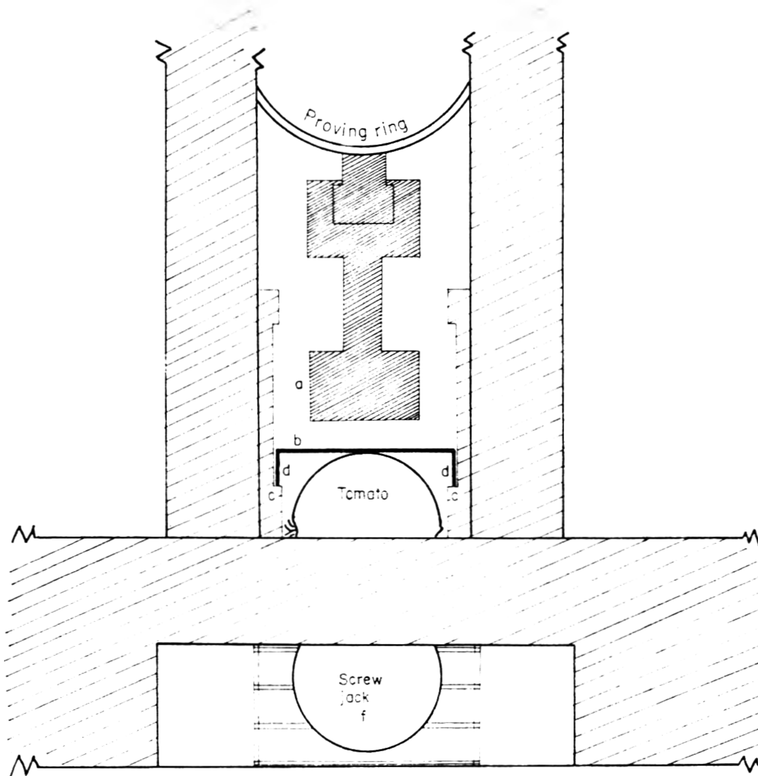

Fig. 2. Shear press compression system.

The arrangement used for compressing the tomato fruit is shown in Fig. 2. A plunger (a) from a standard succulometer cell is attached to the proving ring. A thin piece of sheet aluminium (b), in the form of a bridge, is placed on the ledge (c). The height of the 'legs' (d) is such that the bridge is exactly 5 mm above the point of lowest descent of the plunger (a). The tomato fruit to be tested is placed on the screw jack (f) and is elevated until it touches the bridge (b). The bridge is then removed and the plunger is activated thereby compressing the tomato by 5 mm. The deformation of the proving ring is recorded on a strip chart and the force peak rises to a maximum and falls off again during the compression of the fruit. A ram speed of 4·5 mm per second was used in all tests. The orientation of the tomato fruit during the compression was such that the line joining the stylar scar to the calyx was horizontal. A sample of twenty-five fruit can be tested in a period of 10 min using this system.

Linearity and calibration

A standardized 10-lb Salter spring balance was used to calibrate the system and check it for linearity. The hook of the spring balance was placed under the bottom of the plunger (a) (Fig. 2) and an operator pulled the spring up vertically until the balance was recording 1 lb (proving ring system static). When a steady 1 lb force was attained, as indicated by a horizontal straight line on the strip chart, the height of the straight line from the baseline was measured. The procedure was repeated for spring balance readings of 2–10 lb at 1 lb intervals thus giving a set of parallel lines. The heights of these lines on the recorder were noted, thus measuring the deformation of the proving ring under forces from 1 to 10 lb. Each force (from 1–10 lb) was applied three times, thus giving thirty applications in all. It was necessary to change the span adjustment on the recorder during these tests in order to keep the straight lines on the paper for the different forces applied.

Correlation with finger feel

Correlation of the shear press system with subjective finger feel tests was carried out using tomatoes carefully selected on the basis of colour and size. Twelve fruit at the green/yellow stage were picked (cv. Eurocross BB) every second day from a glasshouse over a period of eighteen days, giving a total of ten batches or 120 individual fruit. Each fruit was individually coded with a small stick-on label. All the fruit were uniformly round and were in the size range 50–52 mm in diameter. They were stored in a room at 18°C after picking. On the eighteenth day the fruit were presented to a twelve-member panel (six males and six females) experienced in tomato production and marketing who were asked to place the fruit in order (1 to 10) from firmest to softest on the basis of finger feel. The rank numbers given to fruit of the same age by the twelve panelists were added, thereby giving a firmness rating to the ten sets of tomatoes. The tests took place in a dark room (to remove the influence of colour, and to make the codes invisible) and each panelist received a set of fruit that ranged from firm (just

picked) to very soft (eighteen days old). When each panelist had completed his or her assessment the same fruit were then compressed individually on the shear press and the peak heights on the strip chart corresponding to each compression were measured. The peak heights for tomatoes of the same age post-harvest were added together and the order of firmness obtained with the shear press was correlated (rank method) with that obtained by the panel.

Acceptability levels

In addition to putting the ten fruit in order from firmest to softest the panelists were also asked to indicate (i) the break point in firmness between suitability and non-suitability for sale at retail level and (ii) the break point between usable and not usable in the home, i.e. too soft to slice, or only suitable for stews or puree. In this way it was possible to assign acceptability categories in terms of grams force for a 5 mm tomato fruit compression.

Fruit size and firmness reading

Tomato fruit were placed in seven categories on a weight basis and were then stored at 18°C for eight days. There were from 27 to 31 fruit in each category, made up as nearly as possible by equal numbers of fruit of three cultivars. The seven categories were from 31–40 g, up to 91–100 g in 9 g ranges. Average firmness values (g force) for the fruit in each size category were calculated from the individual results.

Destructive nature of measurements

The destructive nature of the compression test was investigated by carrying out five successive compressions on individual tomatoes, each one 15 sec after the previous one. The tomatoes used were stored at 18°C. All compressions on individual tomatoes were made without changing the position of the tomato on the platform of the screw jack; it was necessary, however, to raise each tomato slightly in order to make it touch the 'bridge' again after the first compression.

Further tests were carried out on eight day-old tomatoes, where three successive compressions were made on each fruit with an interval of 1 hr between them.

Sample size for a given precision

Nineteen different tests were carried out to find the number of fruit needed to obtain a firmness result of a desired precision. Various combinations of age of fruit post harvest, number of fruit per test, and different cultivars were investigated in order to obtain as wide a range of samples as possible. Details of the different combinations are given in Table 5 in the results section. Fruit used in these tests were taken from 12 lb trays of commercially grown tomatoes. The firmness values for tomatoes in each test were used to calculate the standard deviation, using the range method of Kramer & Twigg (1966)

for that test. This figure was then entered in the variables sampling equation (Kramer & Twigg, 1966):

$$n=\left(\frac{ks}{\epsilon}\right)^2$$

where k = the number of standard deviations, s = standard deviation, ϵ = the desired precision and n = the number of samples that need to be tested for a result of a desired precision. A value of 1·65 (90% assurance) was chosen for k and ±7% for ϵ. These values were used in the above equation when computing the results of all tests. The appropriate value of s was entered in the equation for each test, and the number of fruit per sample (n) that needed to be compressed to ensure that the firmness value would be within ±7% from the true value for the lot 90% of the time was calculated.

The percentage of fruit within a given lot (12 lb) whose firmness fell within ±5, ±10, ±15, ±20% of the mean firmness value for the lot (i.e. the spread of values around the mean) was calculated from the data for the nineteen tests described in Table 5.

Results

Linearity and calibration

The results (Table 1) show that the relationship between applied force and proving ring deformation was linear in the range tested. Since the 100 mV recorder span setting was found most suitable for tomatoes, the system was calibrated using this part of the data. The applied forces (1–6 lb) were added together, as were the corresponding straight line heights. One pound was equivalent to a line height of 32 mm, or 1 mm was equivalent to 14·2 g force.

TABLE 1. Chart recorder line heights (mm, mean values) for different forces applied to the proving ring with a spring balance

Applied force (lb)	Recorder span setting (mV)			
	20	50	100	200
1	163	65 (1)*	32 (1)*	16 (1·000)*
2	—	129 (1·985)	63 (1·969)	32 (2·000)
3	—	193 (2·969)	97 (3·031)	48 (3·000)
4	—	—	128 (4·000)	64 (4·000)
5	—	—	162 (5·063)	80 (5·000)
6	—	—	194 (6·063)	97 (6·063)
7	—	—	—	113 (7·063)
8	—	—	—	129 (8·063)
9	—	—	—	145 (9·063)
10	—	—	—	164 (10·250)

* The figures in brackets are ratios obtained by dividing the top number in each column into the subsequent ones.

Correlation with finger feel

The rank correlation coefficient between tomato fruit firmness as measured by a finger feel panel and the shear press was 0·988. The spread of firmness values for the shear press was satisfactory, and the proximity of one value to the one above or below it (Table 2) was generally of the same order as that in the corresponding panel scores, except in the case of the firmest tomatoes.

TABLE 2. Mean panel and shear press values for tomato fruit firmness

Age of tomatoes post-harvest (days)	Panel firmness score*	Shear press values (g force)**
0	12 (1)	1686 (1)
2	28 (2)	1304 (2)
4	37 (3)	1063 (3)
6	60 (4)	808 (5)
8	62 (5)	822 (4)
10	71 (6)	737 (6)
12	80 (7)	652 (7)
14	90 (8)	638 (8)
16	109 (9)	581 (9)
18	111 (10)	553 (10)
Rank correlation: panel × shear press = 0·988		

* Twelve panelists; firmest sample score 1, softest 10.
** Mean value for twelve fruit.

Acceptability levels

The break point in fruit firmness between suitability and non-suitability for sale at retail level was taken at 680 g force, while that for tomatoes usable in the home, i.e. capable of being sliced easily, was 540 g. These figures are based on the evaluation of the finger feel panel.

Fruit size and firmness reading

The results (Table 3) show that the tomatoes in the two smallest size grades were more compressible than those in the larger grades and suggest that fruit weighing over 50 g are most suitable for testing, if the sample is not size graded.

Destructive nature of test

Even though the test was non-destructive in that it did not damage the fruit visibly, it did cause some permanent change in the shape of the tomatoes. This was discovered when making the second compression, when it was found necessary to re-adjust the

TABLE 3. Effect of tomato fruit* size on the firmness reading

No. of fruit tested	Size (g)	Shear press value (g force)
29	31–40	1006
29	41–50	1020
31	51–60	1063
27	61–70	1105
31	71–80	1148
31	81–90	1091
29	91–100	1162

* The fruit were held for eight days post-harvest at 18°C.

tomato upwards slightly on the screw jack in order to make it touch the bridge. This indicated that the tomato had not returned completely to its pre-compression size. The firmness values for tomatoes that received successive compressions (Table 4) confirm this finding, as the mean firmness value increased considerably between the first and second compressions and to a lesser extent between the others. A similar trend was found for tomatoes that received three successive compressions, each separated by 1 hr, with firmness values of 836, 935 and 1034 g respectively.

TABLE 4. Firmness (g force) of tomatoes subjected to five successive* compressions

Age of fruit post-harvest (days)	Compression				
	1	2	3	4	5
0	1331	1587	1559	1629	1672
4	1275	1629	1601	1573	1615
8	1006	1162	1176	1304	1318
12	1063	1261	1346	1261	1261
16	694	893	878	907	893
20	581	723	751	793	779
Mean	992	1209	1219	1245	1256

* Fifteen seconds between each compression.

Sample size for a given precision

The data (Table 5) show the number of fruit from a 12 lb lot that need to be tested to ensure that the results obtained will be within ±7% of the true firmness value of the lot 90% of the time. The number of fruit (n) required for the sample varied from 8 in

TABLE 5. Fruit number* to be tested for a firmness result of a desired precision**

Test	Age of fruit post-harvest (days)	No. of fruit tested	Size (g)	Cultivar	Mean firmness (g)	Range (g)	Precision $\pm \varepsilon$	SD	n (Sample number)
1	7	30	31–40	Mixed	1008	710	71	170	16
2	7	30	41–50	Mixed	1022	767	72	184	18
3	7	30	51–60	Mixed	1065	724	75	174	15
4	7	25	61–70	Mixed	1108	667	78	160	12
5	7	30	71–80	Mixed	1150	710	81	170	12
6	7	30	81–90	Mixed	1093	894	77	214	21
7	7	30	91–100	Mixed	1164	554	81	133	8
8	4	100	50–90	Mixed	1193	1093	84	262	27
9	8	100	50–90	Mixed	937	834	66	200	25
10	12	100	50–90	Mixed	852	738	60	177	24
11	8	26	50–90	Grenadier	1108	653	78	157	11
12	8	24	50–90	Sonato	1079	586	76	136	9
13	8	21	50–90	Eurocross BB	951	483	67	116	8
14	12	27	50–90	Grenadier	895	525	63	126	11
15	12	24	50–90	Sonato	880	497	62	119	11
16	12	21	50–90	Eurocross BB	795	456	56	109	11
17	16	25	50–90	Grenadier	880	653	62	157	18
18	16	24	50–90	Sonato	809	469	57	112	11
19	16	21	50–90	Eurocross BB	738	525	52	126	16

* From a 12 lb box.
** Ninety per cent assurance ±7% from the mean firmness value.

tests number 7 and 13 to 27 in test 8 (Table 5). On the basis of the data in Table 5 it seems that a sample of twenty-five fruit should be sufficient to predict the firmness of most lots with reasonable accuracy and this sample number will be used in future tests with this system. A sample size of fifteen would probably suffice if the fruit were of one cultivar.

The percentages of fruit within a 12 lb lot (averaged over the nineteen lots in Table 5) whose firmness fell within ±5, ±10, ±15 and ±20% of the mean firmness value for that lot were 27, 50, 60 and 80% respectively. For example, if a lot had a firmness value of 1000 g, 50% of the fruit would be within the firmness range 1000g ± 10% (900–1100) and 80% would be within the range 1000 ± 20% (800–1200 g).

Discussion

The shear press system described in these tests proved very satisfactory for measuring tomato fruit firmness and the instrumentation amplifier allowed the small compression forces involved to be recorded. The system is easy to calibrate and the relationship between applied force and that which was sensed and recorded was excellent. Some of the problems of amplifier sensitivity (see 'Materials and Methods') arose from having a 'heavy' proving ring on the shear press; this one was appropriate for loads up to 58 kg, far in excess of the load required for tomato testing. It should be noted that a lighter ring would be more suitable if one was available.

A fruit compression of 5 mm was chosen in these tests because it seemed similar to the amount of compression that a potential purchaser would use at the point of retail sale. It resulted in firmness readings of 1700 to 550 g, depending on the age of the fruit post-harvest. Voisey & Crete (1973) used tomatoes mounted on a transducer to measure the type of forces consumers were likely to use in evaluating firmness. They found the maximum force (mean) used by thirty-one panelists was 1617 g. This was close to the maximum readings of 1700 g found in practice using the shear press system in these tests. The compression tests were carried out on fruit at right angles to an axis from the calyx to the stylar scar, rather than at the stylar scar itself, as practiced by Stenvers *et al.* (1973), because it was felt that this procedure was closest to consumer evaluation. The decision to use a 5 mm compression and a calyx-stylar scar fruit orientation was endorsed by the high correlation between the shear press system and a finger feel panel.

The firmness readings on tomatoes were influenced by fruit size in that fruit (of a given age post-harvest) less than 50 g in weight gave slightly lower firmness values than those above 50 g. In some cases it may be necessary to grade fruit into different size categories, depending on the accuracy required. Shafsak & Winsor (1964) reported a similar trend though not significant statistically.

The firmness test was destructive in that tomato fruits did not return to their original size after the first compression. The second compression gave a higher firmness reading

because it now seemed more difficult to compress a very slightly deformed tomato by 5 mm than when it was in its undeformed state. This means that duplicate samples of fruit must be available when testing tomatoes over a number of days as any particular sample can only be compressed once.

These data also suggest that consumers may damage tomatoes when fingering them in the store and stress the need for correct packing of tomatoes to avoid bruising and compression caused by their own weight.

The variation in fruit firmness within a given lot was considerable (Table 5) and the results suggest that it is desirable to test at least twenty-five fruit/12 lb box when the cultivars are mixed, or fifteen when fruit is all of the one cultivar, in order to get a result close to the true firmness value for the lot. The fact that in this experiment only 80% of fruit in a 12 lb lot (average for nineteen lots) was within ±20% of the mean firmness value for the lot (see 'Results') is important when deciding on acceptability levels for tomato fruit firmness at retail level or for use in the home. The panel tests suggested minimum firmness levels of 680 g and 540 g force for retail sale or home use respectively. These values are to be regarded only as pointers.

This system could be used to measure the firmness of a range of fruit and vegetables by using different proving rings and choosing a suitable amount of compression for the particular fruit or vegetable being tested.

Acknowledgment

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Autolysis and proteolytic activity of cod viscera

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Summary

This paper describes the autolysis of cod viscera and discusses the importance of protease activity for this process. Autolysis yields a lipid phase, an aqueous soluble phase which contains much protein but very little lipid, and an insoluble sediment of protein and lipid. The amino acid composition of the soluble phase differs markedly from that of the sediment; the latter does not contain hydroxyproline and has relatively high levels of aromatic amino acids and of cysteine/cystine. The natural digestion process in the alimentary tract of cod also leaves an insoluble protein with a similar amino acid composition. The sediment which remains after autolysis is very resistant to the activity of the proteases present in the viscera. The pH curve for protease activity with a model substrate does not correspond to the pH curves for autolysis.

Introduction

Little is known about the mechanism of autolysis of fish tissues in a silage. The liquefaction occuring during the process of ensilage is certainly the result of enzymatic action, since cooked fish does not liquefy. It is presumed that the autolysis in silages made from whole fish is mainly due to gut enzymes which are spread throughout the fish mass after grinding (Meinke & Matil, 1973). This is supported by the fact that a silage of fillets alone liquefies poorly (Tatterson & Windsor, 1974).

Liquefaction of whole fish silages is markedly favoured at acid pH values and at temperatures above room temperature (Koury, Spinelli & Wieg, 1971). Freeman & Hoogland (1956) reported, however, that with cod and haddock viscera there was little, if any, effect of the pH in the range 1 to 8 on the rate of autolysis.

It is noteworthy that an insoluble sediment always remains in a fish silage (Tatterson & Windsor, 1974; Freeman & Hoogland, 1956). Also when commercial enzymes are added in order to solubilize fish tissues, an insoluble residue remains (Hale, 1969; Freeman & Hoogland, 1956; Tarky, Agarwala & Pigott, 1973). It is not known why a certain proportion of the fish tissues resists enzymatic digestion. In practice it is important to minimize the yield of this sediment.

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In order to reveal mechanisms of autolysis, it is essential to study the liquefaction process in relation to the activities of hydrolytic enzymes in the autolysing tissues. Such studies will be dealt with in the present paper.

Materials and methods

Cod (*Gadus morhua*) caught near Tromsö (Norway) was used. Viscera with most of the liver removed, but with variable amounts of roe were immediately frozen and stored at −28°C until required.

Silage. To make the silage which is dealt with here, the viscera were passed through a meat grinder with disc holes of 5 mm and thoroughly mixed with formic acid (0·75 volume %) and propionic acid (0·75 volume %). This silage, which has a pH of about 4·2, remains sterile for several months at 27°C.

When the effect of pH on autolysis was studied, the pH of the minced viscera was adjusted with HCl and NaOH.

Autolysis was estimated in two ways.

(a) As dry weight of material in the lipid phase and the soluble aqueous phase (usually expressed as per cent of total dry weight). The autolysates were neutralized before centrifugation for 10 min at 2000 × ***g*** and the dry weight of the supernatant was determined. The dry weights were corrected for added salts.

(b) As low molecular weight Folin positive material. Autolysis was arrested by 10% trichloro acetic acid (TCA) and the Folin positive material in the supernatant determined (Lowry *et al.*, 1951).

Dry weight was determined after drying at 105°C to constant weight, and *ash* after heating for 17 hr at 580°C.

Amino acids were determined in an acid hydrolysate (6 N HCl for 24 hr at 110°C) of the sample, using an automatic amino acid analyser (Jeol; JLC-6 AH).

Total nitrogen content was determined by the Kjeldahl procedure. *Crude* protein was estimated by multiplying total nitrogen by the factor 6·25.

Carbohydrate was determined by the anthrone reaction (Spiro, 1966) in dried samples hydrolysed in 98–100% formic acid for 24 hr at 110°C, using glucose as standard.

Crude fat was determined gravimetrically after Sohxlet extraction of dried samples with diethyl ether.

Protease activity was determined according to Barret (1972) with 2% haemoglobin as substrate. The concentration of *Folin positive material* in the supernatant after adding TCA (4% end concentration) was determined by Lowry's modification of the Folin method (Lowry *et al.*, 1951) using tyrosine as a standard.

Molecular weight distribution was estimated by ultra-filtration, using the Amicon equipment (Amicon B. V. Holland). Dry weight of the filtrates was determined using Diaflo ultrafiltration membranes UM 10 (MW < 10 000) and UM 2 (MW < 1000).

Binding of protease to the insoluble residue in the silage was determined as follows:

0·5 g dry weight were suspended in 9·5 ml of a 1:50 dilution in water of the soluble phase of a freshly prepared silage and mixed thoroughly. After 1 min the insoluble fraction was removed by centrifugation and protease activity in the supernatant measured. The insoluble residue used for the experiments was washed in water three times by resuspending and centrifuging. A portion of this residue was extracted with isopropanol to remove fat.

Results

Figure 1 shows the autolysis at different temperatures of the silage of cod viscera. The rate of autolysis is strongly enhanced at temperatures above room temperature. The

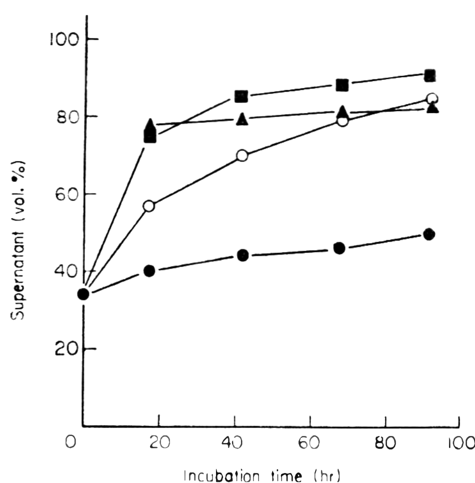

Fig. 1. Autolysis of a silage (pH 4·25) of cod viscera as a function of incubation time at different temperatures. ● 17°C; ○ 27°C; ■ 37°C; ▲ 53°C. Ordinate: Volume of supernatant in per cent of total volume.

autolysis is never complete, i.e. an insoluble sediment remains even after incubation for several months.

The relative amount of the insoluble sediment which remains varies according to the conditions of incubation. This was found also by Tatterson & Windsor (1974). Conditions which cause high initial rate of autolysis do not necessarily give a high yield of soluble material.

The relative yield of solubles in an acid silage (pH 3·5–4·1) was about 80% after 41 hr at 35°C. The yield was not increased by changing the pH to 9–10 after 41 hr. In an alkaline silage (pH 9–10) the yield after 41 hr was only 60%. This yield could not be increased by acidifying the silage to pH 4. (pH was regulated by addition of HCl or NaOH.)

The autolysed silage can be separated by centrifugation at 2000 x *g* for 10 min into a lipid phase, and aqueous soluble phase and an insoluble sediment. The proximate chemical composition of the aqueous soluble phase and the sediment is shown in Table 1.

TABLE 1. Chemical composition of the aqueous soluble phase and of the sediment of the silage of cod viscera (eight days of autolysis at 27°C); figures show percentage of dry weight

	Soluble phase	Sediment
Protein (crude)	77·5	57·8
Fat (crude)	0·4	33·0
Carbohydrate	0·7	0·8
Ash	10·0	4·7
Sum	88·6	96·3

The soluble phase is rich in protein and contains very little lipid. The sediment contains appreciable quantities of lipid besides protein. The size of the lipid phase varies according to the amount of liver residues in the raw material, but the chemical composition of the three phases in not much affected by such variations.

The analytical values of Table 1 do not sum up to 100%. This is partly due to residues of formic acid and propionic acid in the dried material. Another reason is that protein is calculated as total nitrogen x 6·25. This multiplication factor is too low for the aqueous phase because of its high content of low molecular weight peptides. The molecular weight distribution of the soluble phase is shown in Table 2.

TABLE 2. Dry weight (mg/ml) of different molecular weight fractions of the aqueous soluble phase of a silage (pH 4·2) of cod viscera after autolysis at 27°C for different times

Molecular weights	Time of autolysis (days)			
	0	3	6	92
MW > 10 000	35·6	57·2	49·2	38·5
10 000 > MW > 1000	22·1	36·9	30·8	5·2
MW < 1000	39·1	67·2	78·8	113·2
Sum (mg/ml)	96·8	161·3	158·7	156·9

Incomplete solubilization of the silage might be due to inactivation of the tissue degrading enzymes. However, the protease activity in the silage is unchanged after nine days at 27°C (Fig. 2). Even after several months at 27°C this enzyme activity remains high.

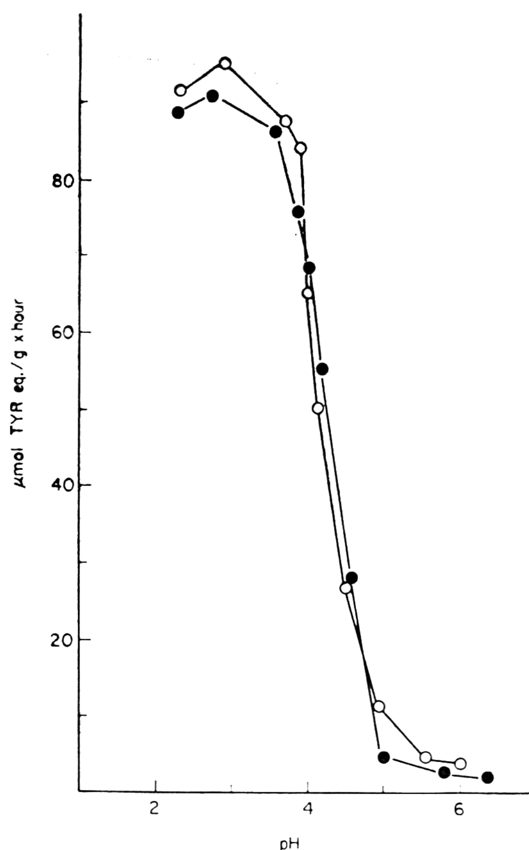

FIG. 2. Protease activity of the homogenated non-preserved fresh cod viscera (●) and in a silage of the same material after nine days at 27°C (○). Samples were withdrawn and diluted fifty times in distilled water and then incubated for 2 hr at 22°C in 0·25 M glutamatebuffers with different pHs, using 2% haemoglobin as a substrate. Abscissa: The pH measured in the incubation mixture. Ordinate: Protein degradation expressed as μmol tyrosine equivalents released as TCA soluble material per hour and gram fresh weight of silage.

Figure 3 shows the solubilization of a sediment from an eight days old silage, when resuspended in the aqueous phase of a freshly prepared silage. There is a significant solubilization the first day after resuspending, indicating that unstable enzymes in the silage are able to degrade the sediment. However, the rate of degradation in the presence of the freshly prepared silage is very low compared with the initial rate of autolysis of complete silage (notice the ordinate values). Removal of most of the lipid from the sediment by extraction with isopropanol rendered it somewhat more susceptible to degradation.

Although not shown in a table we include here that the protease of cod viscera binds

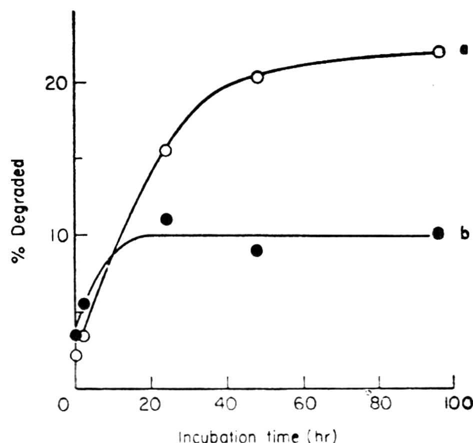

Fig. 3. Solubilization of the sediment of an eight-day-old silage after resuspending in the aqueous soluble phase (pH 4·4) of a freshly prepared silage. Curve a: sediment (80 g) extracted once by suspending in isopropanol (500 ml) and washed twice with water (4·5% lipid). Curve b: sediment (80 g) washed by suspending in water (500 ml) and centrifugation three times (32% lipid). Three hundred milligrams sediment was suspended in 5 ml of the aqueous phase of freshly prepared silage and the increase of dry weight in the supernatant determined gravimetrically. Ordinate: percentage solubilized sediment. Abscissa: incubation time (hr) at 27°C.

very poorly to the sediment. Only about 15% of the protease activity binds to the sediments (with lipids and extracted) at the experimental conditions described.

Table 3 shows the amino acid composition of the soluble phase and of the sediment in the silage. For comparison the table includes values for cod skin and for the fraction of cod skin which is not degraded by the aqueous phase of the silage. The amino acid composition of the protein from the alimentary tract of cod is also shown. The undigestible fractions lack, or contain very little, hydroxyproline, and contain little glycine compared to the soluble fractions. Cystine and aromatic amino acids are present in higher concentrations in the sediment than in the soluble phase.

The insoluble sediment which remains after autolysis at an alkaline pH, has an amino acid composition which corresponds to that remaining after acid hydrolysis. The undigestible protein in the alimentary tract of cod has an amino acid composition which resembles that of the insoluble sediment in the silage.

Figure 4 shows the autolysis of minced cod viscera as a function of the pH. This pH curve, which shows the release of soluble material (dry weight), does not correspond to the pH curve of protease, as estimated with haemoglobin as a model substrate (Fig. 2). This difference is even more pronounced if autolysis is estimated as the release of low molecular material with positive Folin reaction (Fig. 5).

TABLE 3. Amino acid composition of acid hydrolysates of a silage of cod viscera, of cod skin and of the gut content of cod (A: minced cod viscera; B: aqueous soluble phase of a silage after eight days at 27°C; C: the sediment of the silage; D: cod skin; E: undigested fraction of cod skin after exposure for five days to the aqueous phase of a silage; F: insoluble (undigested) fraction of the gut content close to pylorus cecae; G: as F but near anus); figures show moles per 100 mol of amino acids

Amino acid	A	B	C	D	E	F	G
Tryptophane*	0·2	0·2	0·5	—	—	—	—
Lysine	8·1	8·5	5·9	3·5	5·4	5·4	9·4
Histidine	1·5	1·2	1·6	0·9	1·3	1·2	2·2
Arginine	4·8	4·7	4·4	4·6	4·9	4·6	4·9
Taurine	2·5	3·1	0·8	0·6	0·6	5·5	2·0
Hydroxyproline	0·3	0·6	0·0	4·9	0·7	1·3	0·0
Aspartic acid	8·2	7·8	8·7	6·0	10·8	9·2	10·4
Threonine	5·2	4·3	5·9	3·0	4·7	5·4	6·3
Serine	6·4	4·9	6·9	6·0	6·5	6·6	6·7
Glutamic acid	11·5	11·8	10·7	7·8	11·7	10·9	10·8
Proline	6·1	6·6	4·8	8·6	4·0	5·6	5·0
Glycine	9·4	10·1	7·6	32·6	16·3	15·9	10·7
Alanine	9·6	10·6	8·4	10·4	8·2	7·0	7·3
Cystine/2	0·9	0·2	2·7	0·2	0·8	1·4	1·4
Valine	5·6	6·2	6·8	2·4	4·7	4·2	5·9
Methionine	2·6	2·5	2·7	1·9	3·2	2·3	1·5
Isoleucine	4·4	4·5	4·6	1·6	4·0	3·0†	3·2†
Leucine	7·6	7·9	8·0	2·9	7·6	6·0	6·4
Tyrosine	2·3	1·9	4·5	0·8	2·0	2·5	3·4
Phenylalanine	2·9	2·7	4·4	1·3	2·7	2·2	2·7
Ammonia‡	11·5	14·7	12·6	5·2	19·5	24·7	40·8

* Determined after hydrolysis in 4 N NaOH at 100°C for 15 min.

† The gut content contained considerable amounts of glucosamine. Since isoleucine and glucosamine have the same elution volume, the amount of isoleucine was assumed to be half that of leucine.

‡ Ammonia is expressed as mole/100 mol of amino acids in the hydrolysate.

Discussion

An acid silage of cod viscera autolyses quickly and forms a lipid phase, an aqueous soluble phase and an insoluble sediment. These phases separate easily upon centrifugation. As a possible additive to foodstuffs the aqueous soluble phase may be the most valuable, since it is very rich in protein and contains little lipid. Unfortunately the levels of tryptophane and of cystine are very low.

The amino acid composition of the sediment differs significantly from that of the soluble phase. It is noteworthy that the sediment lacks hydroxyproline and contains

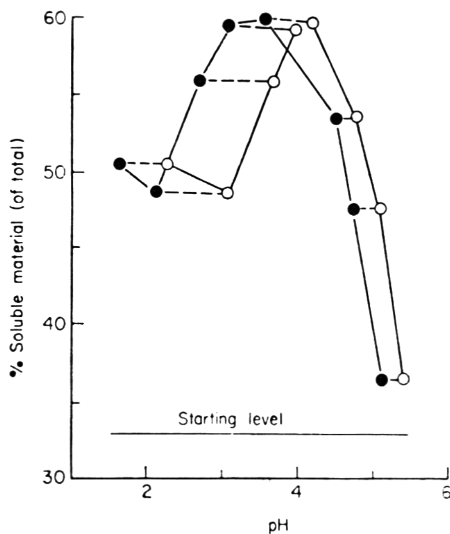

FIG. 4. Autolysis, at different pH, of homogenized cod viscera (incubation for 16 hr at 27°C). Propionic acid was added until pH was 5·1, and then the pH was regulated with HCl. The homogenates were neutralized with NaOH before centrifugation and the dry weight of the supernatant determined. Figures are corrected for formation of salt due to pH regulation and neutralization. ● Start pH; ○ final pH; – – – pH change.

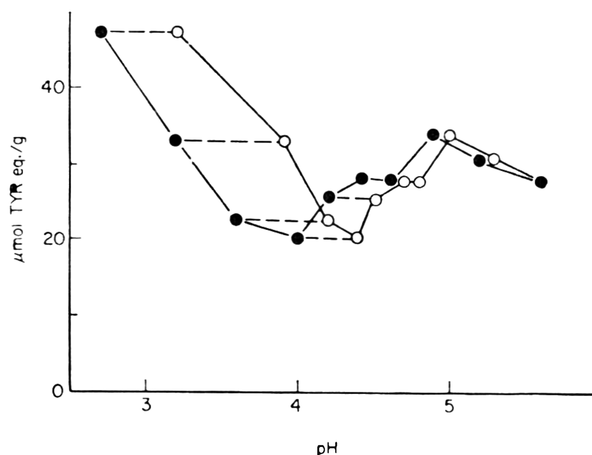

FIG. 5. Autolysis of cod viscera at different pH, measured as increase of Folin positive material soluble in 10% TCA. The curves shows the autolysis and the pH change during the period from 2 to 20 hr after incubation of the homogenized viscera at 27°C. pH was regulated with HCl. ● Start pH; ○ final pH; – – – pH change.

less glycine than the soluble phase. Hydroxyproline is quickly released also from fish skin which is exposed to the soluble phase of a silage. Accordingly we conclude that collagen is quickly solubilized in the silage.

The amino acid composition of the undigested (=insoluble) fraction of the gut content of cod resembles that of the insoluble sediment in a silage. It seems therefore an attractive hypothesis for further studies that the living fish is unable to digest the same tissue fraction which remains as a sediment in the silage.

The activity of the proteolytic enzymes in the silage is unchanged after nine days at 27°C. Incomplete solubilization of the silage can accordingly not be due to inactivation of the proteases which can be recorded with haemoglobin as a model substrate. A slight further solubilization results when the sediment from a silage is resuspended in the aqueous phase of a freshly prepared silage. This suggests that unstable enzymes are able to degrade the sediment slowly. However, since this effect of the fresh silage is very small enzyme inactivation does not offer a plausible explanation of the incomplete solubilization of the silage. We believe that the protein of the insoluble sediment has a structure which hinders the access of proteolytic enzymes. The lipid left in the sediment is one factor which protects the proteins. But even when the lipids are extracted the protein in the sediment is very resistant to hydrolytic enzymes, and still it has a low binding affinity for the proteases. The high proportion of cystine in the sediment suggests that sulphur–sulphur crosslinks may be essential in a protein structure which resists the action of the proteolytic enzymes in the fish silage.

The insoluble sediment may have become resistant to enzymatic digestion during the process of autolysis, or it may be a genuine tissue fraction which is resistant to the fish enzymes. The fact that the relative amount of residual sediment in a silage may vary considerably according to the experimental conditions (pH, temperature) lend some support to the former possibility. Here it may be relevant to point out that complete solubilization of fish tissues is not obtained even with commercial enzymes of microbial or plant origin (Hale, 1969; Tarky *et al.*, 1973).

The initial rate of autolysis is highest at pH 3·5–4·0, if autolysis is measured as increase of dry weight of the soluble phase. If measured as degradation of protein to low molecular components (soluble in 10% TCA) there is a minimum at about pH 4 and optimum at about 5 and below 3. These pH curves differ significantly from that of the protease, estimated with haemoglobin as a model substrate. The pH optimum for the protease may of course vary with the substrate used.

The protein of the sediment has an amino acid composition which is very similar to that of structural glycoproteins extracted from connective tissue (Robert & Comte, 1968). This may indicate that much of the sediment consists of glycoproteins. Accordingly it is possible that autolysis is the resultant of the action of proteolytic enzymes and enzymes splitting those β-glycosidic bonds which interlink the glycoproteins with other structural components. This discussion will, however, be taken up in the continuation of this work.

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A computer method for the analysis of dilatometric measurements on fats

M. F. MURPHY AND J. P. BRADY

Summary

Computer programs were developed for the detailed analysis of raw dilatometric data on fats. The method calculates and plots the best fit polynomial curves of temperature versus the following:

(a) percentage solid fat;
(b) percentage solid change per °C and
(c) specific volume.

The best fit equation of different fats can be used to determine theoretically the melting behaviours of fat mixtures and the ideal proportions of different fats to be blended together to approximate a desired fat in melting characteristics between any prescribed temperature limits.

Curves as high as sixth order can be fitted to utilize shoulder and toe portions of the analytical curves without distorting straight line segments.

Introduction

One of the most important characteristics of butter and margarine is their physical consistency which in turn is closely related to spreadability. The main factor which affects the consistency of a fat is the proportion of solid phase present and this is in turn influenced by temperature (Mattil, 1964). Dilatometry is the classical method for the estimation of percentage solid fat and is accepted as one of the few practical ways for characterising and controlling the consistency of commercial fat mixtures. Differential thermal analysis (Norris *et al.*, 1971) has also been used to determine solid/liquid fat ratios but the method has several drawbacks, the main one being that it is based on dynamic rather than static temperature conditions for its measurement. This results in quite different and less commercially applicable results than those obtained by static means.

More recently, low resolution nuclear magnetic resonance (Wood, Murphy & Dunkley, 1975) has been used to measure percentage solid fat in a mixture with very

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promising and rapid results. The initial cost of the equipment may limit its widespread use.

The dilatometric method is very suitable for routine quality control purposes and fats are usually characterized by dilatometric measurements at two or at most three different temperatures. In this case, manual calculation of results is quite satisfactory.

In order to fully utilize the results of dilatometry for the blending of fats the curve representing the entire melting profile of the fat from 0° to 40°C is desirable. To achieve this, dilatometry readings at 2 to 3°C intervals are needed (i.e. fifteen to twenty readings). The best fit curve can be represented mathematically by a fifth or sixth order polynomial and resort to computer methods become necessary in order to determine its coefficients because of the complexity of the calculations involved.

The purpose of this paper is to outline the computer approach developed by us and to give details of the type of analysis and results obtainable from raw dilatometric data. Details of the practical uses of the fitted equations in the prediction of the properties of fat fractions and fat mixtures are also presented.

Methods

The sample for analysis is prepared and analysed according to the methods of Hannewijk, Haighton & Hendrikse (1964). Dilatometric readings in mm^3 are taken at 2–3°C intervals from 0° to 40°C. Analysis is normally done in triplicate. Samples for which the original and final results at 40°C differ by 10 mm^3 or more are rejected. Dilatation is expressed in mm^3 per 25 g fat.

A specially prepared computer program was used for the analysis of the results which was done on an IBM 1130 computer fitted with an on-line IBM 1627 plotter. The program includes all the calculations required to analyse any set of dilatation data. Instead of using average correction values from tables for the water and oil corrections, these values are automatically calculated by the program. The value for the water correction is evaluated using the specific volume and density of water at the various temperatures while the oil correction value is obtained using the equation proposed by Hannewijk *et al.* (1964).

The data required by the program are input using standard 80 column cards and contain the temperature and corresponding dilatometer readings as well as the weight of fat. A table for each set of temperature and dilatometer readings is produced by the computer giving the corresponding oil and water correction values, dilatation, dilatation per °C, specific volume and % solid and liquid present. A table summarizing the mean values for the replications is then produced, a summarized abbreviated example of which is given in Table 1.

This is followed by the calculation of the best fit regression polynomial curves, up to 6th order, using the Gauss–Jordan method for the solution of the normal equations (Ostle, 1954).

TABLE 1. Abbreviated computer generated results for the means of three replications of dilatometry readings on milk fat

Temperature (°C)	Dilatation mm³/25 g	Specific volume mm³/25 g	% Solid fat
0·4	820	25 788	74·3
2·5	786	25 866	70·3
5·1	753	25 952	66·0
7·8	694	26 067	59·7
10·2	631	26 181	53·3
12·2	548	26 305	45·3
14·0	473	26 418	38·7
16·1	392	26 544	31·7
18·2	327	26 653	26·0
20·3	280	26 744	22·0
24·0	213	26 891	16·3
28·0	134	27 056	10·0
32·1	64	27 215	4·7
36·2	10	27 358	1·0
38·0	0	27 049	0·0

The coefficients of each fitted equation are printed with the correlation coefficient and standard error of estimate. The coefficients of the first derivative of each equation are also provided. The equations relating percentage solid fat (S) to temperature (t) and dilatation (D) to temperature calculated for the data in Table 1 are as follows:

$$S = 73{\cdot}3 + 0{\cdot}2686t - 0{\cdot}3951t^2 + 0{\cdot}1877t^3 - 0{\cdot}0003439^4 + 0{\cdot}000002194t^5$$
$$r = 0{\cdot}999$$

standard error of estimate = $\pm$ 1·1% solid fat

and

$$D = 825 - 21{\cdot}47t + 4{\cdot}320t^2 - 0{\cdot}7647t^3 + 0{\cdot}04489t^4 - 0{\cdot}001106t^5 + 0{\cdot}000009890t^6$$
$$r = 0{\cdot}997$$

standard error of estimate = $\pm$ 29·8 dilatation units.

It is especially necessary to carry at least four significant figures for the coefficients of t because of the magnitude of the t^4, t^5 and t^6 terms at the higher temperatures.

The fitted polynomial equations fit the experimental data very accurately and can, therefore, be the basis for describing the fat quite precisely. This is superior to the traditional method of describing a fat by means of two or three dilatation values at different temperatures.

Tables can be generated from 0°C to the melting point of the fat (i.e. 100% liquid) in steps of 0·1°C giving the (a) percentage solids and (b) percentage solids change per °C.

Computer generated graphs of temperature versus any or all of the following may be optionally obtained:

(a) dilatation,
(b) percentage solid fat,
(c) specific volume and
(d) percentage solid fat change per °C.

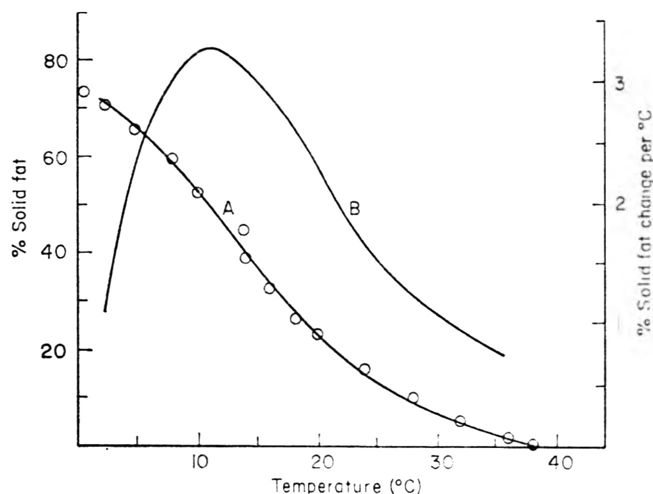

Fig. 1. Best fit polynomial (A) and the experimental points for percentage solid fat in Table 1. Curve B is the first derivative of A and represents the percentage solid fat change per °C.

In the case of (a), (b) and (c) the experimental points and the fitted curves are shown on the computer generated graphs. Figure 1 shows a typical plot from the data, incorporating both percentage solid fat and percentage solid fat change per °C plotted against temperature based on the equation calculated from Table 1. The latter curve indicates how quickly the fat is melting with increasing temperature and the peak of this curve is typically in the region of 10°C for milk fat. The equation of the curve is simply the first derivative of the equation for percentage solid fat.

Discussion

The cumbersome nature of dilatometric calculations associated with the use of average values (i.e. oil and water expansion values) obtained from tables may restrict, especially for research, the degree to which the technique is used in fat analysis. Also, it is not

feasible to calculate manually, or with a desk calculator, best fit equations as complex as fifth or sixth order. Consequently, resort to computer methods is necessary.

Having obtained the equations relating percentage solid fat to temperature, it becomes feasible to mathematically 'mix' fats in any proportions and to derive theoretically the melting curve of any fat mixture made up of fats whose equations are known, as follows:

If two fats have the following equations (relating percentage solid fat (S) to temperature (t)).

$$S_1 = a_1 + a_2 t + a_3 t^2 + \dots$$

and

$$S_2 = b_1 + b_2 t + b_3 t^2 + \dots$$

are to be blended together in the proportions p and $(1-p)$ respectively then the equation of the resultant mixture is

$$S = c_1 + c_2 t + c_3 t^2 + \dots$$

where $c_i = pa_i + (1-p)\ b_i$ and $i = 1, 2, 3$, etc.

This procedure can be extended for any number of fats in a mixture which are present in any proportions.

It should be noted that because of the nature of polynomial curves they should never be extrapolated for temperatures outside the range for which the curve was calculated. In cases where the fat is fully liquid its equation becomes $S = 0$, i.e. all its coefficients are zero.

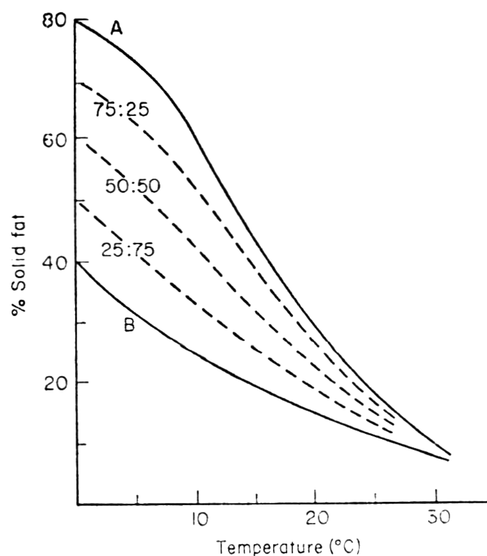

Fig. 2. Theoretically derived equations for different proportions of fat A and fat B.

Figure 2 shows a plot, using the above procedure, for two fats and the calculated solids in different mix proportions obtained from them. We have found that the calculated values correlate well with the experimental results.

An estimation of the proportions of different fats to be mixed together to approximate a desired fat in melting characteristics can also be found reasonably well by theoretical means.

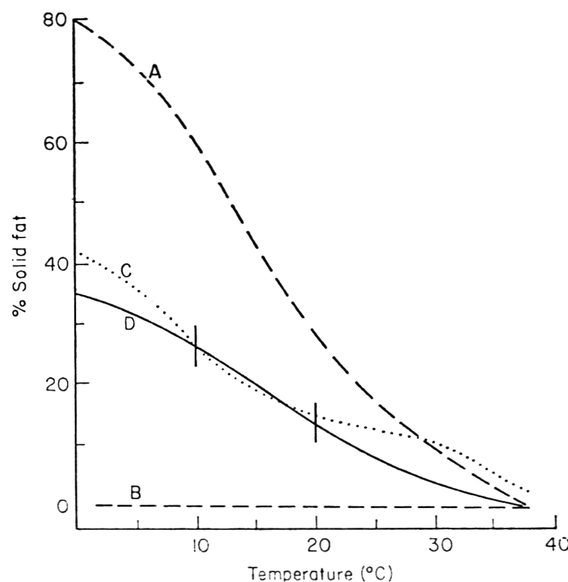

Fig. 3. Optimum blend curve (D) of fats A and B (where B is a liquid oil) to approximate to the desired curve C between 10 and 20°C. Equation D resulted from a 44/56 mixture of A and B.

By means of an iterative process, successively varying blend compositions can be compared, on a least squares basis, with the desired equation and the curve of minimum deviation determined. Figure 3 is an example of this, where the 'best mix' equation (curve D) for a blend of two fats is determined for the range 10 to 20°C. Obviously the narrower the temperature range selected the more accurately can the mixture curve match the desired curve. It is clear that the optimum blend will vary depending on the temperature range selected.

The techniques described for the blending of fats by theoretical mathematical methods take no account of certain physical phenomena (e.g. solubility of solid fat in liquid fat or the formation of eutectics) and, therefore, will not always yield completely accurate results. Provided the fats are compatible (Rossell, 1973) the techniques described can very quickly indicate feasible mixture conditions and thereby reduce very considerably the amount of experimental work necessary to determine optimum blends.

Acknowledgments

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Diffusion phenomena during the decaffeination of coffee beans*

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Summary

The diffusion of caffeine in green coffee beans was investigated at atmospheric pressure using dichlormethane as a solvent.

The diffusion coefficient of caffeine was determined as a function of temperature and water content of the beans. The mathematical treatment was based on Fick's second law of diffusion applied to a spherical model.

The apparent diffusion coefficients are in the range of 0·2–1·1 . 10^{-6} cm^2 sec^{-1}.

Introduction

Caffeine exerts various physiological effects that many people do not tolerate very well. Early in this century processes were patented (German Patent, 1905), by which the caffeine can be extracted from green coffee beans without considerable loss of their organoleptic and stimulant properties.

The procedure consists basically of the following four operations:

(1) swelling the beans;
(2) extraction of the caffeine with an organic (mostly chlorinated) solvent;
(3) driving off the solvent by steam to a tolerable level;
(4) drying the beans.

The present study was undertaken to throw light on the diffusion phenomena during the extraction process. It was of particular interest to find a mathematical model which allows the description of the diffusion of the caffeine in the coffee beans by means of a single quantity, the diffusion coefficient.

To be useful for practical purposes the diffusion coefficient should respond sensitively to changes in experimental conditions such as type of coffee, type of solvent, temperature and water content of the beans, respectively.

* The present work is a short résumé of the thesis work of Mr B. Bichsel presented under the title 'Untersuchungen der Diffusionsvorgänge bei der Entcoffeinierung von Rohkaffee' in 1972 at the University of Berne.

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Materials and methods

Material

Green coffee of the type Arabica from Columbia (medelline excelso) was used with an average initial caffeine content of 1·45 ± 0·03%.

The beans were extracted with dichlormethane as a solvent.

The residual caffeine content of the beans was in the range of 0·01–0·50%, depending on the experimental conditions.

Experimental conditions

The following experimental variables were chosen.

(1) Degree of swelling (initial water content)

High*	44–45% water content
Medium	39–40% water content
Low	32–34% water content

(2) Temperature

High†	37·0°C
Medium	33·5°C
Low	30·0°C

These variables were investigated according to a 3 × 3 factorial experimental plan with several replications. For practical reasons all experiments were carried out at atmospheric pressure (at about 710 torr = 0·947 bar).

Swelling the beans

Practical experience of decaffeination shows that the coffee beans must be swollen with water prior to the extraction with the solvent. This operation was carried out as follows:

(1) weighing 250 g of air dried beans into a 1 litre round-bottomed flask;
(2) immersion of the rotating flask into an oil bath at 90°C;
(3) adding the water through a glass tube directly on to the beans at a rate of twenty-five to thirty drops per minute;
(4) cooling the flask while rotating;
(5) storing the swollen beans for at least 15–20 hr in the refrigerator.

The slow rate of addition of the water allowed the beans to absorb the water readily and without forming a distinct liquid phase. Thus the extraction of water soluble components from the beans as well as the uneven distribution of the water could be avoided.

* Maximum water holding capacity of the beans used.
† Boiling point of the azeotrope $CH_2Cl_2-H_2O$ at the ambient pressure.

Extraction

For the extraction process the apparatus shown in Fig. 1 was used.

The main part of the apparatus consisted of a 1-litre cylindrical flask in which the temperature could be kept constant by means of an ultrathermostat. The solvent, previously saturated with water, was kept flowing through a flowmeter and through the flask. The mixture was constantly stirred during the extraction process.

The batch was initially made up of 500 ml of solvent and 300–370 g of swollen beans. The flow rate of the solvent was generally 2 litres/hr at which the maximum possible rate of extraction was maintained (sink conditions).

Samples were withdrawn by opening the flask for 2–3 min and taking out 50 g of beans necessary for analysis.

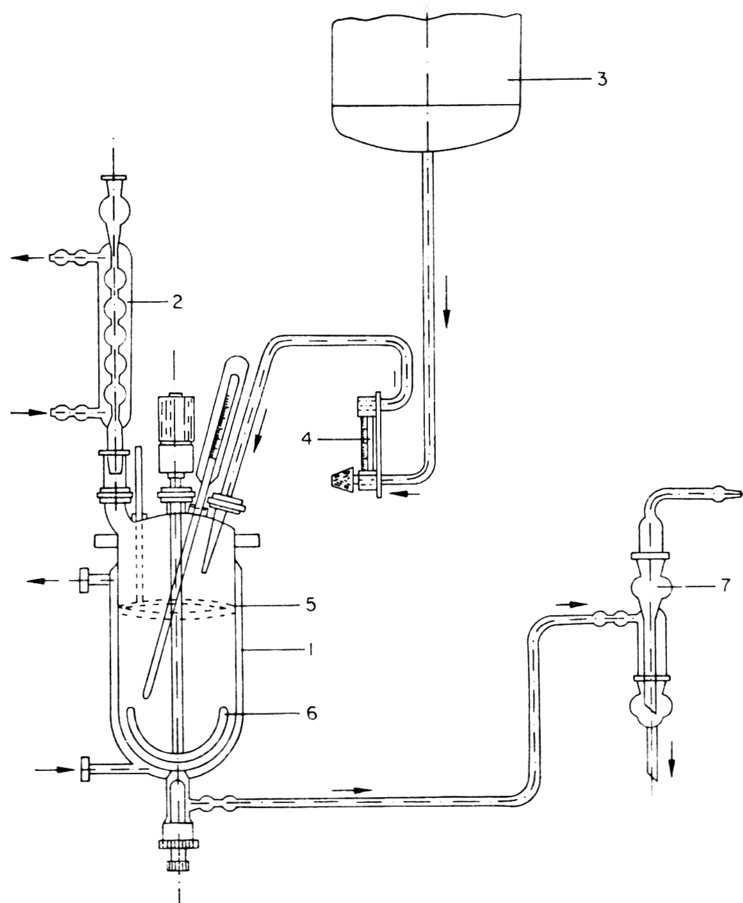

FIG. 1. Extraction apparatus. 1, Thermostatic reaction vessel; 2, cooler; 3, solvent reservoir; 4, flow meter; 5, ring; 6, stirrer; 7, niveau control.

Most of the experiments were conducted for 9 hr, but some were continued for 30 or 60 hr, respectively.

Analytical methods

For the determination of the caffeine content, the solvent content and the water content of the beans, respectively, the analytical plan shown in Fig. 2 was followed.

The caffeine content was determined by the modified Levine-Method in accordance with the Swiss Official Methods of Food Analysis which is in principle identical to the ISO draft method 'ISO/TC34/SC8/GT (France-20) 104 F revisé' (in the meantime accepted with small modifications as an official ISO method).

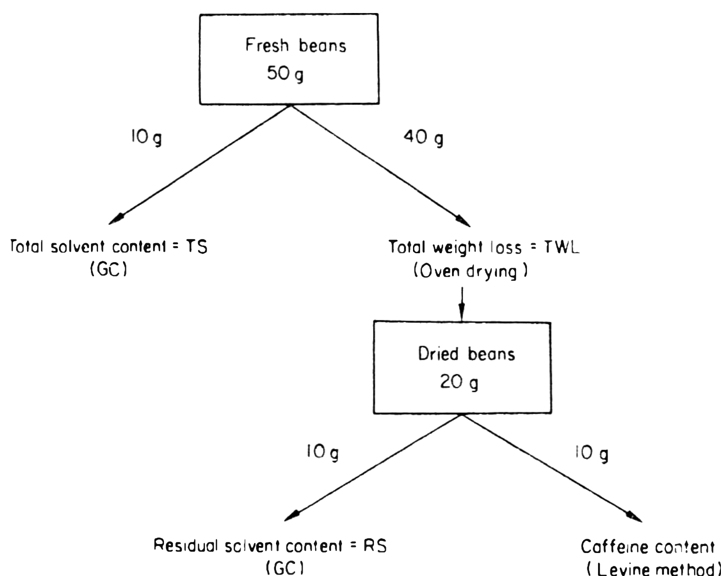

Fig. 2. Methods of analysis. Water content = TWL − (TS − RS).

For the determination of the solvent content of the beans the gas chromatographic procedure described by Gál & Schilling (1971) was applied.

The measurement of the water content was only possible by means of an indirect method consisting of the determination of the total weight loss (TWL) at 105°C (15 hr) and calculating the water content according to the following equation.

$$\text{Water content} = \text{TWL} - (\text{TS} - \text{RS})$$

Where TWL = total weight loss of the extracted beans; TS = total solvent content of the extracted beans; and RS = residual solvent content of the extracted and dried beans.

The water and solvent contents were calculated as a percentage of the total weight of the fresh extracted beans.

The caffeine content is expressed as a percentage of the dry weight of the solvent free beans.

Mathematical methods

The calculation of the diffusion coefficient of caffeine is based on the following assumptions:

(1) the coffee beans are considered to be spheres;
(2) the diffusion is radial, isotropic and independent of the concentration of the caffeine in the beans;
(3) at the beginning of the experiment ($t=0$) the caffeine is evenly distributed in the beans;

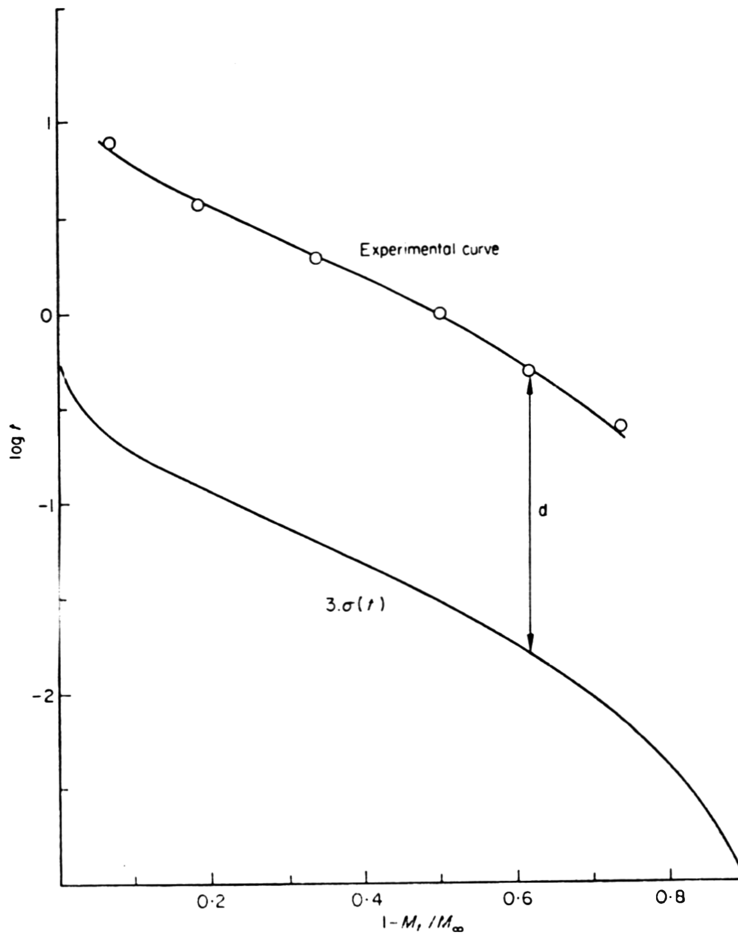

FIG. 3. Sigmoidal graph of the caffeine content

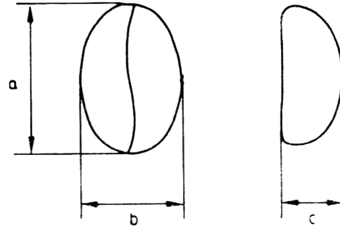

FIG. 4. Geometrical form of coffee beans.

(4) during the whole experiment the caffeine content of the extract phase equals zero.

With these assumptions the caffeine content of the beans as a function of the time can be expressed by the following equation (Crank, 1970):

$$1 - M_t/M_\infty = 6/\pi^2 \sum_{n=1}^{\infty} 1/n^2 \,.\, \exp\left[-Dn^2\pi^2\, t/r^2\right]$$

where M_t = mass of the caffeine diffused out of the beans at time t, M_∞ = total mass of the diffusing species (= initial caffeine content of the beans), D = diffusion coefficient, r = radius of the bean-volume-equivalent-sphere and n = integer.

If we put $D/r^2 = 1$ and $t = x$, then the right side of the above equation becomes identical to the well known Sigma-function except for the factor 3 (Schmidt, 1971):

$$\sigma(x) = 2/\pi^2 \sum_{k=1}^{\infty} 1/k^2 \,.\, \exp\left[-\pi^2 k^2 x^2\right]$$

Plotting $3 \,.\, \sigma(x)$ against $\log x$ one obtains a sigmoidal curve (see Fig. 3).

In the same diagram the experimental values of $1 - M_t/M_\infty$ can be plotted against $\log t$. This also results in a sigmoidal curve which, however, is shifted parallel to the $3 \,.\, \sigma(x)$-curve by the amount of $d = \log (D/r^2)$.

If the radius r of the spherical diffusion model is known, the diffusion coefficient can be calculated by the following equation:

$$D = r^2 \,.\, 10^{-d}$$

The spherical model of the coffee beans

Figure 4 shows schematically the shape of a coffee bean. The unilaterally flat bean is comparable to the one half of an ellipsoid having the half-axes a/2, b/2 and c, respectively. As no mathematical expression for the diffusion in a half-ellipsoid is known the form was approximated by a sphere with an equivalent volume to the half-ellipsoid.

The average bean volume was obtained at different water contents by measuring the axes a, b and c of a large number of beans and also by making pycnometric determinations of their volume.

By equating the average bean volume to the volume of a sphere the radius could be calculated and expressed as a function of the water content with the following formula:

$$r = 3{\cdot}32 + 0{\cdot}0145x$$

where r = average bean radius in millimetres and x = water content in percentage.

The linear regression line is shown in Fig. 5.

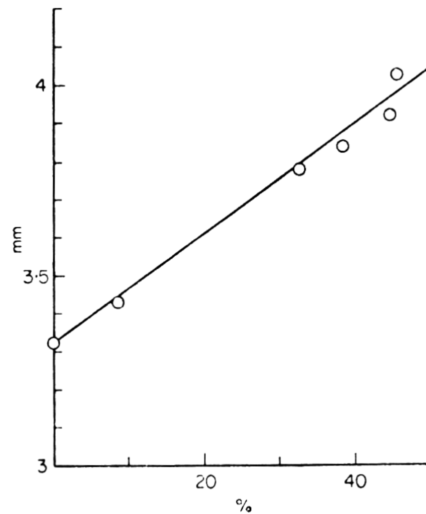

FIG. 5. Sphere radius as function of water content.

Results

The diffusion coefficients of the caffeine in Columbia beans as a function of the initial water content and temperature, respectively, are shown in Table 1.

In comparison the diffusion coefficients of caffeine at room temperature (21–22°C) in pure water and pure dichlormethane, respectively, were determined using the interferometric technique (Table 2).

The values of Table 1 confirm the well known fact in the practice of decaffeination that the diffusion coefficient increases with increasing water content and with increasing temperature.

An estimate of the energy of activation for the diffusion of caffeine in the beans was made using Arrhenius' equation. The values were found to lie in the range of 50–100 kJ/mol which is in reasonable agreement with the activation energy of the

TABLE 1. Diffusion coefficients

Initial water content (%)	Temperature (°C)	D . 10^6 $cm^2 sec^{-1}$	Coefficient of variation (%)
44·7	37·0	0·89	20·6 ($n=4$)
45·7	33·5	0·53	—
44·2	30·0	0·47	4·3 ($n=6$)
39·2	37·0	0·72	—
39·4	33·5	0·50	—
39·2	30·0	0·42	—
34·2	37·0	0·38	—
33·1	33·5	0·31	—
33·2	30·0	0·24	2·9 ($n=2$)

TABLE 2. Diffusion coefficients of caffeine in pure solvents

Solvent	D . 10^6 $cm^2 sec^{-1}$
Water	5·3
Dichlormethane	13·7

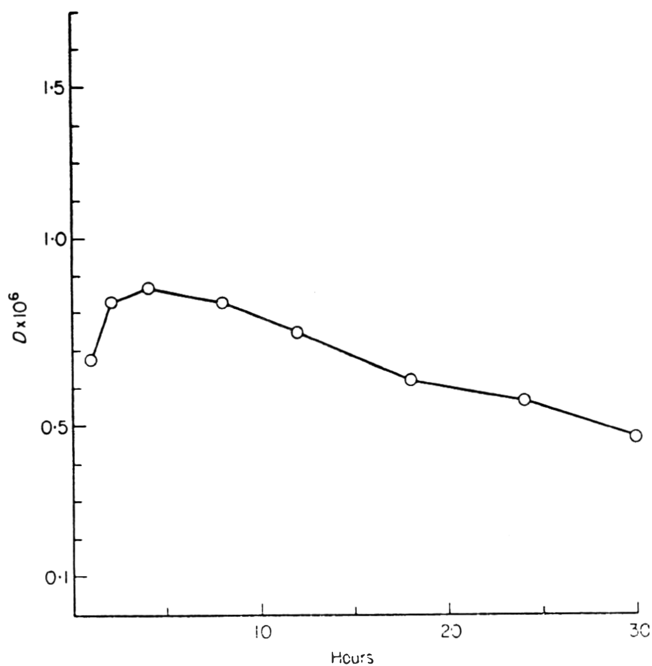

FIG. 6. Change of the diffusion coefficient with time.

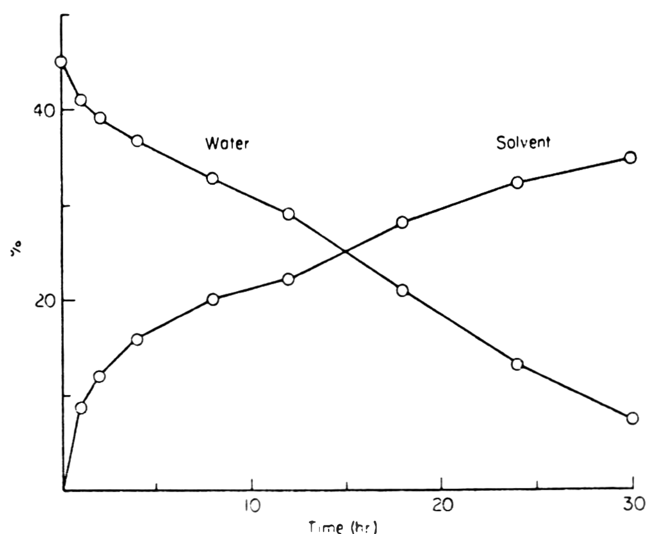

FIG. 7. Time function of water and solvent contents.

diffusion of caffeine in pure water, calculated from data published by Okada & Kawashima (1968).

A thorough examination of the sigmoidal curves of the extraction data shows that the single experimental values are not scattered at random around the hypothetical curve. Rather, there seems to be a systematic variation of the diffusion coefficient with time, which is shown in Fig. 6.

The time dependence of the diffusion coefficient may be explained by the solvent uptake and/or the water loss of the beans during extraction (Fig. 7).

The solvent penetrating into the beans initially promotes the diffusion of caffeine. This increase, however, is soon overcompensated by the inhibiting effect of the water loss and the diffusion coefficient decreases again after having reached a maximum (Fig. 6).

Discussion

The coffee bean behaves with respect to the diffusion of caffeine almost as perfectly as a spherical model system. This behaviour is a consequence of at least two opposite effects, namely the taking up of solvent and the water loss. The overall change of the diffusion coefficient caused by these factors remains, however, small as compared with the shifts brought about by the parameters investigated: water content and temperature. It is therefore reasonable to express the effects of these variables by using a single quantity, namely the average diffusion coefficient.

As for the expelling the water out of the beans, it must be assumed that this is a direct consequence of the penetration of the solvent into them. The solvent molecules go

presumably into the lipoide structures of the beans, thus increasing the volume of this phase. An additional swelling of the beans by a non polar solvent, however, is only possible at the expense of the water phase with the total volume remaining unchanged.

The investigations presented in this paper do not allow definite statements to be made regarding the mechanism of the migration of caffeine in the beans. The caffeine resides in the cells and the diffusion must proceed by passing from cell to cell through the pits in the cell wall. It is reasonable to assume, therefore, that the movement of the caffeine molecules is favoured by widening the pits during swelling of the beans. The increase of the rate of the diffusion by the solvent entering the beans may come about by establishing communicating diffusion channels composed of the fat/solvent phase in which the rate of diffusion is even larger than in the water phase.

By expelling the water out of the beans the number and/or the cross-section of the passages decreases and the movement of the caffeine molecules slows down again.

Acknowledgment

We are grateful to Haco Ltd, Gümligen, Switzerland for financial support to one of us (B.B.) while carrying out the investigations reported in this paper.

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Influence of extraction medium pH on the protein content of some legume starches

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Summary

Starches of lentil, garbanzo and field bean were isolated according to a detailed separational scheme at several pHs in the range of 5·5 to 9·5 and nitrogen determinations made on the purified samples. Nitrogen determinations and protein solubility profiles were also determined on the legume flours in order to facilitate a comparison of the data obtained for the starches. The relationship between protein solubility and starch protein content is discussed. The protein content of garbanzo starch decreased from a value of 0·51% at pH 5·5 to a value of 0·13% at pH 9·5. For field bean starch the protein content was 0·55% at pH 5·5 and 0·18% at pH 9·5. In the case of lentil starch the protein content decreased from 0·42% at pH 5·5 to 0·25% at pH 7·5 with very little change up to a pH of 9·5. It was found that extraction medium pH has no effect upon the yield of starch.

Introduction

Currently there is a great deal of enthusiasm among food technologists to determine the functional properties of legume protein isolates in order to evaluate their potential and usefulness both as foodstuffs and as ingredients in manufactured foods. Ultimately, the results derived from their research taken in conjunction with the current knowledge of soybean technology will serve as a basis for the industrial preparation of all types of legume protein isolate. We feel, however, that before any new general industrial process is devised for legume protein isolate production, the effect of the protein extraction parameters upon the starch fraction, another potentially valuable product to be derived from some legumes, should be investigated.

In reviewing the literature relating to legume starches we found that although a considerable amount of work has been done in the area of characterization of legume starches, there has been no vigorous research conducted to determine how different physical factors associated with protein starch extraction methods affect the yield,

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purity and physical properties of purified legume starches (for literature pertaining to legume starch characterization see, for example, Kawamura & Fukuba, 1957; Kawamura & Tada, 1957; Correa, Rosenthal & Tolmasquim, 1965; Tolmasquim *et al.*, 1965; Schoch & Maywald, 1968; Kawamura, 1969; Lineback & Ke, 1975).

In our investigations concerning the isolation of protein and starch from Chilean varieties of lentil bean (*Lens culinaris*), garbanzo bean or chickpea (*Cicer arietinum*) and field bean (*Phaseolus vulgaris* var. Coscorron) we have observed that changes in protein–starch extraction methodology sometimes produce drastic changes in the physical appearance of isolated starches. For example, lentil starch prepared by extraction of the flour at a pH of 5·5 is almost pure white, whereas, lentil starch prepared by extraction at pH 9·5 is a pale cream colour.

In view of the above we have begun a series of experiments which are designed to determine how various physical factors such as size of flour particle, solvent/flour extraction ratio, temperature, time, rate of stirring during extraction, size and shape of settling container, concentration of starch suspension during sedimentation and extraction medium pH influence the yield, purity and physical properties of purified legume starches. In this communication we report the results of our first study dealing with the effect of extraction medium pH on the nitrogen content of starches derived from the legumes mentioned above.

Materials and methods

Materials

Samples of Chilean grown lentil, garbanzo and field beans were purchased in November 1975 at a local market. All chemicals were reagent grade unless otherwise specified.

Extraction procedure

The beans were screened and washed with cold water to remove contaminating material and the dried beans first ground to a coarse powder in an Alpine Augsburg 25a Mill and further ground to pass a 0·3 mm sieve in an Alpine Augsburg 160z Mill. Portions of each of the legume flours were extracted at pHs of 5·5, 6·0, 6·5, 7·0, 7·5, 8·0, 8·5, 9·0 and 9·5 ± 0·05 pH units according to the procedure described below and the purified starches analysed for nitrogen by the Kjeldahl procedure according to Smith (1967). Nitrogen determinations were also performed on the flours utilizing this procedure; however, smaller quantities of sample were used.

A 200 g sample of legume flour was wetted with 10 ml of toluene and suspended in 2 l of distilled water with the aid of a three-bladed immersion type stirring apparatus driven at 750 rev/min. Toluene was added to the extraction mixture to preclude the possibility of fermentation (Schoch & Maywald, 1968). The pH of the solution was adjusted to the appropriate value by adding small amounts of either concentrated HCl or NaOH solution. The resulting suspension was stirred for exactly 2 hr with the

temperature maintained at 25 ± 2°C. The pH of the suspension was determined every 10 min and any deviation from the original pH corrected by the addition of more HCl or NaOH solution.

The suspension was filtered consecutively through 60, 140, 200 and 250 mesh phosphor bronze sieves and twice through a 325 mesh sieve. The residues remaining on the sieves were washed with a total of 500 ml of distilled water adjusted to the appropriate pH and the washings combined with the original filtrate. The crude starch suspension was allowed to settle in a 4 litre beaker for 30 min and 2200 ml of supernatant decanted. The starch was resuspended in 2 litre of distilled water adjusted to the proper pH, allowed to settle for 20 min and 2000 ml of supernatant decanted. The 20 min sedimentation procedure was repeated two additional times followed by one sedimentation in 2 litre of pure distilled water for 20 min. Finally, after decanting 2100 ml of supernatant, the purified starch was suspended in 500 ml of distilled water and isolated by suction filtration.

The starch was dried in a thin layer for 24 hr at room temperature, powdered to pass 100 mesh and dried at room temperature for an additional 24 hr. Kjedahl nitrogen determinations were performed in duplicate on 12-g samples dried at 120°C for 18 hr. That the starches were substantially free of fibre was established by staining with an acidic solution of methylene blue and examining under a microscope.

It should be noted that in order to obtain reproducible results, the time necessary for the extraction–purification process must be carefully controlled. In these experiments the sieving procedure required 25 min, the sedimentation procedure required 130 min including 5 min for decanting the supernatant, and the final filtration required 5 min yielding a total time of 135 min. In all experiments this value was strictly adhered to within ± 10 min.

Determination of protein solubility profiles

In order to facilitate a comparison of the data obtained from the nitrogen determinations on the various starches, protein solubility profiles were determined for each of the legume flours. The reference method for determination of protein solubility described by Romo, Lakin & Rolfe (1975) proved satisfactory for this purpose.

Results and discussion

Although legume proteins exhibit maximum solubility at both low pH and high pH and therefore can be extracted from contaminating substances at either of these values, the pH range of 5·5 to 9·5 was chosen for this study because moderately acidic extraction of proteins can cause some hydrolysis of the starch fractions, thus altering the physical properties of the purified starches. In addition, in our laboratory protein isolates are without exception prepared by extraction at neutral to basic pHs. Extraction at the mildly acid pHs of 5·5 to 7·0 was performed for the sake of comparison and presented

no problem with respect to starch hydrolysis, since Schoch & Jensen (1940) demonstrated that starch hydrolysis does not occur in mildly acidic (pH 5·5) solutions at room temperature. It is conceivable, although not likely, that enzymatic hydrolysis could occur at these pH values by amylases indigenous to the legumes; however, no attempt was made in this study to determine the amylolytic activity of the extraction mixture.

Yield of starch as a function of pH

From the data presented in Table 1 it is apparent that the yield of starch from a specific legume is not affected by the pH of the extraction medium. The variation in

TABLE 1. Protein content (%N × 6·25) and yields of starches as a function of pH, and nitrogen content of flours, dry basis

Sample	Extraction pH	% Protein	% Yield
Garbanzo flour	—	20·8	—
Field bean flour	—	20·9	—
Lentil flour	—	28·8	—
Garbanzo starch	5·5	0·51	26·2
Garbanzo starch	6·0	0·39	25·1
Garbanzo starch	6·5	0·25	25·4
Garbanzo starch	7·0	0·26	27·3
Garbanzo starch	7·5	0·19	26·0
Garbanzo starch	8·0	0·20	24·7
Garbanzo starch	8·5	0·20	25·3
Garbanzo starch	9·0	0·19	27·2
Garbanzo starch	9·5	0·13	26·8
Field bean starch	5·5	0·55	26·0
Field bean starch	6·0	0·45	27·8
Field bean starch	6·5	0·36	25·4
Field bean starch	7·0	0·34	27·8
Field bean starch	7·5	0·27	25·3
Field bean starch	8·0	0·24	26·1
Field bean starch	8·5	0·27	26·3
Field bean starch	9·0	0·22	25·0
Field bean starch	9·5	0·18	24·7
Lentil starch	5·5	0·42	28·5
Lentil starch	6·0	0·31	29·9
Lentil starch	6·5	0·31	27·8
Lentil starch	7·0	0·26	28·0
Lentil starch	7·5	0·25	26·9
Lentil starch	8·0	0·27	28·6
Lentil starch	8·5	0·25	29·5
Lentil starch	9·0	0·26	27·4
Lentil starch	9·5	0·26	27·8

yield as the pH changes appears to be random and is presumably due to the inability of the investigator to exactly reproduce the conditions for each extraction. Most of the variation was probably introduced at the sieving stage of the extraction procedure, where it was extremely difficult to quantitatively wash the starch from contaminating material on the sieves with the small amount of wash water utilized (500 ml total for seven sievings).

The average yield of Garbanzo and field bean starch was 26·0% with standard deviations of ±1·0 and 1·7, respectively, while the average yield of lentil starch was 28·3% with a standard deviation of ±0·9. The higher yield of lentil starch was due to the fact that lentil starch sedimented more rapidly than either the field bean or garbanzo starch.

It should be pointed out that no attempt was made utilizing the extraction procedure described in this paper to obtain maximum yields of starch, but rather to obtain reproducible yields of starch substantially free of fibre in a relatively short period of time. In the case of lentil and garbanzo bean the starch content is probably in the neighbourhood of 40% (Schoch & Maywald, 1968; Lineback & Ke, 1975); however, this is conjecture upon our part. We have estimated the starch content of field bean to be 35–40% based upon the difference in weight after determination of protein, fats, soluble carbohydrates, ash and crude fibre.

Protein content of flours

The protein contents (%N × 6·25) of the legume flours are also given in Table 1. The protein content of garbanzo and field bean flour are 20·8 and 20·9%, respectively. The value reported for garbanzo bean is in good agreement with the previously reported values of 20·4% (Lineback & Ke, 1975), 21·0% (Fan & Sosulski, 1974) and 20·1% (Amino Acid Content of Foods and Biological Data on Proteins, 1970) and that for field bean agrees fairly well with the value of 22·1% reported in the latter reference cited above.

The 28·8% protein content of Chilean grown lentil is much higher than the value of 21·7% reported for *Lens culinaris* by Fan & Sosulski (1974) and significantly higher than the value of 24·2% reported in Amino Acid Content of Foods and Biological Data on Proteins (1970). Possibly, these differences are due to intervariety variations in the protein content of the legumes and/or differences in climate and soil conditions under which the beans were grown.

Protein content of starches

The most obvious feature of the data presented for the protein content of the starches in Table 1, is that in all three starch samples, i.e. garbanzo, field bean and lentil starch, the nitrogen content more or less decreases as the pH of the extraction medium increases. The protein contents of garbanzo and field bean starch were reduced from 0·51% and 0·55% at pH 5·5 to 0·13% and 0·18% at pH 9·5, respectively, while the protein content

of lentil starch decreased from 0·42% at pH 5·5 to 0·25% at pH 7·5 with little change in the protein content between pH 7·5 and pH 9·5.

At first glance it would appear that the lentil starch protein content is not as sensitive to changes in pH as is the protein content of garbanzo and field bean starch, since the protein content of lentil starch is not significantly reduced above a pH of 7·5. To a certain extent this is true; however, when one compares the protein contents of all three starches at pH 7·5 (the midpoint of the pH range) with those at the extremes (pH 5·5 and 9·5) it quickly becomes apparent that for all three legume starches the major decrease in protein content occurs in the region of pH 5·5 to 7·5.

For example, at pHs of 5·5, 7·5 and 9·5 the protein content of garbanzo starch is 0·51%, 0·19% and 0·13%, respectively, therefore the absolute decrease in protein content from pH 5·5 to 7·5 is equal to 0·51–0·19% or 0·32% and from pH 7·5 to 9·5 the decrease equals 0·19–0·13% or 0·06%. When these values are converted to relative percentages the result is obtained that 84% of the protein removed over the entire pH range of 5·5 to 9·5 is removed on attaining a pH of 7·5, whereas, there is only a 16% reduction between pH 7·5 and 9·5. When this argument is applied to field bean starch the decrease from pH 5·5 to 7·5 is 76% and from 7·5 to 9·5, 24%.

This substantial decrease in protein content in all three legume starches in the pH range of 5·5 to 7·5 is not surprising when the solubility behaviour of the legume proteins in this pH region is considered. From Fig. 1 it can be seen that the solubility of the legume proteins increases very rapidly in the pH range of 5·5 to 7·5 and only slowly in the pH range from 7·5 to 9·5, therefore, one would expect to see a rapid decrease

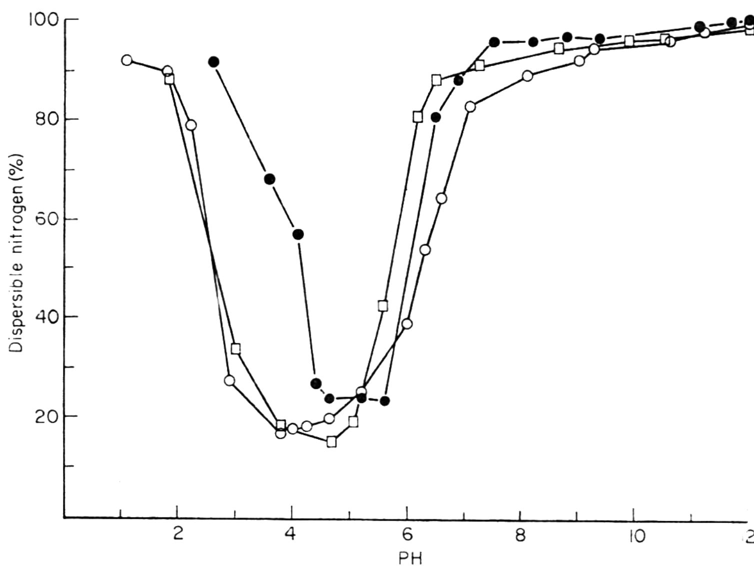

Fig. 1. Protein solubility profiles for the three legume flours. ○ Field bean; ● Lentil; □ Chickpea.

in the amount of contaminating protein present in the starch samples as the pH of the extraction medium is increased from 5·5 to 7·5 and little or no decrease in the region of pH 7·5 to 9·5.

It is interesting to note that although there is considerably more protein present in lentil flour than either garbanzo or field bean flour and the solubility of lentil protein is less at pH 5·5 than the solubility of proteins present in the other two flours, the protein content of the lentil starch prepared at pH 5·5 is unexpectedly lower than the protein content of garbanzo or field bean starch prepared at the same pH. One would expect the converse to be true. At high pHs (9·0–9·5) where little difference in protein solubility exists between the three flours, less contaminating protein is present in both garbanzo and field bean starches than in lentil starch as would be predicted. Unfortunately, we have no explanation for the results obtained at pH 5·5.

Comments on the protein solubility profiles

Since Kjeldahl nitrogen determinations were used as the basis for construction of the protein solubility profiles, total soluble nitrogen was determined rather than soluble protein nitrogen. The difference in these two values (perhaps as much as 15%) presents no real problem with respect to this study, because the general shape of the curve and the region of most rapidly increasing protein solubility were the aspects of protein solubility which we were most interested in, rather than the absolute concentration of protein at any given pH. It should be kept in mind, therefore, that the actual concentration of dissolved proteins represents only a portion of the dispersible nitrogen at any point on the curves depicted in Fig. 1.

Fan & Sosulski (1974) have published data on the protein solubility profiles of lentil and garbanzo flour, but no published data could be found for field bean flour. Their results for garbanzo flour protein solubility agree well with ours and the basic pH region of their solubility curve for lentil is similar to ours; however, there is a major difference in the shape of the curves for lentil protein in the acid region. Fan & Sosulski reported that lentil protein is only partially soluble in moderately acidic solutions (pH 2–3), but more soluble than in the region of minimum solubility (approximately 3–5). In contrast we report that the solubility of lentil protein rapidly increases from a low value at pH 4·4 (28% dispersible nitrogen) to a high value at pH 2·6 (90% dispersible nitrogen).

It should be noted that we report no values for dispersible nitrogen below a pH of 2·6 for lentil flour, because we have observed that the amount of dispersible nitrogen is a very sensitive function of extraction time below a pH of 2·5. Furthermore, the only major difference between Fan & Sosulski's method for determination of protein solubility profiles and ours is that in their method an extraction time of 2 hr is employed, while with our method the sample is extracted only 20 min. The conflicting results are presumably due to this difference in extraction times; however, we are currently studying the phenomenon exhibited by lentil protein in order to quantify

the relationship between nitrogen dispersibility and extraction time at moderately acidic pHs and this will be the subject of a future communication.

Conclusions

The amount of contaminating protein present in garbanzo, field bean and lentil starches was shown to be a function of the pH of the extraction medium. Substantial reduction in the protein content of these starches is obtained by increasing the pH of the extraction-medium from 5·5 to 7·5. The protein content is either not reduced or reduced very little in the pH region of 7·5 to 9·5. It was determined that the reduction in protein content is related to the protein solubility in the pH regions described above. It was also found that the yield of the legume starches mentioned above is not influenced by the pH of the extraction medium.

Acknowledgments

We thank Judy King for her technical assistance and Juan Retamales for performing the large number of Kjeldahl analyses required by this study.

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Book Reviews

Food from Waste. Ed. by G. G. Birch, K. J. Parker and J. T. Worgan. London: Applied Science Publishers, 1976. Pp. xii + 301. £16.

In this volume are published the twenty papers (and discussions thereon) which formed the basis for a symposium at the National College of Food Technology, Weybridge in 1975.

Following an introduction by E. J. Rolfe and a general review by S. R. Tannenbaum and G. W. Pace, the contributions were arranged in four sessions. Although these were not designated by subject headings, a common aspect of the nine papers in Sessions I and II is the use of microorganisms and algae in converting cheap and abundant waste, either directly or following chemical treatment, into material which could be used for feed or food. The microorganisms considered include fungi, yeasts and bacteria and, among the substrates, are the wastes from crop plants (including potatoes), molasses, manure, paper and palm oil.

The four papers in Session III outline the chemical and physiological processes involved in recovering protein from leaves, from the effluents of potato starch mills and flesh food processing plants and from whey.

The final five papers, which constitute Session IV, cover a variety of topics: a discussion of world food problems, a nutritional and toxicological evaluation of novel feeds, a consideration of the socio-economic implications of processing food from waste, a description of an ion-exchange press for protein recovery and a resumé of the 'Bioplex Concept'. The latter is concerned with the benefits of treating organic wastes 'by a stream of processes so that the waste from one can become the raw material for another'.

The diverse nature of the papers in Session IV reflects the overall impression generated by the volume, namely that its undoubted value—and the significance of the topic it presents—would have been enhanced by a more systematic arrangement of the contents.

In the thoughtful contribution of L. G. Plaskett, which forms the penultimate chapter, he comments as follows. 'It is certain that, if total organic matter is going to be scarce, it will not be possible to indulge prejudices against using some organic materials which are disgusting to us.' This could well be taken as the essential philosophy underlying the concept for food from waste.

Although the volume is not as all-embracing as the statement on the inside of the dust cover suggests—'This book represents the pooled viewpoints of those scientists throughout the world who are in the forefront of the food from waste protein'—the fact that it gives a most useful and authoritative coverage of this important topic is not in dispute. The text is well produced with no overt typographical errors (one might

mention, however, that in Chapter 4, p. 71, 'Alamine' appears instead of 'Alanine'), but £16 seems a somewhat elevated price for a volume of this kind.

R. A. LAWRIE

Nutrition Technology of Processed Foods. Food Technology Review No. 25.
By NICHOLAS D. PINTAURO.
New Jersey, Noyes Data Corporation, 1975. Pp. x + 332. U.S.$36.

For those who are unfamiliar with the review series of patents from N.D.C. this publication is based on 125 U.S.A. patents dating from 1956 to 1974, the greater proportion being post-1969. The author has made an effort to make the patent provisions more readable though whether these patents are indeed a main source of nutritional information as implied in the Introduction is open to question. The value and economic applicability of the patents has been left to the reader to assess and care is required in considering the formulations and processing techniques since they have been written for the purposes of patenting and the majority are in accord with American practice. The reader should take into account the legislation of the country involved as well as in some cases the side effects of the processing techniques on components other than those which are the subject of the patent.

The subjects have been assembled under various food and medical headings and includes salt substitutes, complete diets and special processes, these were presumably derived from the patent listings which unfortunately is not as useful in this context as in the other books by the company. In the absence of a subject index one has to peruse the volume carefully if it is required to assess the techniques used; the use of Vitamin A, for instance, is well covered on p. 213 but there are also some twenty-five other references at approximately ten-page intervals to consider. Protein fractionation enrichment and enzymic treatment is covered under a number of headings as are fats and carbohydrates.

In spite of the criticisms the book is a useful volume for the library shelf to reduce the tedium of patent search for the technologist who wishes to use it as a starting point in his investigation. Critical reading of the book can provide information, stimulate the inventive mind and raise questions of possible problem areas in product development.

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Second Edition, 1975. 120 pages. £12.80.

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Edited by Thomas E. Furia and Nicolo Bellanca, *Dynapol, Palo Alto, California*

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

SI UNITS

gram	g	Joule	J
kilogram	kg = 10^3 g	Newton	N
milligram	mg = 10^{-3} g	Watt	W
metre	m	Centigrade	°C
millimetre	mm = 10^{-3} m	hour	hr
micrometre	μm = 10^{-6} m	minute	min
nanometre	nm = 10^{-9} m	second	sec
litre	l = 10^{-3} m³		

NON SI UNITS

inch	in	= 25·4 mm
foot	ft	= 0·3048 m
square inch	in²	= 645·16 mm²
square foot	ft²	= 0·092903 m²
cubic inch	in³	= 1·63871 × 10^4 mm³
cubic foot	ft³	= 0·028317 m³
gallon	gal	= 4·5461 l
pound	lb	= 0·453592 kg
pound/cubic inch	lb in^{-3}	= 2·76799 × 10^4 kg m^{-3}
dyne		= 10^{-5} N
Calorie (15°C)	cal	= 4·1855 J
British Thermal Unit	BTU	= 1055·06 J
Horsepower	HP	= 745·700 W
Fahrenheit	°F	= 9/5 T°C + 32

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

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Contents

555 The detection and estimation of soya protein in food products by isoelectric focusing
J. W. Llewellyn and B. Flaherty

565 Equilibrium moisture contents of air dried beef. Dependence on drying temperature
Hector A. Iglesias and Jorge Chirife

575 The protein of intermediate moisture meat stored at tropical temperature. IV. Nutritional quality
Z. A. Obanu, Ruth J. Biggin, R. J. Neale, D. A. Ledward and R. A. Lawrie

583 Iron availability from intermediate moisture beef
Z. A. Obanu, Ruth J. Biggin, R. J. Neale, D. A. Ledward and R. A. Lawrie

589 Wiltshire curing with and without nitrate. II. Vacuum packed collar bacon and vacuum packed bacon from pigs with high ultimate pH
A. A. Taylor, B. G. Shaw and P. D. Jolley

599 Supplementation of bread with soybean and chickpea flours
Soad A. M. Youssef, Ali Salem and Abdel Hamid Y. Abdel-Rahman

607 A method for measuring tomato fruit firmness using a modified shear press
T. R. Gormley and D. Keppel

619 Autolysis and proteolytic activity of cod viscera
Jan Raa and Asbjorn Gildberg

629 A computer method for the analysis of dilatometric measurements on fats
M. F. Murphy and J. P. Brady

637 Diffusion phenomena during the decaffeination of coffee beans
Bernard Bichsel, Stefan Gal and Rudolf Signer

647 Influence of extraction medium pH on the protein content of some legume starches
C. G. Anderson and C. R. Romo

655 Book reviews

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