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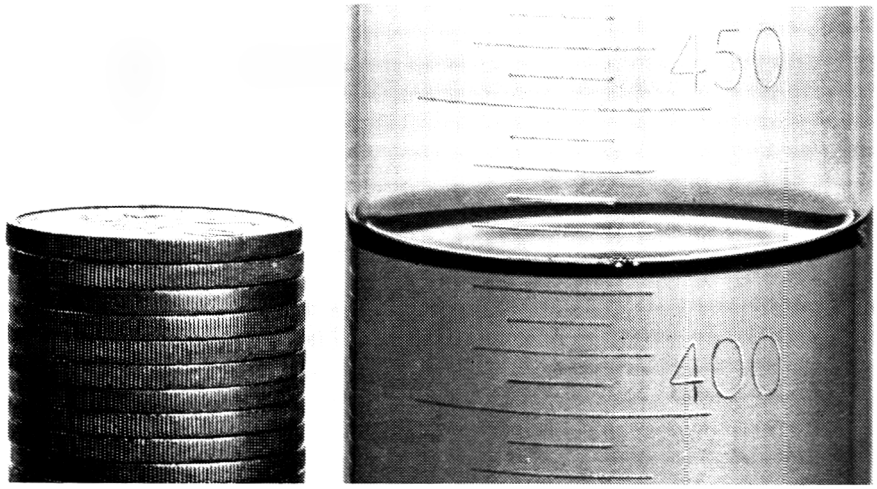

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Editorial

One of the aims of the Institute of Food Science and Technology of the United Kingdom, laid down when it was founded ten years ago, is 'to promote the dissemination of knowledge and ideas amongst scientists and technologists'. The development of its *Journal of Food Technology* in the first eight years of its existence has confirmed that there is a real need for a specialist journal to serve this truly multi-disciplinary field. The wide scope of the subjects covered by the Journal is now attracting contributions not only from all English speaking countries but from many other parts of the world.

The increase in contributions has brought problems in its train, not the least amongst them an unduly long delay between acceptance of a manuscript and its appearance in print. This is clearly undesirable. The Council of the Institute has decided, therefore, to increase the number of issues of the Journal from four to six per year beginning in 1975.

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Fully automated analysis of amino acids in foods by ion exchange chromatography

M. G. DAVIES

Summary

A fully automated system suitable for the analysis of amino acids is described. A standard Technicon autoanalyser was modified by means of an automatic sample loading and programming device. The time for the analysis of a complete amino acid mixture has been reduced so that two analyses are completed in 4 hr. A data acquisition system was incorporated which enabled results to be calculated using an off-line computer.

Introduction

There have been rapid developments in the use of ion exchange chromatography for the analysis of amino acid mixtures and the technique is being applied increasingly to the investigation of the composition of food proteins. Moore and Stein were the first to describe a method for the ion exchange separation of amino acids in a series of papers (Moore & Stein, 1951, 1954a, b). Their procedure used two columns (150 cm × 0·9 cm and 15 cm × 0·9 cm) of polystryrene sulphonic acid resin. The amino acid mixture was loaded on to both columns which were eluted with buffers of increasing pH. The acidic and neutral amino acids were separated on the long column and the basic amino acids on the short column. Amino acids were estimated by collecting fractions of the column effluent and reacting them individually with ninhydrin. Moore, Spackman & Stein (1958) realized that there was a need for a more rapid method of monitoring the column effluent which was more accurate and less laborious. They devised a system in which ninhydrin was continuously pumped into the stream of buffer emerging from the chromatography column. The mixture was pumped through a coil of tubing in a boiling water bath and then passed through a colorimeter. The absorbance of the blue colour produced in the reaction of amino acids and ninhydrin was read continuously and plotted on a recorder chart. Different types of ion exchanger were investigated by Spackman, Stein & Moore (1958) and by Hamilton (1958). They concluded that the

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best separations were obtained on a polystyrene sulphonic acid and cation exchanger which was cross-linked with about 8% divinylbenzene.

In the two-column separation method, the eluting buffers were contained in separate reservoirs connected to the column by a manifold of tubing and taps. Peterson & Sober (1959) developed a nine-chambered device (Varigrad) which produced a pH and sodium ion gradient when the chambers were filled with the appropriate buffers. The Varigrad enabled the gradient to be adjusted more precisely and it was incorporated into an automatic analyser system by Piez & Morris (1960). Their system used one ion exchange column (140 cm × 0·9 cm) for the separation. Since only one sample was required for analysis it avoided the volumetric errors which arose due to the application of samples to separate columns. Hamilton (1963) suggested modifications to improve the sensitivity and resolving power of the systems then in use. He employed ion exchange resin which was fractionated to give uniformly sized particles close to 17 μm diameter and he reduced the column dimensions to 127 cm × 0·6 cm. Several types of analysers based on both single column and dual column operation have been manufactured and are available commercially. The time required for a complete analysis has been reduced from several days in the original Moore and Stein technique to less than 24 hr, by pumping the eluent under pressure through the columns.

In these laboratories a standard amino acid analyser (Technicon Instrument Co. Ltd Type NC-1) was originally used and performed one analysis of a protein hydrolysate in 20 hr. A single column (140 cm × 0·6 cm) of cation exchanger, in the form of 22 μm diameter beads was used to separate the amino acids. A pH gradient was produced by means of a device similar to the Varigrad. Sodium citrate buffers of pH's ranging from 2·88 to 5·00 were made up separately and dispensed into the chambers of the Varigrad. Elution of the amino acids was achieved by pumping buffers through the column at a pressure of about 150 lb in^{-2} starting with pH 2·88. The column was thermostated at 60°C by means of a circulating heating bath and water jacket. The column effluent and ninhydrin were pumped into a manifold of tubing using a peristaltic pump and mixed together. The reaction mixture was led into a glass coil immersed in an oil bath set at 95°C, which gave a residence time of 15 min. Three colorimeters linked to a three-point dotting recorder monitored the absorbance of the liquid stream at 570 nm and 440 nm.

The analyser was modified to perform four analyses per day by the addition of another chromatography column and a pump controlled by an adjustable camshaft timer. However, the increasing numbers of samples produced by surveys of raw materials for animal feeding stuffs and the screening of novel protein sources, exceeded the capacity of this system. A greater analysis rate could be achieved either by (a) several columns operated sequentially or (b) automatic sample loading. The former alternative entails a high cost and has limited potential for the development of accelerated analysis. Therefore, modifications were made to the analyser in order to automate sample loading. In addition the analysis time was reduced and the calculation of results was automated.

Apparatus and methods

Automatic programmer

The pH gradient elution device on the NC-1 analyser has to be filled with the correct buffer mixture prior to the chromatography of an amino acid mixture. The system described by Thomas (1970) was used to automate both sample loading and the buffer elution sequence and full construction details are given in his paper. It consists of an electronic programming unit and a modification to a Technicon automatic sampling module.

Sample loader. Two complete amino acid mixtures are analysed simultaneously each on an ion exchange column 40 cm × 0·6 cm. A Technicon high pressure minipump is used for each column and is connected by 0·034 in i.d. polythene tubing to a sampler probe made of a stainless steel hypodermic needle (2 in, 25 gauge). Both probes are fitted in the sampler arm. The sampler modification consists of a box attached to the top of the sampler which contains three cams in place of the standard timing cam. These cams operate micro switches connected to the column pumps. A voltage signal from the program switches on a relay which turns on the sampler, which rotates the cams. The column pumps are switched on in sequence to load a sample on each column.

Programming unit. The programming unit contained a photoelectric scanner and a power supply for six solenoid valves. A stepping motor drives a scanning head with six photoelectric cells at a speed of 2 in/hr. One photocell scans a white cylindrical drum which is rotated by a motor at 2 rev/min. The other five photocells scan four light tracks. Both the light tracks and the drum are illuminated by 8 in fluorescent tubes. Each photocell is connected to circuits which are designed to give a change of voltage when the light intensity at the photocell alters. The alteration of light intensity is produced by blanking off the illuminated surface with Fablon.

The solenoid valves are operated in three pairs so that when one of a pair is energized by a signal from the programming unit the other is de-energized. When energized, each valve acts as a clamp and exerts sufficient pressure to close a plastic tube.

Reagents. Three stock sodium citrate buffers are used for gradient elution and are made up from Analar grade materials.

Buffer I: pH 2·20, 0·2 M Na^+ (238 g sodium chloride, 210 g citric acid, 100 ml thiodiglycol, 20 g phenol, 200 ml Brij 35 (British Drug Houses Ltd), 19,700 ml distilled deionized water).

Buffer II: pH 2·20, 0·2 M Na^+, is made up as for I, except that 2 l of water are replaced by 2 l Analar methanol.

Buffer III: pH 12·25, 0·4 M Na^+ (238 g sodium chloride, 210 g citric acid, 160 g sodium hydroxide, 20 g phenol, 200 ml Brij 35, 19,800 ml deionized distilled water).

Ninhydrin: 200 g in 10 l methyl cellosolve, 5 l 4 N sodium acetate buffer (330 g anhydrous sodium acetate and 100 ml glacial acetic acid made up to 1 l with water), 5 l deionized distilled water.

Hydrazine sulphate: 5·24 g in 10 l methyl cellosolve and 10 l deionized distilled water.

Oxygen-free nitrogen is bubbled through both the ninhydrin and hydrazine sulphate solutions, which are allowed to stand overnight before use.

Operation of the system. Figure 1 shows the layout of the chromatographic and analytical system. For ease of handling the reagents are stored at floor level and pumped to bench level through the solenoid valves by a Technicon peristaltic pump. The tubing is connected using standard autoanalyser fittings. Two tygon tubes (0·65 in i.d. 0·030 in wall thickness) from buffers I and II are passed through valve pair A. After the valve the two tubes are joined by a glass T-piece to give one acidic buffer stream. Valve pair A is thus used to select either one of buffers I and II. The acidic buffer and buffer III are passed through valve pair B using tygon tubing which is then joined by a T-piece to a 0·056 in i.d. autoanalyser pump tube (flow rate 1·20 ml/min). The other end of the pump tube is joined via a connector containing a magnetic stirrer bar (Portex Ltd M630/40) operated by a stirrer (Toyaseibakusho MS-16B), to the reservoir of the sampler module. In this way valve pair B regulates the proportion of acidic and basic buffers and is controlled by the photocell scanning the rotating drum.

Ninhydrin solution and a 50% aqueous solution of methyl cellosolve are connected through valve pair C using 0·065 in i.d. Solvaflex (Technicon Instrument Co. Ltd)

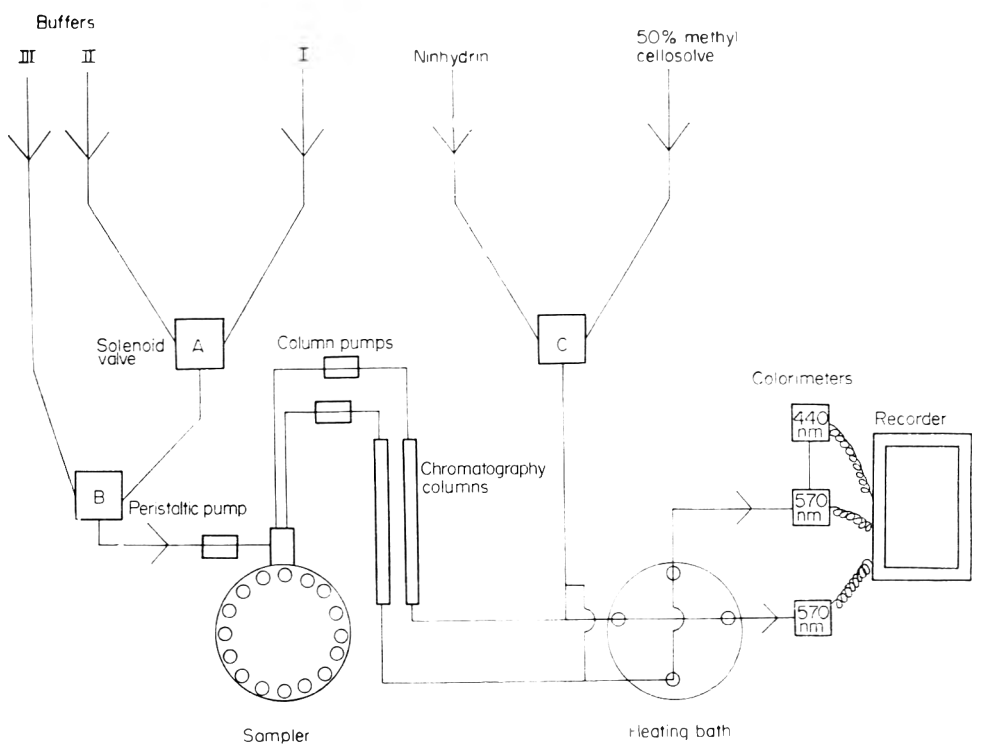

FIG. 1. Amino acid analyser flow diagram.

tubing to a 0·090 in i.d. (2·42 ml/min) Solvaflex pump tube. This enables a wash of 50% methyl cellosolve to be pumped through the autoanalyser analytical system between chromatograms. Hydrazine sulphate solution is connected with Solvaflex directly to a 0·056 in i.d. (1·06 ml/min) pump tube.

The light tracks on the programming unit control the valve pairs A and C, the sample loader and the analysis time. The period for each of these operations is controlled by blanking off the tracks with black Fablon strips. The relative positions are shown in Fig. 2. The pH gradient of the eluting buffer is controlled by scanning the rotating drum surface and the shape of the gradient is shown in Fig. 2. This gradient is reproduced by cutting out the corresponding curve in black Fablon, which is secured to the drum. The drum rotates once every 30 sec and as the scanning head moves along its axis the proportion of acidic and basic buffers is varied. The buffer is mixed by the magnetic stirrer as it is pumped into the sampler reservoir.

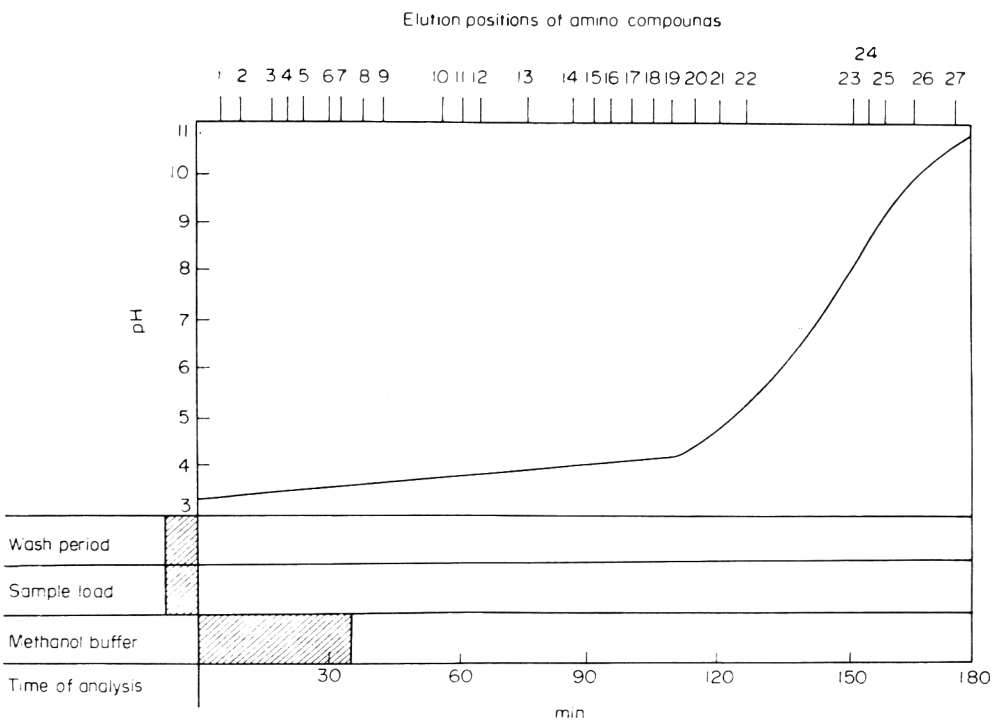

FIG. 2. Diagram of the elution programme used for the separation of amino acids by ion exchange chromatography. Key to the identification of the amino compounds: 1, cysteic acid; 2, phosphoethanolamine; 3, 4-hydroxyproline; 4, methionine sulphoxide; 5, aspartic acid; 6, threonine; 7, serine; 8, glutamic acid; 9, proline; 10, glycine; 11, alanine; 12, cystine; 13, valine; 14, methionine; 15, glucosamine; 16, isoleucine; 17, leucine; 18, norleucine (internal standard); 19, tyrosine; 20, phenylalanine; 21, γ-aminobutyric acid; 22, ε-aminocaproic acid (internal standard); 23, histidine; 24, lysine; 25, tryptophan; 26, ammonia; 27, arginine.

The ion exchange columns are packed with a slurry of three parts resin and one part buffer I. The slurry is poured into the column in sections and packed down using the high pressure pump. This is repeated with stirring until the resin bed is up to 0·5 in below the top of the column. The columns are thermostated at 60°C and the flow rate of 0·42 ml/min giving a pressure of 350 lb in^{-2}. Samples for analysis are made up in buffer I and placed in 2 ml cups in the sampler tray. The sample loader is set to load 0·6 ml of sample mixture for each chromatogram.

Preparation of high resolution ion exchange resins

Hamilton (1958) showed that resin particle size is an important factor and that a relatively restricted range is necessary for optimum column performance at high flow rates. The cost of uniform size ion exchange resin is high, especially for sizes up to 20 μm which are the most convenient for high-speed chromatography. However, a broad size range was obtained (Zeo-Karb 225 beads, 8% D.V.B. Permutit Co. Ltd) and fractionated by elutriation. Resin was separated by using a vertically supported V-shaped vessel with the sides straightened out for the top 10 cm (Fig. 3). Water was pumped through a sinter (porosity 2) with a peristaltic pump (Watson-Marlow type MHRE). A gradient of linear velocity was established with the minimum at the top of the vessel where the resin particles were discharged by overflowing into a sintered filter (porosity 4). The input flow rate was selected from a calibration graph calculated from the relationship between minimum linear velocity and particle radius (input flow rate, ml/min = 0·952 a^2 where a = radius of resin bead in microns). The size of each fraction

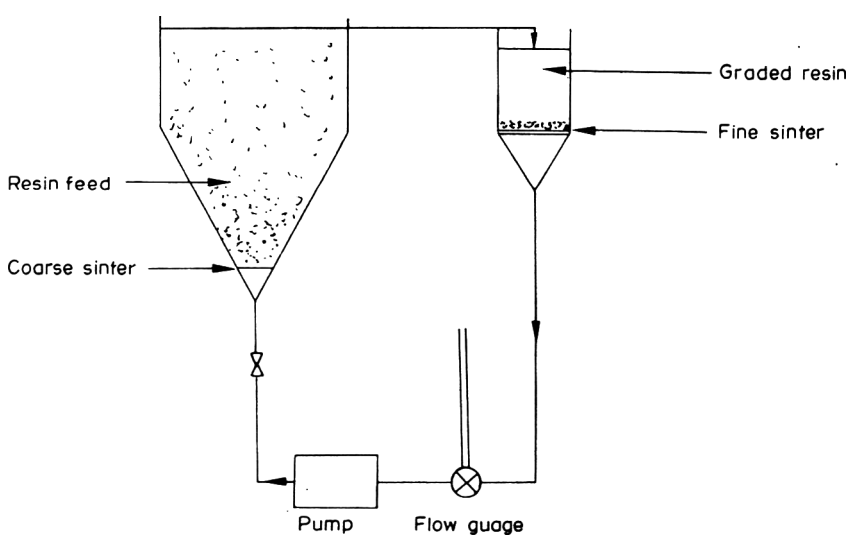

FIG. 3. Elutriation system for the production of high resolution ion exchange column packings.

was checked from enlarged photomicrographs and was found to agree closely with the calculated size. A batch of diameter 10 ($\pm$ 1) is used for chromatography.

Data acquisition system

The standard three-point recorder on the analyser was adapted so that the voltage could be sampled, by a data logger (Solartron Electronic Co. Compact logger LY 1470). An extra (retransmitting) slide wire is fitted to the recorder in such a way that it follows the movement of the existing slide wire and produces voltages in the range 0–100 mV. The selector switch mechanism in the recorder is adapted so that it can be used to control the rate at which voltage readings are taken from the slide wire. These modifications enable the recorder to be connected to a data logger and are described in more detail elsewhere (Davies & Watts, 1971). The data logger (Fig. 4) consists of a digital voltmeter punch drive unit and a paper tape punch and receives data from the analyser as follows.

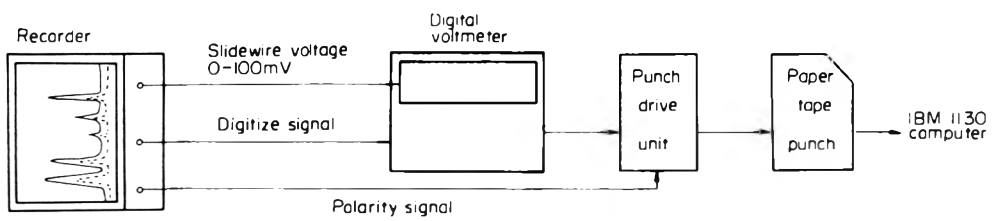

FIG. 4. Block schematic diagram of the data acquisition system used for amino acid analysis.

The voltage readings from the retransmitting slide wire correspond to the movement of the pen on the chart recorder as it traces out the chromatogram. The recorder produces a chromatogram from both amino acid analyses simultaneously. The digital voltmeter measures the voltage from the slide wire at a rate of one every 7·2 sec for each chromatogram and also translates the readings into a code suitable for processing by a computer (binary coded decimal). Next the punch drive unit sorts the readings from the digital voltmeter into a form suitable for punching out on tape. In addition it inserts a plus or minus symbol before the voltage reading depending upon the chromatogram to which it refers. The tape is processed by means of an IBM 1130 computer using a Fortran IV program (Taylor & Davies, 1973) which gives a print-out showing the area of each peak.

Results and discussion

The coefficients of variation for the elution times and norleucine ratios of each amino acid are shown in Table 1. The norleucine ratio was calculated by dividing the amino acid peak area by the peak area of norleucine, which is used as an internal standard.

TABLE 1. Reproducibility of twenty analyses of a standard mixture containing 10 μg/ml of each amino acid

Amino acid	Norleucine ratio		Elution time	
	Mean value	Coefficient of variation (%)	Mean value (min)	Coefficient of variation (%)
Aspartic acid	0·834	1·4	45·1	0·4
Threonine	0·949	1·6	52·7	0·5
Serine	1·122	2·1	57·5	0·7
Glutamic acid	0·759	2·2	65·9	1·1
Proline (440 nm)	0·165	3·5	72·5	0·4
Glycine	1·591	1·6	88·7	0·3
Alanine	1·377	2·5	92·6	0·3
Cystine	0·585	1·5	100·8	2·0
Valine	1·020	2·1	111·5	0·7
Methionine	0·792	2·1	125·2	0·8
Isoleucine	0·963	1·5	136·2	0·3
Leucine	0·913	1·5	141·3	0·3
Norleucine	1·000	—	145·7	0·2
Tyrosine	0·668	2·1	150·7	0·6
Phenylalanine	0·766	2·1	155·5	0·2
Histidine	0·775	2·7	184·1	0·2
Lysine	0·923	1·6	188·6	0·1
Arginine	0·903	3·2	203·8	0·3

Proline shows the largest variation in norleucine ratio as it is less sensitive to ninhydrin than the other amino acids, and also the absorption is measured at 440 nm compared to 570 nm in the case of the others. The variation in arginine is relatively high since the baseline varies at this point in the chromatogram.

The peak elution times are measured from the time at which the sample is loaded to the mid point of each peak. They are sufficiently consistent to enable the peaks to be identified automatically by a computer (Taylor & Davies, 1973).

The time for the complete analysis of one sample and the regeneration of the ion exchange column is 4 hr. During this time two samples are analysed simultaneously which enables twelve amino acid chromatograms to be produced in 24 hr. The rate of consumption of buffer solutions and reagents for each analyser system is about the same as that for the standard single column analyser, so that the usage per chromatogram is reduced by 80%. This leads to a saving in the time required for making up solutions as well as for the operation of the analyser. The buffer solutions for the pH gradient are simpler to make up. The three solutions give consistent pHs between batches and titration to adjust the pH is usually unnecessary. In case of analysers using the Varigrad

or step-wise elution, about six buffers are usually required and each has to be titrated to the appropriate pH. The use of shorter ion exchange columns makes this part of the system more compact and requires less ion exchange resin.

Amino acid analysers using automated sample loading and step-wise elution are commercially available but are relatively expensive. The system described by Ertingshausen, Adler & Reichler (1969) produces an amino acid chromatogram in 70 min using a two-column separation method. However, cysteic acid is not detected by this method, and γ-aminobutyric acid and the amino sugars overlap with tyrosine and phenylalanine, so that the system has limitations for the analysis of some foods such as egg and yeast protein.

Conclusions

The modifications described here have enabled the analysis rate to be increased and have reduced the tedium in the calculation of results. The addition of automatic sample loading plus data acquisition adds 40% to the cost of a two channel amino acid analysis system, whereas commercially available systems of this type cost up to three times as much, without automatic calculation facilities. A more economic use of the existing equipment has been made and also there is a saving in operator time and cost per analysis. In addition the system is quite flexible. The amino acid analyser is arranged in modules, which could be used for other autoanalyser determinations. The programming unit can be reset by the alteration of the positions of the blanking strips on the light tracks and by re-drawing the pH gradient curve on the rotating drum. Therefore it could be adapted for other column chromatographic analyses such as gel filtration.

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Migration of styrene monomer from packaging material into food. Experimental verification of a theoretical model

J. T. DAVIES

Summary

A standard equation for a diffusion process has been adapted to cover the conditions of certain simple extractability experiments in which components migrate from polymers into foods. Data obtained during previous work on the migration of styrene monomer from polystyrene into foods have been re-examined in order to confirm the validity of the adapted equation. A computer was used to assist in evaluating the constants of the diffusion process using the derived mathematical expression. It was possible to relate the change in the diffusion coefficients to the interaction of certain foods with the polymer.

Introduction

Many different types of foods are currently stored and sold in polymeric (i.e. plastic) packaging materials. The polymeric material usually consists of the base polymer, of very high molecular weight, dispersed or dissolved in which are smaller amounts of lower molecular weight components such as additives and oligomers. The additives e.g. antioxidants or antistatic agents are deliberately added to improve the properties of the polymer. The use of such polymeric systems are controlled by laws and/or codes of practice, which may require extractability tests. These tests are often done with raised temperatures to accelerate migration, and may involve solvents such as heptane rather than a real food. Relatively little data has been published to explain quantitatively the interaction of food and polymer. The various interactions and resultant migrations of these lower molecular weight components into foods that might be possible are listed by Katan (1971) while Garlanda & Masoero (1966) and Knibbe (1971) consider theoretically the contact of polymers with aqueous and fatty foods respectively.

Model systems that are devised to examine the migration of substances from polymeric packaging materials into foods can be very complex. The parameters involved include the diffusion coefficients of the migrant in both the polymer and the food. The diffusion coefficient in the polymer may not be constant if there is migration of a food component into the polymer and both diffusion coefficients will vary with temperature

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changes due to both filling of containers or changing storage conditions. There have been various approaches to the mechanisms of such migrations.

This paper examines in detail a standard mathematical expression derived from Fick's Laws of diffusion (Crank, 1957) that is applicable to certain simple extractability models. For this expression to hold the migrating material is assumed to move freely in an extractant of limited volume and this extractant does not migrate into or react with the polymer. This means that the rate controlling stage is diffusion within the polymer.

In order to see if the derived equation could be verified experimentally, and to detect any anomalies when the conditions defined above did not hold, data from extractability studies was required. Data obtained when extractions were done for reasons of biological safety was not very useful. As described above this usually related to exaggerated or accelerated storage for a single fixed time. Recent taint and odour studies in the author's laboratory and partially reported in conjunction with the Monsanto Chemical Co. (Cartwright, Davies & Phillips, 1970) have provided many data concerning the migration of styrene monomer from polystyrene into a wide range of real and model foods.

This data is used in this paper to produce 'best fit' curves for the equation derived below.

Experimental

Polystyrene sheets of appropriate dimensions were immersed in the real or model foods at constant temperature, and the extractant was analysed at intervals for styrene monomer. The analytical methods used varied according to the extractant, but all depended upon a gas chromatographic estimation of styrene. These methods have been described by Davies & Dunn (1973). The final equilibrium concentration was not measured, since this was not required for the original aims of the experiments. In some cases it would not have been possible to establish, because the food would have deteriorated before equilibrium was set up. For all of these experiments the 'polystyrene sheets' consisted of pieces of injection moulded beakers stored in contact with the extractant in stoppered flasks.

Mathematics

A solution of Fick's diffusion equation with the relevant boundary conditions for the absorption or desorption of a diffusing material between a solid sheet and a 'stirred' solution of limited volume is given by Crank (1957).

$$M_t = M_{\text{inf}}\,(1+b)\,(1-\exp\,(T/b^2)\,\text{erfc}\,(T/b^2)^{1/2}) \qquad (1)$$

T $= Dt/l^2$;
b $= a/Kl$;
D = diffusion coefficient of the material in the sheet;
t = time;
l = half thickness of sheet;

a = half apparent thickness of solution, i.e. a = vol. of solution/surface area of both surfaces of sheet;

K = partition factor, i.e. concentration just within the sheet is K times that just outside the sheet;

erfc is the complement of the error function, i.e. a mathematical term.

M_t = Mass of material that has migrated after time t;

M_{inf} = mass of material that has migrated after infinite time;

M_t and M_{inf} are expressed as a concentration (i.e. in ppm).

A more detailed description is given by Carman & Haul (1954) who offer this equation as a possible means of measuring diffusion coefficients. For food in contact with a polymer, this equation might be expected to be valid if two conditions hold; first the migrating material is always evenly dispersed in the food, by stirring or rapid diffusion. Secondly there is no interaction between the food and the polymer (e.g. fat or other components of the food did not diffuse into the polymer and change the diffusion coefficient of the material within the polymer during the migration process). As explained in the introduction, the best data available to verify this equation and to find its limitations was that concerning the migration of styrene monomer from polystyrene into a range of real foods. The equation may be modified as follows for this case.

For the conditions of these particular experiments b was small compared with 1 and thus the term $1+b$ could be taken as unity. M_{inf} and K were not known for each experiment and would have been difficult or impossible to measure—particularly with short shelf life foods. As the amount of styrene monomer migrating was small compared with the amount remaining in the polymer, the following relationship was used:

$$K = \frac{S}{M_{\text{inf}}} \tag{2}$$

where S = ppm of styrene monomer in the polystyrene (for convenience it is expressed as % on the figures).

Thus equation (1) becomes:

$$M_t = M_{\text{inf}} \left\{ 1 - \exp \frac{Dts^2}{{M_{\text{inf}}}^2 a^2} \operatorname{erfc} \left(\frac{Dts^2}{{M_{\text{inf}}}^2 a^2} \right)^{1/2} \right\}. \tag{3}$$

D and M_{inf} are unknown constants for each experiment and there is no way in which a function of M_t (the styrene concentration of the food) could be plotted against t to produce a straight line to confirm the validity of (3), or to evaluate D and/or M_{inf}. Thus a curve fitting technique was required to see how well experimental data would fit equation (3). This was done as follows.

When the known constants on the right-hand side of equation 3 were inserted, together with values for the unknowns D and M_{inf} M_t could be calculated on an interactive computer for any value of t. In order to check the accuracy of the computer in handling the extreme values encountered in the exponential and error function terms,

these terms were also evaluated manually using mathematical tables. For all the given values of $Dts^2/M_{inf}{}^2a^2$ the computer obtained the correct exponential figure, but the error function term had an error of +0·5% at $Dts^2/M_{inf}{}^2a^2 = 9$, and this error increased progressively. When $Dts^2/M_{inf}{}^2a^2 = 28{\cdot}5$, the error in the erfc term was +370%, resulting in M_t being only 68% of the true figure. The computer subroutine used for calculating error functions was modified by introducing the constants used by Abramowitz & Stegun (1965). It was of interest to use this equation to demonstrate the effect of varying one of the parameters on the shape of the curve of M_t plotted against t. Figure 1 shows a model system with curves for various values of D.

Probably of more practical interest was the calculated effect of varying the volume of extractant/surface area ratio. This is shown for a range of values in Fig. 2.

The curve fitting was done by a manual process using the programme described above. A value for M_{inf} was selected and by manual hunting the best value of D was obtained, i.e. when at least one of the computer calculated values for Mt equalled the

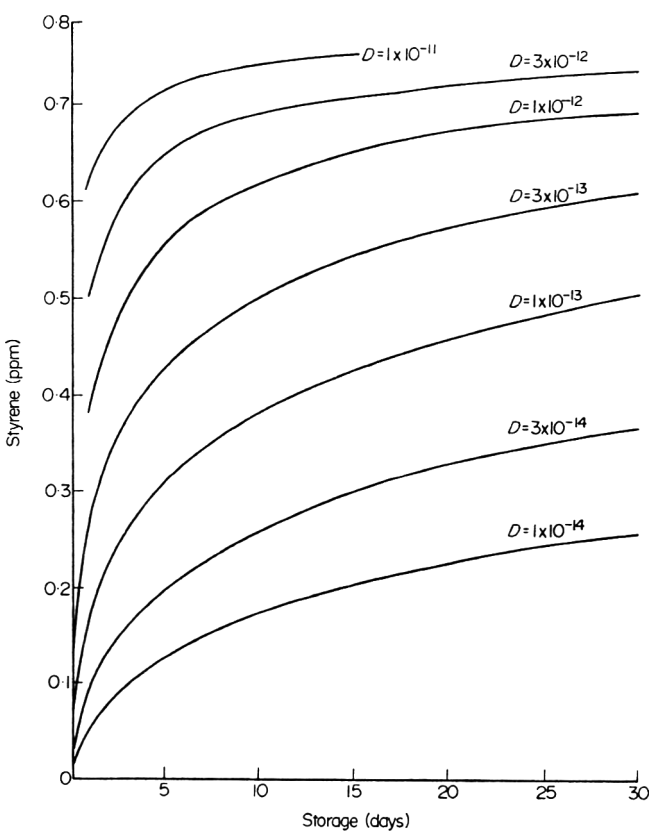

FIG. 1. An example of the effect of varying D. Values $M_{inf} = 0{\cdot}8$ ppm; $S = 0{\cdot}2\%$; $a = 1{\cdot}3$ cm.

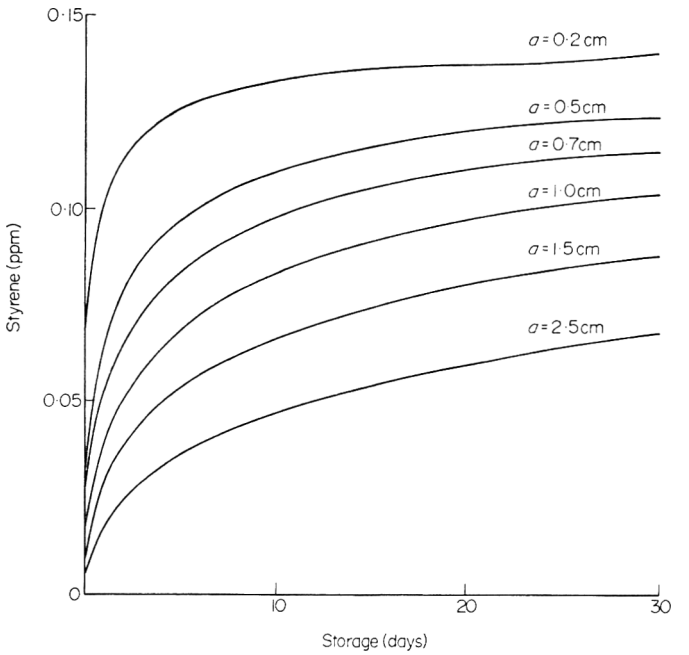

FIG. 2. An example of the effect of varying the volume/surface ratio, i.e. '*a*'. Values $M_{inf} = 0{\cdot}15$ ppm; $S = 0{\cdot}15\%$; $D = 1 \times 10^{-14}$.

corresponding experimental value. M_{inf} was varied until a value of D was obtained that produced a curve that fitted all of the experimental data, if indeed it was possible. No attempt was made to automate the curve fitting procedure although the computer used could have handled some kind of iterative process.

Results

Three of the groups of experiments from the recent taint and odour studies on polystyrene provided suitable data. In each group the amount of styrene monomer migrating from polystyrene into water was measured and the amount migrating into foodstuffs under similar conditions was measured. Within each group similar polystyrenes were used with up to three different levels of styrene monomer.

In the first group, water was compared with single and double cream at 5°C. The fat contents of the creams were 19·5% and 53% respectively and both were oil in water emulsions. Toughened polystyrene with monomer levels of 0·05%, 0·15% and 0·25% was used. The polymers were experimental grades provided by the Monsanto Chemical Company. The results for the water extractions are shown in detail in Fig. 3. The best fit curves are the continuous lines and the experimental data is shown as discrete points. The estimated values of D and M_{inf} are shown on the figures.

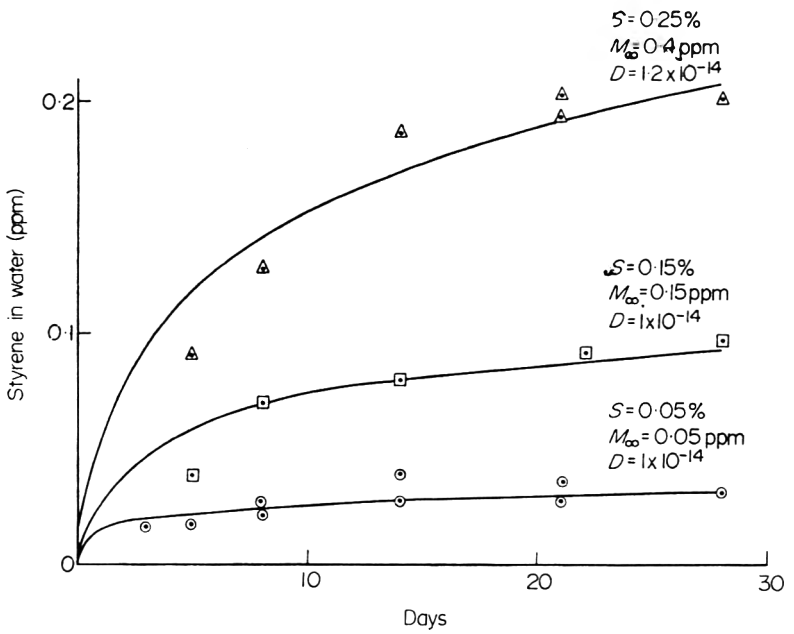

FIG. 3. Toughened polystyrene stored in contact with water at 5°C. Polymer with three different monomer levels was used. The estimated values of D and M_{inf} are given ($a = 1{\cdot}3$ cm) The points are experimental data and the continuous lines are best fit curves.

The second group of experiments compared water and a 1 : 2 sunflower oil/soft margarine mixture stored in contact with crystal polystyrenes of three monomer levels (0·41%, 0·24% and 0·07%) at 20°C. The results of the water extractions are shown in Fig. 4. These were also experimental grades of Monsanto polymers.

The third group compared orange juice diluted as a 'ready to drink' squash and water, stored in contact with a toughened polystyrene at 20°C. (This was a different grade to that used for the cream experiments.) The results are shown in Fig. 5. When attempts were made to use the values of D obtained from the aqueous extractions to fit curves to the cream and oil/margarine mixtures, by varying only M_{inf} no fits could be obtained. (See Fig. 6 for an example.) This indicated that D had altered when the polystyrene was in contact with these systems. Indeed, curve fitting arbitrarily on the basis of equation 3 showed that to fit the data, values of D ten-fold greater than the values for the aqueous extractant were required for the creams and approximately one hundred-fold greater for the oil/margarine would be required. These values have little quantitative significance, but do indicate that when the product contains fatty material that may interact with the polymer, diffusion coefficients of the migrating substance will increase, as expected.

With the orange squash/water experiments fits can be obtained for both extractants using the same value of D. This indicates that the increased extraction of styrene into

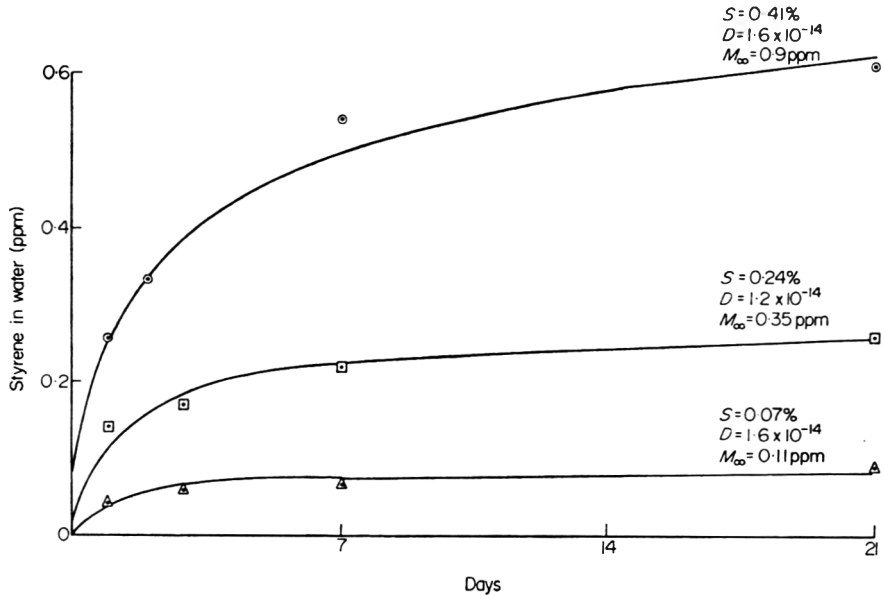

FIG. 4. Crystal polystyrene stored in contact with water at 20°C—three monomer levels. The estimated values of D and M_{Inf} are given ($a = 0{\cdot}50$ cm). The points are experimental data and the continuous lines are best fit curves.

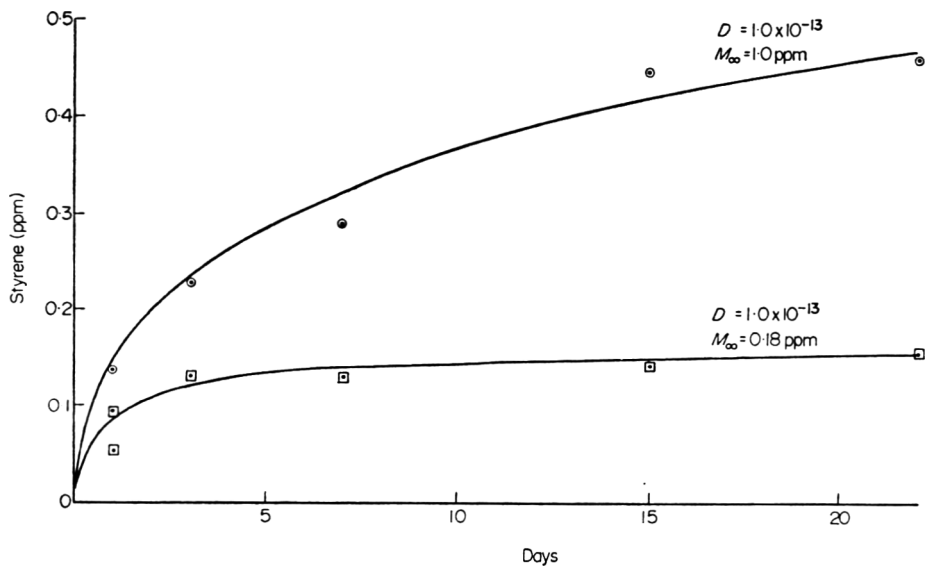

FIG. 5. Toughened polystyrene—one monomer level stored in contact with water (□) and diluted orange squash (○) at 20°C. The points are experimental data and the continuous lines best fit curves. Estimated values of D and M_{Inf} are given ($S = 0{\cdot}11\%$; $a = 0{\cdot}69$ cm).

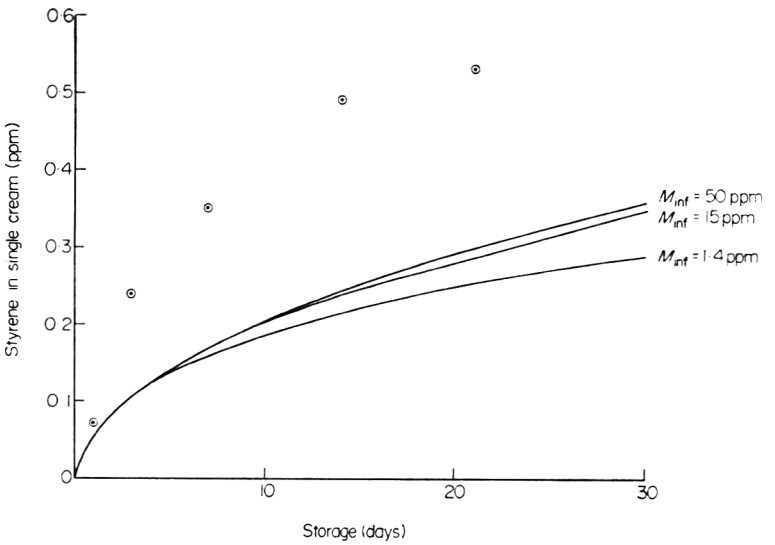

FIG. 6. Storage of single cream in contact with toughened polystyrene. The points are experimental data. The continuous lines are attempts at curve fitting using the value for D, i.e. $1{\cdot}2 \times 10^{-14}$ obtained from water storage experiment. There is no value of M_{inf} that will produce a fit.

the orange squash is due to a higher solubility of the styrene in orange squash rather than interaction of polymer and orange oil. This is an unexpected result as the anomalous behaviour of orange juice usually observed in extractability experiments has been attributed to the penetration of the polymer by the orange oil.

Conclusions

An equation has been adapted to cover the migration of some substances from polymeric materials into food.

It has been shown that the experimental data from certain extractability experiments with aqueous foods will fit the derived equation. This does not prove absolutely that this is the correct equation for this particular model, but provides confirmation. Further confirmation could be obtained if values of D and M_{inf} were obtained from independant experiments and were similar to the derived values. Also more complex experiments could probably be devised in which the other parameters i.e. S and a were plotted in turn against M_t with t constant and a similar curve fitting technique applied.

The limitations of this equation using fatty foods have been shown.

Acknowledgment

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Kinetic considerations of the permeation of organic vapours through a flexible packaging film

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Summary

A new method is presented for measuring the rates of permeation of organic vapours through flexible packing films with the specific intent of studying flavour deterioration from packaged foodstuffs. The method is convenient, relatively rapid, quantitative and the results are presented as first order rate constants for the permeation of individual chemicals through a film. This allows ready calculation of such potentially useful data as the temperature effects on permeation rates, the half-life of retention of a vapour by a film barrier or the energy necessary for permeation through a particular film. As an example of the method, data for the permeation of eight organic compounds through 50 μm polyethylene film over the temperature range -25°C to $+35$°C are given as well as the half-lives of retention of these compounds and the permeation energies required. The permeability constants may also be readily calculated from the rate data.

Introduction

The flavour or aroma of a food product is comprised of a particular and often delicate balance of organic chemicals. Since flexible films are extensively used for food packaging, a knowledge of the protection these films provide is of great concern to both food research and industry. In our laboratories and in others, it has been common practice to collect fresh foods in season, preserve them by methods such as freeze drying, and store them at low temperature in flexible film packages until the flavour and other qualities have been investigated. It therefore becomes natural to wonder about the possible flavour deterioration during storage either by loss of flavour contributors or contamination by foreign chemicals which may be present in the storage atmosphere. It is also of obvious interest to food packagers that their products reach the consumer with as near a natural fresh flavour as possible.

The permeation of flexible films by pure gases and water vapour has been studied extensively. Unfortunately, film permeation by organic vapours has received only

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limited attention. A variety of permeation cells have been used, but most of these have been designed to measure liquid transport or maintain a pressure difference across the membrane. The studies presented thus far vary in sophistication from measuring the weight loss of liquid filled polyethylene bottles by Bent & Pinsky (1955) to the use of radioactive tracers in liquid transport by Coughlin & Pollak (1969). Hoffmann, Kramer & Linowitzki (1965) also used a radiometric technique for the permeation of labelled volatile compounds. Wientjes, Maarse & Van Straten (1967) studied the permeation of coffee volatiles, entraining the permeate with a nitrogen flow and collecting it on a precolumn for subsequent analysis. Smit (1968) used the same system to study the permeation of aroma from fruit juices. They were able to observe only changes in the ratios of permeating compounds and no quantitative permeation data were measured. Popovskii, Lipis & Soboleva (1968) measured the relative permeation of some fruit aroma components, but again only ratios were determined and there was no method of calculating temperature dependence. Laine & Osburn (1971) have recently measured the weight gain of silica gel filled polyethylene bags suspended in organic vapours and attempted a correlation with Hildebrand solubility parameters.

A practical and convenient method for determining permeability of flavour components through flexible films should fulfill all of the criteria outlined below.

(1) The data must be quantitative and reproducible.

(2) The procedure must be relatively rapid so that repeated determinations can be made in relatively short time.

(3) The apparatus must be inexpensive or normally found in a research or development laboratory.

(4) The technique must be of such a nature that a technician can easily obtain and handle the data.

(5) An isostatic condition must be maintained at all times during measurements to assimilate the conditions normally found in packaged foods.

(6) The data must be presented in such a form that comparative evaluation of films may be readily accomplished.

(7) The data must be of such a nature that meaningful interpretations of temperature variation and energy required for permeation may be made.

The method described below appears to comply with these requirements.

Description of method

Permeation cell

The peremation cell was constructed of two modified flange-lipped belljars of 1 l capacity each. This size jar appeared to be optimum since intimate mixing of components was not good in a larger (2 l) jar and repeated sampling could affect the concentration in a smaller unit. To the side of each jar a suitable opening was made for the attachment of a teflon valve and septum inlet. A similar teflon septum valve is

commercially available under the trade name 'Mininert', but offers the disadvantage of short septum life with repeated penetration. The teflon valve reduces the possibility of loss of any component due to absorption into or permeation through the silicon rubber septum material.

Two concentric 45° angle cut teflon rings were constructed to hold the test film between the flanges of the belljars. These teflon rings provide a positive seal for the film to separate the two chambers of the cell and provide a seal for the edge of the film to the outside of the cell. This eliminates the possibility of loss of components from the cell due to permeation through the film edge. The flange lips of the jars, with the teflon rings and film in place, are then tightly held together with screw type metal clips.

The test film inside the cell has an exposed circular surface with a diameter of 10·5 cm and a total exposed surface area of 86·6 cm². Figure 1 depicts the assembled permeation cell and components.

General experimental procedure

The cell is assembled as described above with the film to be tested in place. Both chambers of the cell are flushed with purified N_2 before the septum inlets are secured. An appropriate sample of a chemical reference standard is then injected into each

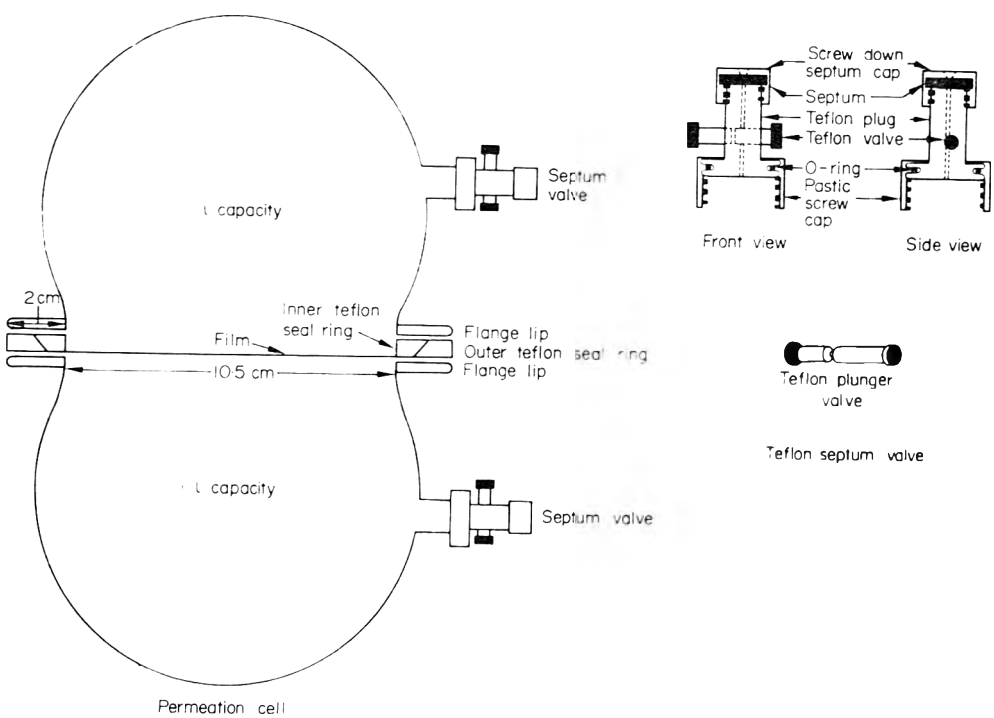

FIG. 1. Diagram of permeation cell and components.

compartment through the septum inlets. The reference standard is necessary to assure a quantitative estimation of the relative concentration of the chemicals whose permeation rates are to be measured. The cell is then allowed to equilibrate in a constant temperature environment. When equilibrium has been obtained, an appropriate sample of the chemical or mixture of chemicals to be tested is introduced into one chamber (A) through the septum. The amounts of sample and reference are such that they exist entirely in the vapour state inside the cell.

Gas samples are withdrawn from time to time from each chamber and analysed by gas chromatography. The syringe used for sampling is flushed with N_2 then placed in an evacuated drying oven at 50°C between sampling. An amount of N_2 identical to the amount of sample taken is injected into the chamber each time a sample is withdrawn, to ensure the isostatic condition on the film.

Thus the disappearance of the vapour from the initial chamber (A) and the appearance of the vapour in the other chamber (B) is measured with respect to time and may be graphically represented.

There exists a possibility of absorption or adsorption of the compounds tested on the walls or film within the chamber causing noticable concentration changes and thereby affecting the accuracy of the kinetics. To assure that this is not the case, one can check to see that the total vapour measured, A + B, remains constant throughout the measurement period. The data have shown that A + B is constant for the compounds listed in this paper with 50 μm polyethylene film. It is suggested that a similar check be made whenever new compounds or films are used.

Mathematical considerations

The permeation process was considered mathematically analogous to a reversible first order chemical reaction. Two model systems appeared as likely possibilities for describing the transfer of material from one side of the film to the other. The first of these is an

$$\begin{array}{ccccc} & \overset{k_a}{\rightarrow} & & \overset{k_d}{\rightarrow} & \\ A & & F & & B \\ & \underset{k_b}{\leftarrow} & & \underset{k_c}{\leftarrow} & \end{array} \qquad (1)$$

equilibrium where A and B are the relative concentrations of the chemical in the respective chambers, F is the relative concentration in the film, and k_a, k_b, k_c and k_d are the respective rate constants. This model system could describe the process if, after the film becomes saturated, it contains a significant amount of the permeating material. However, though consistent values for k_a and k_c could be measured, values for k_b and k_d were not reproducible. Thus this model was considered invalid, and it seems unlikely that it would become valid for the situation of packaged foods.

Another simpler equilibrium system

$$A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} B \tag{2}$$

where A and B have the same meaning as above, and k_1 and k_2 are the respective rate constants, was then considered. Physically that is to say that the film acts as an 'energy barrier' in the transfer process. If it is assumed that $A+B=A_0=$ the initial amount of a particular chemical injected, and further that from the symmetry of the permeation process, i.e. the same chemical permeating the same film from opposite directions, that $k_1=k_2$ then it may be shown that

$$\frac{A}{A_0} = \tfrac{1}{2}\exp(-2k_1t)+\tfrac{1}{2} \tag{3}$$

$$\frac{B}{A_0} = -\tfrac{1}{2}\exp(-2k_1t)+\tfrac{1}{2} \tag{4}$$

$$\frac{A-B}{A_0} = \exp(-2k_1t) \tag{5}$$

$$\log_e (A-B) = -2k_1t+\log_e A_0. \tag{6}$$

Thus a graph of the natural logarithm of $(A-B)$ plotted against time should yield a straight line with a slope of $-2k_1$.

In practice the general procedure has been to take the measurements as described above and prepare graphs of A versus time and B versus time for each compound. From a suitable section of these curves, corresponding values for A and B at various times are graphically determined. Then a plot of $\log_e (A-B)$ versus time is made and from the slope of this straight line graph the value of k_1 is determined. An example of such graphs for the permeation of *n*-butyl acetate through 50 μm polyethylene film at 25°C appears in Fig. 2.

Only a relatively small portion of the curves are necessary to obtain reproducible data and therefore only a relatively short period of time is necessary to get a complete kinetic description of the permeation process, even for slowly permeating material.

It has generally been the custom to report permeation data in terms of a permeability constant, P, which is defined as

$$P = \frac{\text{moles}}{\text{time}} \times \frac{\text{standard molar volume}}{} \times \frac{\text{film thickness}}{\text{film area}} \times \frac{1}{\text{vapour pressure}} \times 10^9 \tag{7}$$

and P has the units (std cm^3) (cm)/(sec) (cm^2) (cm Hg). The permeability constants may be calculated from the first order rate constants of permeation in the following

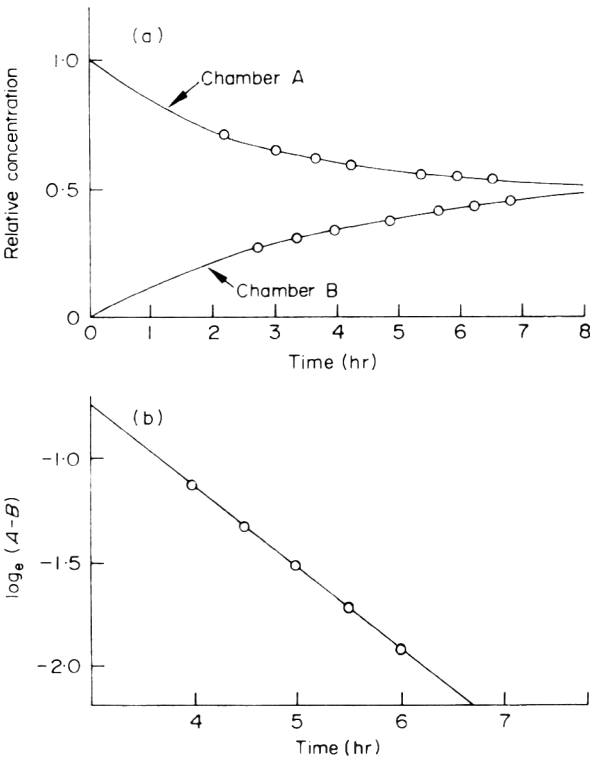

FIG. 2. (a) Graph showing typical curves for the loss of vapour from chamber A and gain of vapour in chamber B for the permeation of *n*-butyl acetate through 50 μm polyethylene film at 25°C. (b) Straight line graph of $\log_e (A-B)$ versus time for the permeation of *n*-butyl acetate through 50 μm polyethylene film at 25°C.

manner. Assume the situation of the permeating vapour in equilibrium with liquid on one side of the membrane passing into an open atmosphere on the other side at a rate defined by the measured first order rate constant. The concentration of the vapour in the initial chamber remains constant and the number of moles of vapour may be estimated by

$$n_0 = \frac{P^0 V}{RT} \tag{8}$$

where n_0 = number of moles of vapour within the chamber, P^0 = vapour pressure, V = volume of chamber, R = gas constant and T = temperature in °K. The number of moles of material passing through the film to the atmosphere is then given by the first order expression

$$n = n_0 (1 - \exp(-k_1 t)) \tag{9}$$

where n = number of moles permeating the film. Combining equation (8) and (9), and

calculating the amount of material entering the atmosphere in 1 sec leads to

$$\frac{n}{\text{sec}} = \frac{P^0 V}{RT}\,\frac{(1-\exp(-k_1))}{t} \tag{10}$$

where n/sec = moles sec^{-1} permeating the film, k_1 = first order rate constant in sec^{-1}, and $t = 1$ sec. If the volume of the chamber is assumed to be 1 l and the vapour pressure is in atmospheres, then R has the units litre-atm/deg-mol. The expression for the permeability constant P can be derived from equations (7) and (10).

$$P = \frac{V}{RT} \times \frac{(1-\exp(-k_1))}{t} \times \frac{\text{std cm}^3}{\text{mole}} \times \frac{L}{A} \times \frac{1}{76} \times 10^9 \tag{11}$$

where $V = 1$ l, $t = 1$ sec, std cm^3/mole = standard molar volume at the proper temperature, L = film thickness (cm), A = film area in cm^2, and $\frac{1}{76}$ = atm/cm Hg.

Temperature dependence

If the first order rate constant for a particular compound and film are measured for at least three temperatures, an Arrhenius plot ($\log_e k_1$ versus $1/T$ or $\log_e P$ versus $1/T$) may be constructed and the rate of permeation at any temperature where it is assumed the Arrhenius equation is valid may be extrapolated.

Thus a knowledge of the permeation rates over the complete range of food handling and storage temperatures may be readily obtained. Also the activation energy of permeation (*Ep*, analogous to the activation energy of a chemical reaction) may be calculated and these energies necessary for permeation could be an excellent criteria for evaluating a film.

Other information obtainable from rate constants can be calculated as particular needs dictate. An interesting item among these is the half-life for retention of a vapour by a film barrier. This assumes that the permeation proceeds essentially in only one direction and could well approximate a vapour escaping from a package to the atmosphere.

The half-life of retention $t_{1/2}$ may be calculated from the expression

$$t_{1/2} = \frac{0{\cdot}693}{k_1}. \tag{12}$$

Experimental and results

As an example of the method, the results of the study of the permeation of eight organic vapours through a commercial polyethylene film of 50 μm thickness are reported here. Runs were made on several of the chemicals individually and in groups of four or five components at a time. The rate constants obtained from a particular vapour did not vary significantly from run to run. The internal standard was in each case 0·5 μl of

TABLE 1. First order rate constants ($k_1 \times 10^2$ hr^{-1}) for permeation through 50 μm polyethylene film

(T°C)	Acetone		2-Butanone		2-Pentanone		2,4-Dimethyl-3-pentanone		1,4-Dioxane		Ethyl acetate		*i*-Butyl acetate		*n*-Butyl acetate	
	obs.	calc.	obs.	calc.	obs.	calc.	obs.	calc.	obs.	calc.	obs.	calc.	obs.	calc.	obs.	calc.
35	2·93	2·98	7·84	7·96	14·6	14·4	14·2	14·2	13·3	12·8	8·80	8·65	15·7	15·4	41·8	39·3
25	1·50	1·45	4·18	4·08	7·68	7·84	6·73	6·75	5·91	6·38	4·77	4·93	8·05	8·34	20·4	23·0
15	0·660	0·671	1·98	2·00	4·13	4·08	3·05	3·05	3·15	3·04	2·74	2·70	4·40	4·33	13·7	12·9
5	—	0·293	—	0·934	—	2·03	—	1·30	—	1·37	—	1·41	—	2·14	—	6·99
0	—	0·190	—	0·625	—	1·40	—	0·829	—	0·899	—	1·01	—	1·48	—	5·05
−5	—	0·121	—	0·412	—	0·958	—	0·520	—	0·582	—	0·706	—	1·00	—	3·61
−15	—	0·0464	—	0·170	—	0·426	—	0·194	—	0·231	—	0·334	—	0·444	—	1·77
−25	—	0·0165	—	0·0655	—	0·178	—	0·0668	—	0·0853	—	0·149	—	0·184	—	0·818

benzene in each chamber of the cell. The amount of sample used was 1 μl per component. The experiments were conducted in the manner described above. Slopes and intercepts of straight line graphs were determined by the method of least squares.

Gas chromatography was done on a Perkin-Elmer model 990 instrument with an FID detector and the peak areas were measured with a Perkin-Elmer model D-26 electronic integrator. An analytical column 3 mm × 3 m was prepared using 10% di-*iso*-decylphthalate stationary phase on Chromosorb P 80/100 mesh solid support. The carrier gas was N_2 and carrier flow and column temperature were adjusted to give good base line separation between all components on an individual run.

TABLE 2. Relative rates of permeation through 50 μm polyethylene film (with acetone vapour at −25° as 1·00)

T (°C)	Acetone	2-Butanone	2-Pentanone	2,4-Dimethyl-3-pentanone	1,4-Dioxane	Ethyl acetate	*i*-Butyl acetate	*n*-Butyl acetate
35	180·6	481·2	873·7	860·6	775·8	524·2	933·3	2382·0
25	87·9	247·3	475·2	409·1	386·7	298·8	505·5	1394·0
15	40·7	121·2	247·3	184·8	184·2	163·6	262·4	781·8
5	17·8	56·6	123·0	78·8	83·0	85·5	129·7	423·6
0	11·5	37·9	84·8	50·2	54·5	61·2	89·7	306·1
−5	7·33	25·0	58·1	31·5	35·3	42·8	60·6	218·8
−15	2·81	10·3	25·8	11·8	14·0	20·2	26·9	107·3
−25	1·00	3·97	10·8	4·05	5·17	9·03	11·2	49·6

TABLE 3. Half-life (hr) for permeation through 50 μm polyethylene film

T(°C)	Acetone	2-Butanone	2-Pentanone	2,4-Dimethyl-3,-pentanone	1,4-Dioxane	Ethyl acetate	*i*-Butyl acetate	*n*-Butyl acetate
35	23	8·7	4·8	4·9	5·4	8·0	4·5	1·8
25	48	17	8·8	10	11	14	8·3	3·0
15	103	35	17	23	23	26	16	5·4
5	237	74	34	53	51	49	32	9·9
0	365	111	49	84	77	69	47	14
−5	573	168	72	133	119	98	69	19
−15	1490	407	163	357	300	207	156	39
−25	4200	1058	390	1037	812	465	376	85

TABLE 4. Permeability constants calculated for permeation through 50 μm polyethylene film

$$P = \frac{(\text{std, cm}^3)\ (\text{cm})}{(\text{sec})\ (\text{cm}^2)\ (\text{cm Hg})} \times 10^9$$

T(°C)	Acetone	2-Butanone	2-Pentanone	2,4-Dimethyl-3-pentanone	1,4-Dioxane	Ethyl acetate	*i*-Butyl acetate	*n*-Butyl acetate
35	6·33	16·9	30·6	30·2	27·2	18·4	32·7	83·5
25	3·08	8·67	16·6	14·4	13·6	10·5	17·7	48·7
15	1·43	4·25	8·68	6·49	6·46	5·73	9·20	27·4
5	0·625	1·98	4·32	2·77	2·92	3·01	4·56	14·8
0	0·405	1·33	2·99	1·77	1·92	2·14	3·15	10·7
−5	0·258	0·873	2·04	1·11	1·24	1·51	2·14	7·63
−15	0·099	0·361	0·909	0·414	0·493	0·714	0·950	3·73
−25	0·035	0·138	0·379	0·143	0·182	0·319	0·394	0·546

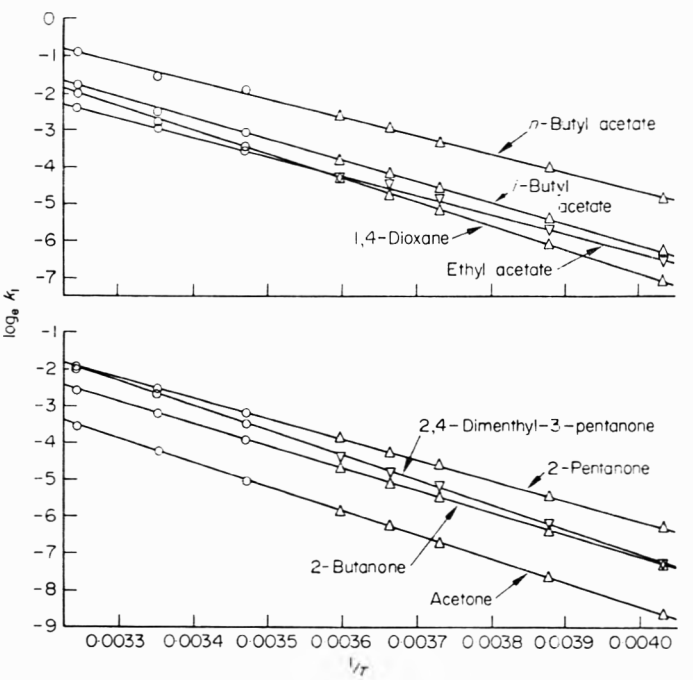

FIG. 3. Arrhenius temperature dependence graphs ($\log_e k_1$ versus $1/T$) for the permeation of various organic vapours through 50 μm polyethylene. ○, Measured points; △, calculated points.

TABLE 5. Activation energies of permeation and pre-exponential factors for the permeation of organic vapours through 50 μm polyethylene

Compound	Ep (Kcal/mol)	Pre-exponential factors	
		$\log_e S$	$\log_e Po$
Acetone	13·1	17·967	23·309
2-Butanone	12·1	17·295	22·677
2-Pentanone	11·1	16·238	21·571
2,4-Dimethyl-3-pentanone	13·6	20·194	25·552
1,4-Dioxane	12·7	18·647	23·996
Ethyl acetate	10·3	14·339	19·670
i-Butyl acetate	11·2	16·431	21·57
n-Butyl acetate	9·8	15·072	20·462
	$K_1 = S\exp(-Ep/RT)$	$P = Po\exp(-Ep/RT)$	

The temperature of a run was controlled within ± 1 °C in an oven. For lower temperature measurements the entire oven was placed in a constant temperature cold room.

The first order rate constants measured and calculated for these compounds appear in Table 1. The relative rates of permeation (relative to the slowest permeating compound, acetone at -25°C) appear in Table 2. The half-lives in hours for the permeation of the various compounds at the temperatures indicated appear in Table 3.

In Table 4 is a list of the permeability constants calculated from the rate constants. The Arrhenius temperature dependence graphs for each of the compounds appear in Fig. 3, and the activation energies of permeation as well as the pre-exponential factors are listed in Table 5.

The trends expected from theories on permeation, such as increasing permeation with increasing non-polar character and decreasing permeation with branching, are evident from the data. However, no speculation on these type inferences will be made in this paper. It should be noted that part of the data in Table 1 as well as the calculated data of Tables 2–4 has been obtained by interpolation or extrapolation of graphs such as Fig. 3. Since the possibility of passing transition points is always present with extrapolated data, one must take care not to over interpret the results for predicting trends.

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Dielectric loss as a parameter for the study of polymorphism in trierucin

S. PARKASH AND J. M. V. BLANSHARD

Summary

The temperature dependence of the dielectric loss of trierucin has been used to confirm the existence of five polymorphic forms. Comparable studies of the temperature dependence of the permittivity were less informative.

Introduction

The exact number of polymorphic forms of trierucin and their melting points have been the subject of conflicting reports by Malkin (1954) and Chapman (1965). Recently, Hagemann & Tallent (1972) have reinvestigated the problem by differential scanning calorimetry and have concluded that trierucin possesses five polymorphic forms, the α_L-crystal form melting at 12·0°C and not at 6·0°C as previously reported.

Little effort has been devoted to the use of dielectric measurements for the study of polymorphism in triglycerides other than that reported by Crowe & Smyth (1950, 1951). Their investigations were confined to an examination of the temperature dependence of the permittivity of a limited number of monoacid and diacid triglycerides. This communication reports a study of the temperature dependence of the dielectric loss as a technique for examining the polymorphic behaviour of triglycerides generally and trierucin in particular.

Experimental

Materials

Glycerol (Analar grade) was redistilled over calcium hydride at 1 mm pressure and directly esterified at 125°C with zone-refined erucic acid (purity > 99·95% by GLC) in the presence of *p*-toluene-sulphonic acid as catalyst at 0·5 mmHg pressure. The resultant trierucin was demonstrated by GLC and TLC to have a purity of greater than 99·9%. The ir spectrum of a liquid film of the trierucin showed no absorption band near 963 cm^{-1} indicating the absence of any trans isomer.

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Methods

The permittivity ϵ' and loss factor ϵ'' were measured using a three terminal dielectric cell jacketed for temperature control (designed and constructed in these laboratories) and a Wayne Kerr Universal Bridge (type B221) provided with an internal detector; these were used in conjunction with an external source (Marconi R-C Oscillator type TF 1370 A) at 7 kHz such that the capacitance and conductance could be measured to an accuracy of 0·1% and 0·2% respectively. The temperature of the material in the cell was ascertained to an accuracy of $\pm 0{\cdot}1$°C with a calibrated copper-constantan thermocouple.

The α_L-form of the pure trierucin was prepared in the dielectric cell by rapid chilling of the melt (about 8 g at 40°C) with a coolant at -14°C. The crystal form was confirmed as α_L-crystalline from the ir spectrum of an independent sample subjected to the same temperature regime. The dielectric cell and enclosed sample (α_L-form) were gently warmed, the average rate of heating of the sample being no greater than 0·2°C per minute. The sample thickness between the electrodes was 0·50 mm. The permittivity ϵ' and the loss factor ϵ'' were determined every 2–3 min.

Results and conclusion

It can be seen from the results plotted in Fig. 1 that as the temperature increased, the permittivity decreased due to the transformation of the α_L-form (the rotator phase) to higher melting forms which possess less molecular mobility. The permittivity assumed a minimum value at about 25°C though the exact temperature at which the minimum occurs may vary with heating rate. On heating the sample further, the permittivity

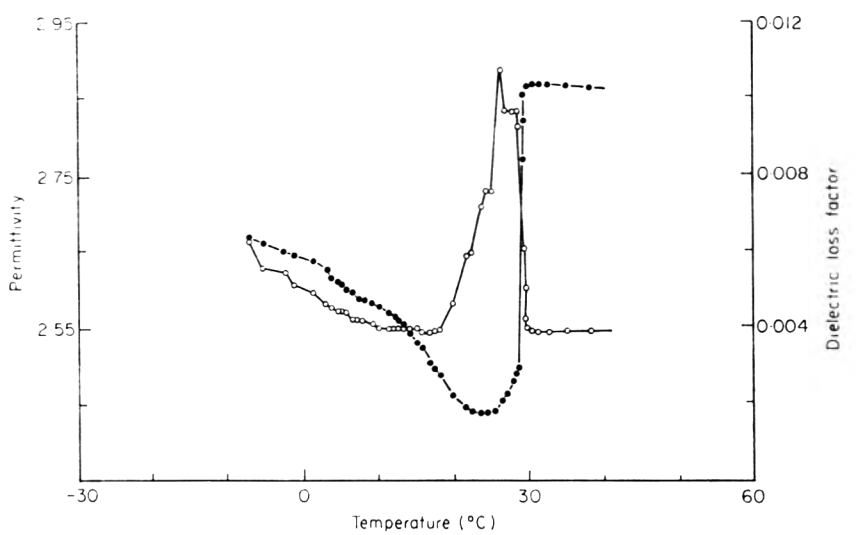

FIG. 1. Temperature dependence of the permittivity ϵ' (●—●) and the dielectric loss factor ϵ'' (○—○) for trierucin at 7 kHz.

rises sharply due to the loosening of the molecular crystal lattice until the material melts between 31·5 and 32·0°C (the melting point of the stable β_L-form).

An inspection of the loss factor versus temperature curve immediately reveals more discontinuities than are evident in the plot of permittivity versus temperature. As the temperature increases, the loss factor ϵ'' decreases and then remains substantially constant from 9·9°C to 14·1°C. The gradual decrease in loss factor can be interpreted in terms of premelting of the α_L-form and the progressive formation of the β'-polymorph. The different melting points for the α_L-form reported in the literature are presumed to reflect the different heating rates used. On further heating the loss factor increases (probably due to the acquisition of a slightly higher degree of rotational freedom by the molecules in the crystal lattice during the transformation), rises to a maximum and falls to a minimum value at about 31·5–32·0°C. This is the melting point of the stable β_L-form. The three significant shoulders on that region of the loss factor versus temperature curve intermediate between the α_L- and β_L- forms provide evidence for the existence of three intermediate forms with melting points at 22·0, 25·0 and 28·5°C. These are in good agreement with those reported by Hagemann & Tallent (1972).

These results confirm that trierucin has five different crystal forms, the lowest phase transition ($\alpha_L \rightarrow \beta_L'$) being substantially complete at 9·9°C, and indicate that dielectric loss studies may give more extensive information than permittivity measurements when examining the polymorphic behaviour of triglycerides.

Acknowledgments

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Nutritional and chemical changes in heated casein

I. Preliminary study of solubility, gel filtration pattern and amino acid pattern

R. C. OSNER* AND R. M. JOHNSON†

Summary

Samples of commercial casein were processed either in solution or dry at temperatures between 100° and 130° for up to 8 hr. Denaturation (loss of solubility) occurred mainly at 120° and 130° and was preceded by darkening and by changes in gel filtration pattern, which indicated that molecular association had taken place. In samples subjected to severe heat treatment there were significant losses of aspartic acid, threonine, serine, cystine, histidine and lysine but apparent increases in glycine and alanine. The relationships between the various changes are discussed.

Introduction

Within the human and animal food industries today there are many situations in which materials with a high protein content are heated to temperatures at which denaturation (insolubilization) may occur. During the manufacture of protein concentrates for animal consumption, the traditional canning of low acid products, the de-toxification of oil-bearing seeds and in methods of processing such as texturization, proteins may be heated to above 100°C for various lengths of time. Dry proteins denature at different rates when heated (Mecham & Olcott, 1947): the solubilities of casein, egg white, denatured egg white, gluten, hoof, soybean globulin and zein have been determined after heating for 18 hr at a number of temperatures between 60° and 203°C and the temperature of minimum solubility for most proteins was 153°C. The present study should contribute towards the understanding of heated protein systems; commercial casein was chosen as it is a typical soluble animal protein system. There are several chemically distinct proteins in commercial 'casein'.

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Experimental

Simple analytical techniques

Moisture present in the samples was determined by drying to constant weight at 105°. For nitrogen determinations, the A.O.A.C. (1965) micro-Kjeldahl method was used. The colour of the samples was measured in a Standard Lovibond Tintometer using a pure magnesium carbonate block as the standard white surface. To determine the solubility of heated casein, sodium hydroxide (10 ml, 0·1 N) was added to casein powder (0·1 g) and the mixture shaken mechanically for an hour, before centrifuging at 37,000 ***g*** for 10 min. Duplicate samples of the supernatant liquid (1 or 2 ml) were taken for the determination of nitrogen content.

Total amino acid content

The method selected (Blackburn, 1968), was most suitable for hydrolysis and preparation of pure proteins for ion exchange chromatography. The amino acids were determined in duplicate on the hydrolysates with a Locarte amino acid analyser (The Locarte Company, London) by a fully automatic ion exchange column procedure (Spackman, Stein & Moore, 1958). Calculations were based on a comparison with a mixture containing all the amino acids in the concentration of 0·05 μmol/ml.

Gel filtration

This technique (Andrews, 1964) was used to give an indication of changes in molecular weight that occur when casein is heated. Samples in sodium hydroxide (0·5 ml, 0·1 N) were applied to a column of Sephadex G-100 (Pharmacia Fine Chemicals Ltd) suspended in sodium hydroxide of the same strength at room temperature (20 ± 3°). Before collection of the eluate in 2·5 ml fractions, its absorption was measured continuously using a u.v. absorptiometer (absorption at 254 nm). By calibrating the column with 'markers' of known molecular weight (cf. Andrews, 1964), it was possible to assess what changes had taken place in heated casein.

Preparation and heat treatment of samples

The acid-precipitated casein (6·3% moisture) was packed under nitrogen in cans and stored at −20° before use. Casein solution (5%) was made by dissolving casein powder (53·2 g) in phosphate buffer (1 l, 1%) at pH 7. The oxygen-free solution was filled into 1 oz screw-capped bottles, which were closed and processed in a retort for 1, 2, 4 or 8 hr at 100, 110, 120 or 130°. One bottle each of dry casein packed in air and nitrogen, and one bottle of casein solution (5%) were processed for each variable. After processing, all samples were stored at −20° until analysis.

Results and discussion

Effect of heat on solubility and colour of casein

Because of their similarity, the results for casein heated in air or under nitrogen were combined and the Lovibond units for red, yellow and blue were added to give an index of 'total darkening'. The colour of casein changed markedly as a result of heating in the dry state, especially at 120 and 130° (Table 1). There was a loss of solubility for some dry air-free samples heated in air or under nitrogen at 120 and 130°, and as the presence of air did not have a noticeable effect, the results were combined (Table 1).

TABLE 1. Changes in colour and percentage solubility of heated casein

Process temperature	Time (hr)	'Dry' casein 'total darkening.	Percentage solubility	
			'Dry' casein	5% Casein solution
0	0	2·8	100	100
100°	1	2·7	100	100
	2	2·8	99	110
	4	3·2	100	100
	8	4·1	100	100
110°	1	3·1	99	99
	2	3·4	99	99
	4	3·8	97	99
	8	4·4	95	99
120°	1	4·0	99	100
	2	4·5	98	100
	4	6·0	60	89
	8	7·1	39	85
130°	1	5·2	95	100
	2	5·9	57	100
	4	8·2	38	87
	8	8·7	28	70

Processing casein solution at 100 and 110° affected its odour but not its solubility. After severe processing at 120 and 130°C (Table 1) three layers were present in the bottle: a supernatant, clear layer; a middle, opaque colloidal layer, soluble in sodium hydroxide solution (0·1 N); and a layer of coagulated, red, translucent gel insoluble in sodium hydroxide solution (0·1 N). As the first two layers merged into each other, they were both decanted into a clean boiling tube and mixed thoroughly before sampling in each case for total nitrogen determination. Although samples became progressively

darker as the conditions of processing became more severe, it was not possible to obtain an accurate measurement of the colour changes because of the different layers present in the partly denatured solutions.

Effect of heat on amino acid composition and gel filtration pattern

After heating casein for 8 hr at 130°, there were significant losses of cystine, aspartic acid, threonine, serine, lysine and histidine, although glycine content had apparently increased (Table 2). Possible mechanisms for these losses have recently been reviewed (Bjarnason & Carpenter, 1970), although there is no satisfactory explanation for the observed increase in glycine content.

The gel filtration pattern of commercial casein is shown in Fig. 1, together with the estimated molecular weights of the peaks. Association of the molecules of casein presumably occurs during commercial preparation, as the molecular weights of the

TABLE 2. Total amino acids in control and heated casein

Amino acid	Control casein mean* and s.d.	120°		130°	
		4h	8h	4h	8h
Cysteic acid§	36 ± 2	—	—	—	25
Aspartic acid	520 ± 8	500†	500†	490†	480†
Threonine	370 ± 13	360	350	390	330†
Serine	580 ± 17	520†	500†	510†	470†
Glutamic acid	1550 ± 23	1570	1540	1510	1580
Proline	880 ± 45	890	860	830	890
Glycine	250 ± 10	260	240	260	280‡
Alanine	330 ± 13	340	240	340	330
Valine	520 ± 22	500	540	500	500
Methionine	180 ± 8	190	170	190	180
Isoleucine	360 ± 13	370	370	240	350
Leucine	650 ± 14	650	640	650	640
Tryosine	330 ± 9	330	320	320	310
Phenylalanine	300 ± 9	290	290	290	280
Histidine	180 ± 6	180	170	170	160†
Lysine	520 ± 11	490†	480†	480†	440†
Arginine	210 ± 8	210	200	210	220
Tryptophan¶	50 ± 3	—	—	—	48

* Mean of 6 hydrolysates, with standard deviation corrected for small sample.

† Significantly lower than control.

‡ Significantly higher than control.

§ Cystine was determined, as cysteic acid after performic oxidation for control casein and the sample processed at 130° for 8 hours only.

¶ Tryptophan was only determined for control casein and the sample processed at 130° for 8 hours.

monomeric forms of any of the caseins are not above 30,000 (review by McKenzie, 1967) and the alkaline conditions of gel filtration would not have encouraged association. After the heat processing of dry casein, changes in molecular weight appeared before any decrease in solubility and these changes were demonstrated by the patterns obtained by gel filtration (Fig. 2): as the processing time was increased for samples heated at 100° there was a sharp increase in the higher molecular weight peak A at the expense of peak B, whilst there was little change in peaks C and D. At 110°, this change occurred sooner; thus processing at 100° for 4 hr gave a similar pattern to that obtained by processing at 110° for 2 hr and there was a similarity in the pattern obtained by processing at 110° for 8 hr, 120° for 2 hr and 130° for 1 hr. As casein became insoluble at 120 and 130°, so peak A became smaller; after processing at 130° for 8 hr, there was very little of either peak A or B left. However, during the processing of casein at 120° and 130° for 4 or 8 hr, some material of comparatively low molecular weight (3500) was produced and this was illustrated by applying equal concentrations of soluble nitrogen to the gel column (Fig. 3). For the production of this material, there must have been cleavage of peptide bonds within protein molecules and this could be the result of the destruction of threonine, serine or cystine (Bjarnason & Carpenter, 1970).

All the changes in gel filtration pattern appeared to be much more sharply defined in casein heated dry than when casein was heated in solution. The higher molecular weight material (peaks A and B) eventually disappeared with increasing severity of heat treatment, probably because further molecular association resulted in insolubilization. For casein heated dry, the relationship between decreasing solubility and

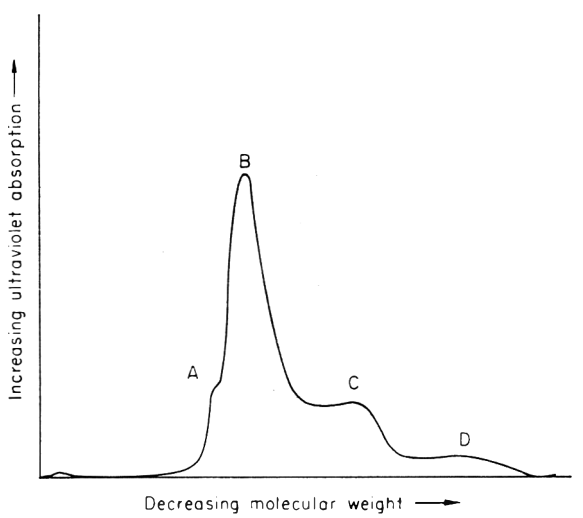

FIG. 1. Gel filtration pattern for commercial casein. Approximate molecular weights are: for peak A, 67,000; peak B, 48,000; peak C, 19,000; peak D, 7700.

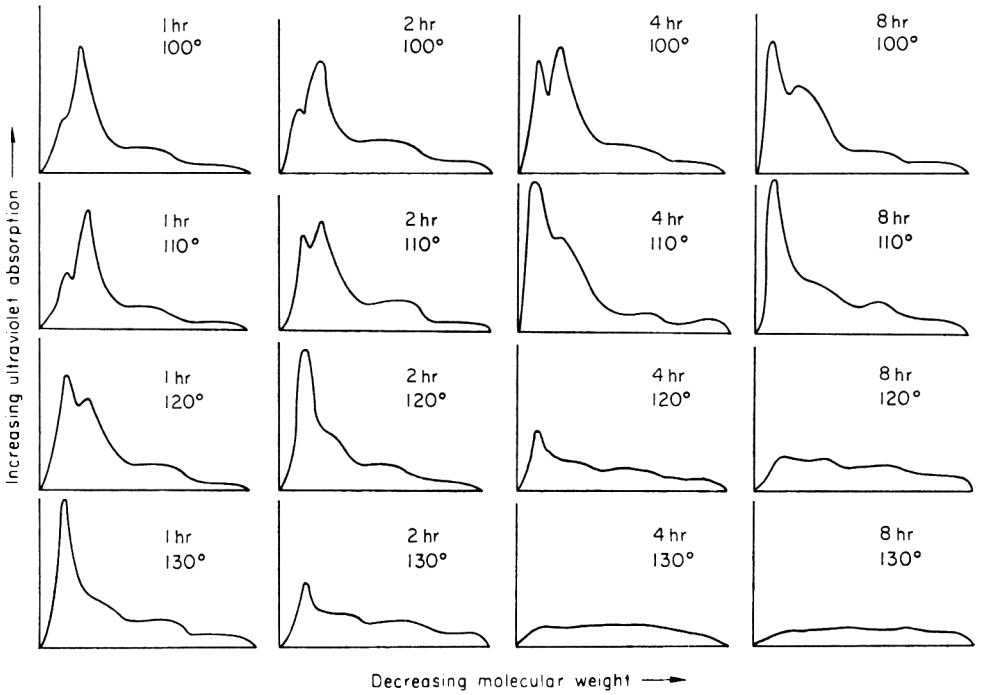

FIG. 2. Gel filtration patterns for heat treated commercial casein (6% moisture).

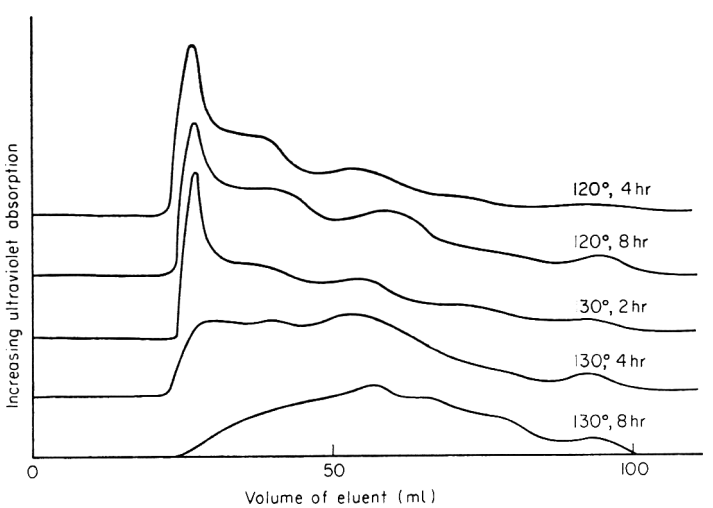

FIG. 3. Gel filtration patterns for heat treated commercial casein, where an equal concentration of soluble nitrogen was added to the column in each case.

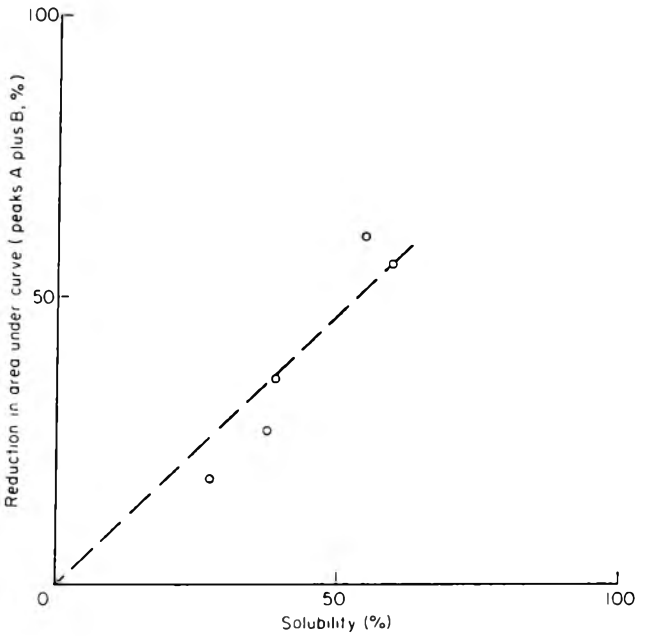

FIG. 4. Relationship between the solubility of heat treated casein and its gel filtration patterns.

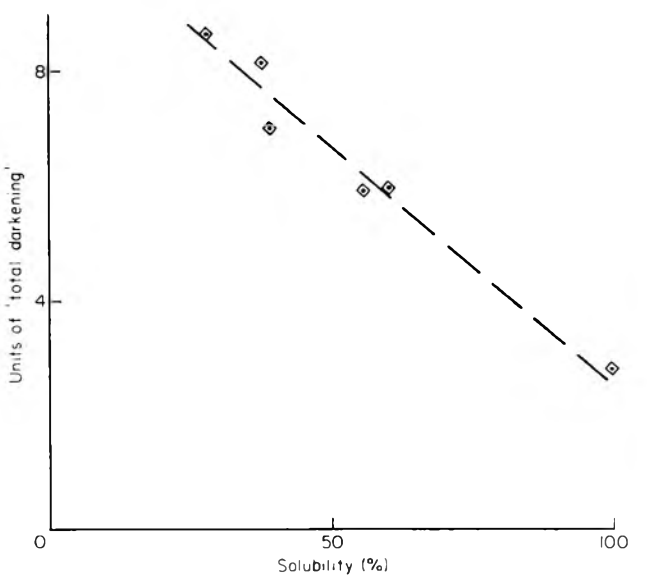

FIG. 5. Relationship between the solubility of heat treated casein and the 'total darkening'.

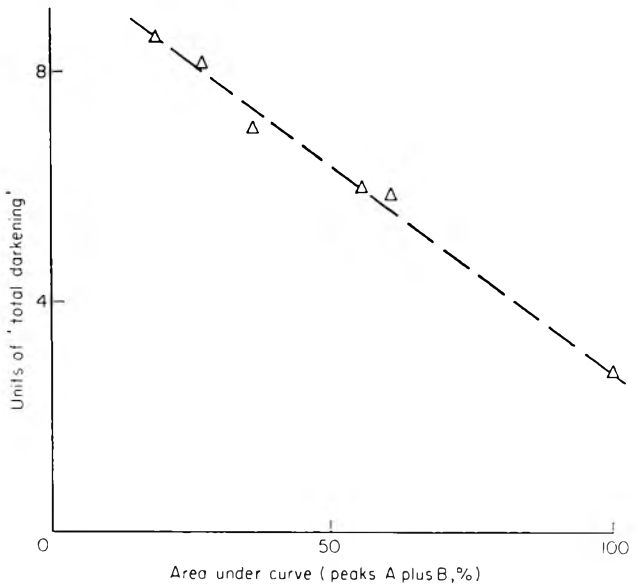

FIG. 6. Relationship between 'total darkening' and the gel filtration patterns.

peak area (for peaks A+B) has been shown in Fig. 4. There was also a clear relationship between 'total darkening' and both solubility and peak area after the onset of denaturation (Figs 5 and 6), as 'total darkening' seems to give an indication of how far the reactions causing insolubilisation have proceeded. It is hoped that more detailed studies on the chemical and nutritional changes taking place during the heat treatment of dry casein will be reported later.

Acknowledgments

We thank the Directors of Swift Ltd for a gift of Commercial Casein and Mr H. J. Palmer for helpful discussions.

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Starch gelation as a function of water content

R. COLLISON AND W. G. CHILTON

Summary

A dye staining method has been used to determine the proportion of starch granules which are damaged as a function of initial water content during (a) microwave heating and (b) baking in a forced-air-convection oven.

Although the granules are damaged much more rapidly on microwave heating, it was found that the proportion of damaged granules depends on the amount of water in the sample rather than on the method of heating. Samples of potato starch containing up to 30% water suffer no measurable damage. Beyond this point, damage increases with increasing moisture content, and all the granules are damaged provided the samples contain initially 55% or more water.

Introduction

This investigation was carried out with two aims in mind:

(a) to study the significance of starch-water ratio in starch gelation,
(b) to study the significance of water content in relation to microwave cooking systems.

The behaviour of many starch containing foods depends on the interaction between starch and water. One of the most important features of starch is its ability to gelatinize on heating with water. Although many aspects of starch gelation have been extensively studied, comparatively little work has been reported on the significance of starch-water ratio. Since this varies enormously amongst different food commodities we have examined a wide spectrum of mixtures from 'dry' starch powders containing a few per cent water to suspensions of starch in water. Two heating methods were used: a microwave oven and a forced-air-convection oven.

The extent of starch damage was measured by a staining technique using chlorazol violet R. A number of dyes have been used in the past to estimate starch granule damage. The classical method of distinguishing intact starch granules from those damaged during milling involves the use of the dye congo red (Jones, 1940) which

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preferentially stains the damaged granules. Jones suggested that starch granules damaged in milling suffer peripheral cracking which allows the dye to enter the granule. Congo red is also absorbed by gelatinized granules but does not stain non-gelatinized granules unless they are mechanically damaged. Recently chlorazol violet R which has a more intense colour has been suggested (Flint & Moss, 1970) as an alternative to congo red.

Experimental

Sample preparation

Starch samples of different moisture contents were prepared from a stock of potato starch of known moisture content. Samples of starch were spread in to a thin film on a metal tray and then sprayed with a fine spray of water until a weight corresponding to a particular moisture content was reached. Above 50% water fluid pastes were obtained and satisfactory homogeneous samples were easily prepared. It was more difficult to prepare homogeneous samples containing less than 50% water, and these had to be left for about 70 hr at 5°C in order to equilibriate. The final water contents of these prepared samples were determined by drying in a vacuum oven at 110°C for 16 hr.

Heating methods

One gram samples of starch in specimen bottles were heated in

(a) a Sharp (Model R-1501) Microwave oven at 1·3 kw power and a microwave frequency 2450 MHz for up to 150 sec and
(b) a Blodgett forced-air-convection oven (type CTB 1) set at 204°C (400°F) for up to 20 min.

Staining experiments

From each experiment a small sample of heat-treated starch was placed on an embryo dish and mixed with two drops of distilled water and two drops of chlorazol violet R. After standing for 60 sec the proportion of damaged granules, as indicated by staining, was counted for a given field at × 100 magnification. A total of 600 granules was examined from each batch.

Results

The samples of potato and wheat starch used in these experiments contained a small proportion of damaged granules before heating. These values were subtracted from the values obtained after heating, so that the recorded results represent damage caused during heating in the microwave or forced-air-convection (F.A.C.) ovens.

Figures 1 and 2 show the effects of microwave heating and F.A.C. heating on starch water samples containing 75% water. All the granules are damaged after 30 sec in the microwave oven and comparatively little water has evaporated. By contrast it

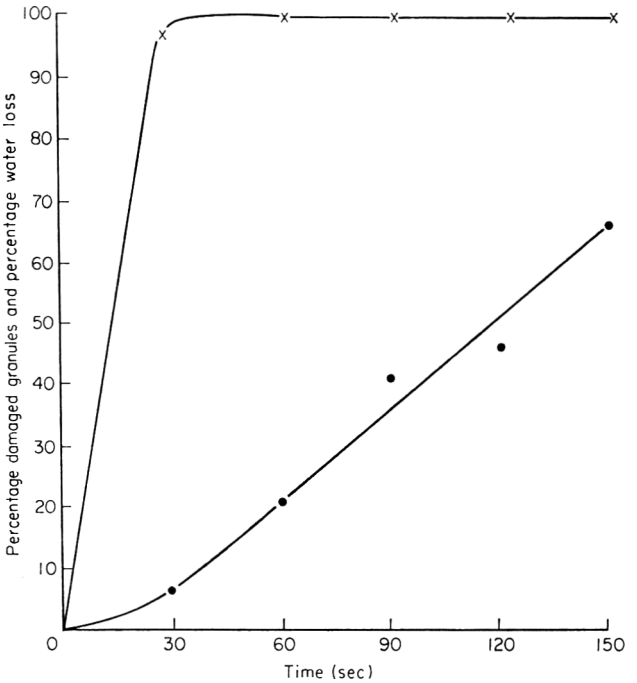

FIG. 1. Microwave heating of potato starch containing initially 75% water. Percentage damaged granules (×) and percentage water loss (●) plotted against time.

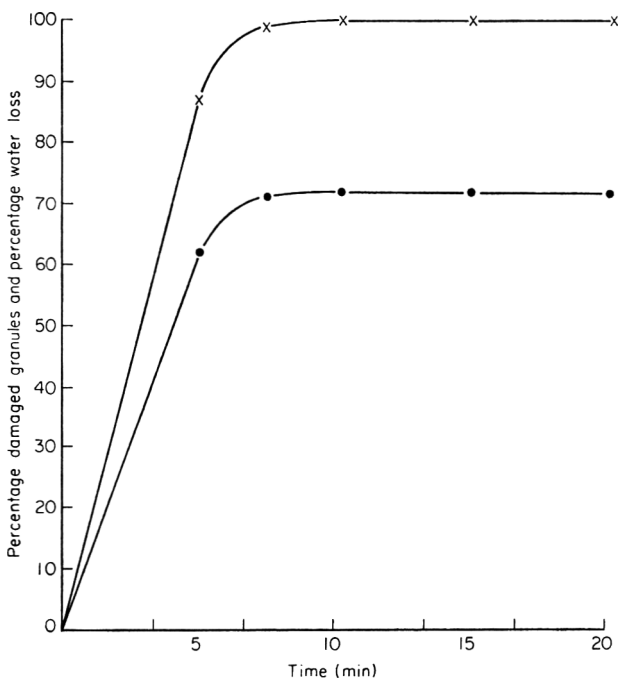

FIG. 2. F.A.C. heating of potato starch containing initially 75% water. Percentage damaged granules (×) and percentage water loss (●) plotted against time.

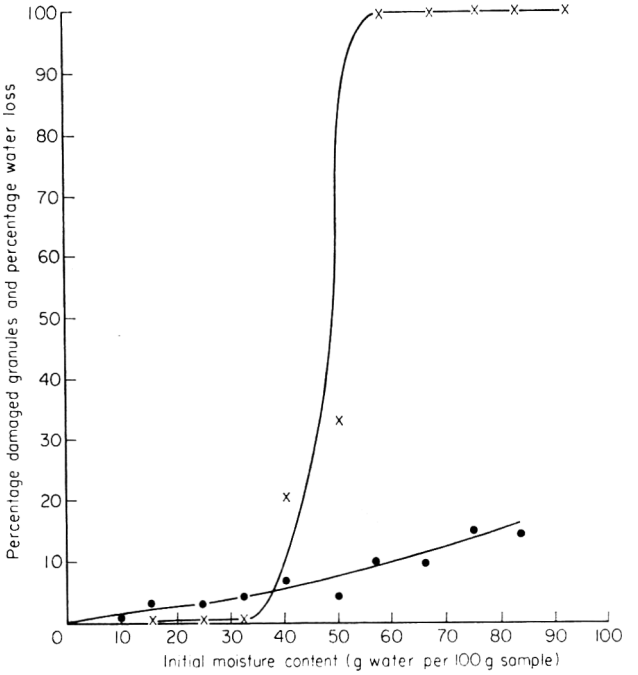

FIG. 3. Percentage damaged granules (×) and water loss (●) as a function of initial water content for potato starch after 30 sec microwave heating.

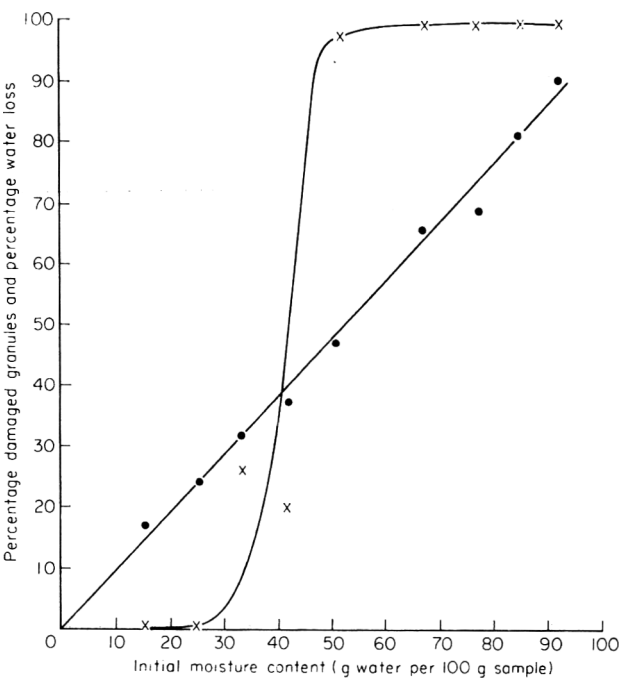

FIG. 4. Percentage damaged granules (×) and water loss (●) as a function of initial water content for potato starch after 7 min F.A.C. heating.

requires 7 min heating in the F.A.C. oven to damage all the granules and by this time most of the water has been lost.

The proportion of damaged granules depends on the starch-water ratio rather than on the method of heating. The plot of percentage damage granules against initial water content (Figs 3 and 4) is essentially the same for both heating methods. Samples containing over 55% water undergo complete granule damage, provided sufficient heating time is given. Samples containing less than 55% water on examination after heat treatment are found to contain both damaged and un-damaged granules. Below 30% initial water content no measurable granule damage is found.

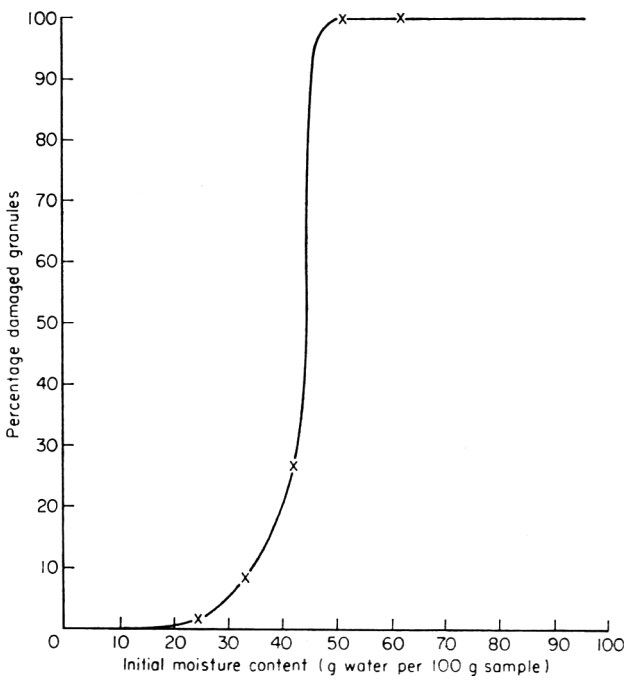

FIG. 5. Percentage damaged granules as a function of initial water content for wheat starch after 30 sec mirowave heating.

The results for wheat starch after 30 sec microwave heating (Fig. 5) follow the same pattern of behaviour as for potato starch.

Discussion

(a) *Significance of starch-water ratio*

Starch granules contain amylose and amylopectin molecules which are hydrogen bonded to form regions of crystalline material interspersed with regions of less ordered structure. On gelation, some of the hydrogen bonds are broken down (Leach, McCowan

& Schoch, 1959) allowing the granules to absorb more water and swell. Among other changes, the granules loose their birefringence and acquire the ability to absorb certain dyes including chlorazol violet. Microscopic examination showed that the stained granules were always non-birefringent; since loss of birefringence is often used as a measure of starch gelation (Freke, 1971), then by definition damaged granules can be regarded as gelatinized granules. The results in this paper show that water content is a critical factor in starch gelation. No gelation occurs below 30% water; this represents four water molecules per anhydroglucose unit and is close to the amount of unfreezable water for potato starch (Duckworth, 1971). It is likely that some, if not all of this water, is heavily bound on to starch chains (Collison & Dickson, 1971; Chilton & Collison, 1974). Only at higher water content will there be sufficient 'free' water available for gelation, and not until 55% water is there sufficient free water to loosen up every granule in the sample.

(b) *Gelation by microwave heating*

Microwave heating depends on the absorption of microwave radiation and its conversion to thermal energy by the molecules of the sample. Water is the most active constituent of food, and the heating capacity of a food sample is largely dependent on its water content. Because of the penetrative power of microwaves—up to 2 in.—rapid uniform heating occurred throughout the samples used in this experiment.

By contrast, heating by the F.A.C. oven depends on the transfer of heat from the circulating air to the surface of the sample, followed by slow conduction from the surface to the interior. This is a much slower process and produces a less uniform heating effect.

The experimental results presented in this paper show that starch will gel on microwave heating provided there is sufficient water, and provided the sample is given sufficient time to reach the gelation temperature. All samples containing over 30% water show evidence of some gelation. The only noticeable difference between the two methods is due to the speed of microwave heating which allows the sample to retain much more of its moisture and thereby produce a much softer and less brittle product.

It is quite possible that samples containing less than 30% water will absorb microwaves, because it is known that microwaves will cause protein denaturation in samples of flour containing 20% or less of water (Edwards, 1964). However, as gelation does not seem to occur below 30% water, any changes which could occur at lower moisture contents would not be reflected by the staining technique.

Acknowledgment

We would like to thank Dr Olga Flint of the Proctor Department of Food and Leather Science, Leeds University, for the gift of a sample of dye, and for allowing us the use of her photographic facilities during the course of this work.

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Kinetics of moisture movement during air drying of sugar beet root

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Summary

The effect of several variables on the drying rate of sugar beet root slices has been investigated. The standard solution of the nonstationary-state diffusion equation was used to analyse the experimental results during the initial phase of the falling rate period of drying.

It was found that Fick's law can be used to predict average drying time, internal moisture distributions and sample temperature during dehydration with reasonable accuracy, despite the complex nature of the mechanisms of moisture transport.

Introduction

Although drying is sometimes a preliminary step to sugar extraction from sugar beet root, limited information is available in the literature on kinetics of water removal from sugar beet root.

Any improvement in the methods for drying would require a better understanding of the drying mechanism and how this is affected by various controllable factors. Consequently, in the present work experimental data on rates of air drying of sugar beet root slices were obtained.

It is also the purpose of this paper to discuss the application of Fick's law of diffusion to describe the rate of moisture movement during the initial phases (when the average moisture content has a vapour pressure close to that of pure water) of food dehydration in general, with particular emphasis to sugar beet root.

Many papers have been published on the application of Fick's law as a useful way to predict average drying times of food materials (Becker & Sallans, 1955; Fish, 1958; Jason, 1958; Saravacos & Charm, 1962; Lawrence & Scott, 1966; Salas & Labuza, 1968; King, 1968; Chen & Johnson, 1969; Labuza & Simon, 1970; Chirife, 1971).

However, the mechanisms of moisture transport are so varied and complex that although a practical semi-empirical description is sometimes easy, a theoretical interpretation of the exact nature of the flow mechanisms is generally difficult.

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For this reason, in this work Fick's law is employed as a mathematical description of the process, rather than a physical model, in an attempt to predict the moisture distribution and temperature evolution of the specimen during dehydration.

Recently, it has been suggested (Labuza, 1972) to use computer solutions to optimize the nutrient retention during food dehydration. This requires, among other factors, the knowledge of the moisture and temperature distributions in a food undergoing drying. Fick's law, as is shown here, may represent an easy and convenient way to fulfil part of that requirement.

Material and methods

Drying equipment

The laboratory drier consists of a centrifugal fan which blows the air over twelve 1 kW electric bar elements into a chamber at the base, then upwards through a vertical duct at the end of which the sample is suspended from a precision balance. The vertical duct has a flow-smoothing section of small glass spheres. The inlet dry bulb temperature is regulated by a thermostat and relay which controls the heaters; wet and dry bulb thermometers are fitted in the drying chamber. The air velocity is varied by using a slide valve at the fan outlet and is measured by an orifice plate connected to an inclined manometer.

For the measurement of product temperatures during drying, fine wire thermocouples (0·02 cm diameter) were inserted in the sample at different depths.

Materials

Medium size sugar beet roots from Miramar, Argentina, were kept refrigerated at 2–3°C until use. Mean values of moisture content on samples taken at intervals over several months were between 2·5 and 3·6 g water/g dry solid.

The sugar beet roots were sliced mechanically, in most cases transversally to the main axis, and cut in 6 cm square pieces with thickness varying from 0·4 to 1·0 cm. At least ten measurements of the thickness were made at different points with a dial micrometer; only slices that fell within a 5% range of the average thickness were used.

To simplify mathematical interpretation of the experimental results, when the thickness was greater than one tenth of the size, the edges of the slice were sealed with resin and aluminium foil. In this condition drying took place only from the two major faces.

Measurement of moisture profiles during drying

After a given drying time, the sample was withdrawn from the drying chamber. Three or four cylinders were obtained from the sample by using a sharp end metal tube and sectioned rapidly by means of a hand microtome and a sharp smooth-surfaced blade into six to ten slices. The moisture contents of the individual slices were determined gravimetrically using a vacuum oven.

Results and discussion

A constant rate period was not observed in any of the experiments of this work, hence, the entire drying process for sugar beet root occurs in the range of the falling rate period. This is in agreement with observations made by Saravacos & Charm (1962) and Labuza & Simon (1970). Similarly, Chirife & Cachero (1970) reported that the drying of tapioca root—which also has a relatively low initial moisture content—occurs only in the falling rate period.

When the experimental data of moisture content versus time were plotted on semi-logarithmic coordinates a straight line portion was obtained followed by a curve which was concave downward. This is illustrated in Fig. 1, in which values of log $(\overline{W} - W_e)/(W_0 - W_e)$ versus time/(thickness)^2 were plotted for three different dry bulb temperatures. The factor (thickness)^2 was introduced for purposes of comparison to allow for

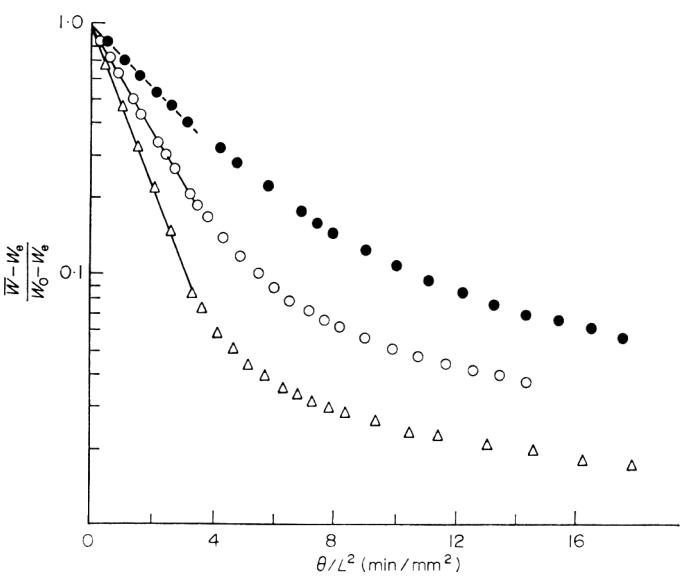

FIG. 1. Drying behaviour of sugar beet root slabs. ●, 47°C; ○, 60°C; △, 81°C.

differences in the thickness of the samples. Actually, it was previously found that the drying time in the straight line portion showed a thickness dependence somewhat lower than 2. However, this latter value was used since the error introduced is not relevant.

A drying behaviour similar to that shown by curves in Fig. 1 was found by other authors during dehydration of biological materials (Jason, 1958; Chen & Johnson, 1969; Chirife & Cachero, 1970); the falling rate period is divisible into two distinct phases, the first and second falling rate periods. In the present study we are concerned with the first falling rate period for which a linear relationship is observed between the logarithm $(\overline{W} - W_e)/(W_0 - W_e)$ and the time factor θ/L^2.

By studying the effect of different velocities of air on the moisture content of various thicknesses of material during drying it is possible to determine which resistance (internal or external) controls the rate of drying. From the results shown in Fig. 2 it can be seen that internal moisture movement is the main resistance to the rate of loss of moisture especially in the thicker samples for which the drying rate is not affected by air velocity.

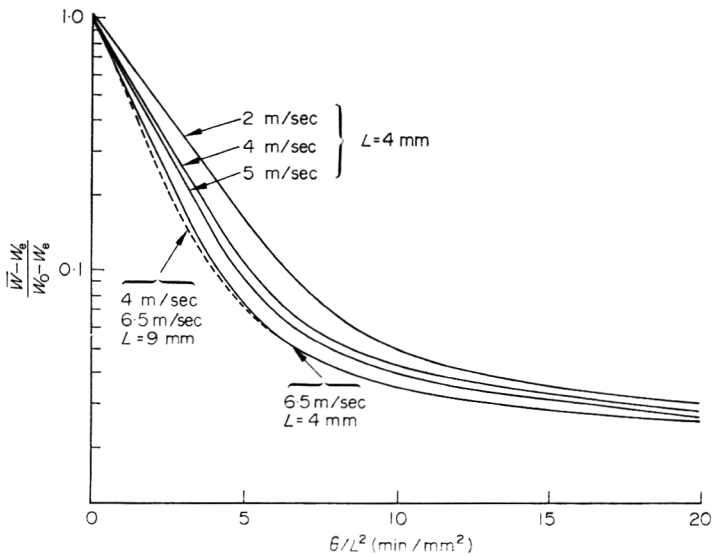

FIG. 2. Relative effect of air flow rate on drying curves.

The first phase of the falling-rate period of sugar beet root may be represented by the solution of Fick's law for unidimensional flow:

$$\frac{\delta W}{\delta \theta} = D \frac{\delta^2 W}{\delta x^2}$$

with the appropriate boundary conditions,

$$\theta \to 0^+ \quad W = W_0 \quad 0 < x < L$$

$$x \to 0^+ \quad W = W_e \quad \theta > 0$$

$$x \to L^- \quad W = W_e \quad \theta > 0$$

Under these conditions the solution expressed as average moisture content for the slab is:

$$\frac{\bar{W} - W_e}{W_0 - W_e} = \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \exp\left\{-(2n+1)^2 \pi^2 \frac{D\theta}{L^2}\right\}. \qquad (1)$$

Equation (1) allows us to calculate the diffusion coefficient from the slope of the straight line representing log $(\overline{W}-W_e)/(W_0-W_e)$ versus time, with the equilibrium moisture contents obtained from the literature (Iglesias, 1973).

Linear regression was employed to obtain values of diffusion coefficients for different dry bulb temperatures, which are plotted in Fig. 3 as log D versus $1/T$. It can be seen that the plot is essentially a straight line, from which the activation energy for diffusion may be estimated using an Arrhenius type equation:

$$D = Ae^{-E_a/RT}. \qquad (2)$$

The activation energy was calculated from the slope of the line in Fig. 3 and was found to be, $E_a = 6900$ cal/g mol. This value can be compared with 5400 cal/g mol obtained by Chirife (1971) for tapioca root, 7100 cal/g mol reported by Jason (1958) for fish muscle, and 4310 cal/g mol found by Chen & Johnson (1969) for tobacco leaf, all these values corresponding to the so called 'first falling rate period' (Chen & Johnson, 1969).

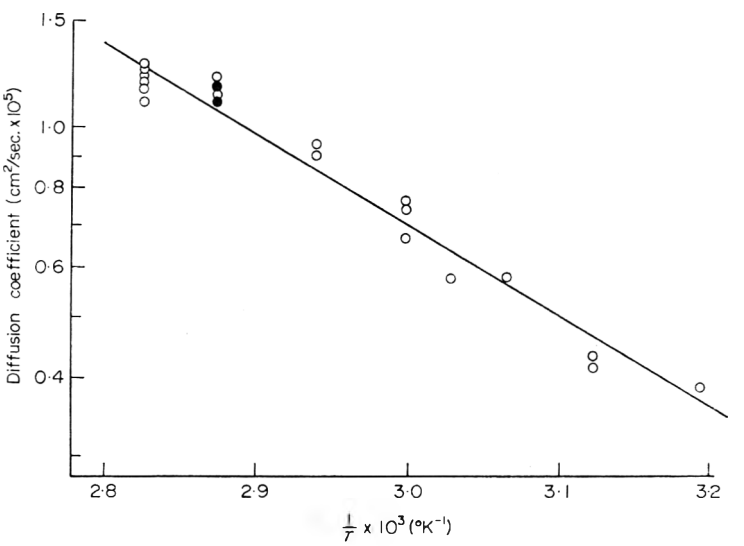

FIG. 3. Effect of temperature on the diffusion coefficient of water in sugar beet root. ○, transversal; ●, longitudinal.

We believe that a theoretical explanation of the value of the activation energy is rather difficult in view of the varied and complex mechanisms which can account for the moisture transport in the moisture range here considered (Van Arsdel & Copley, 1963). A successful analysis of the drying mechanism for foodstuffs which allows the prediction of activation energies for diffusion is that of King (1968). However, this model is only applicable to foodstuffs at moisture contents where the equilibrium relative humidity is below 100%, which of course, is not the situation studied in the present work.

The isotropic behaviour of sugar beet root was examined in the following experiment: samples were sliced longitudinally to the main axis of the root instead of transversally and dried at one dry bulb temperature. Diffusion coefficients were calculated in the usual way and plotted in Fig. 3 for purposes of comparison. It can be seen, that within the limits of experimental error, the points for the longitudinally sliced samples lie on the same line indicating that sugar beet root is isotropic with respect to moisture transport.

We also investigated the effect of blanching on the drying rate of sugar beet root. Blanching is considered in the literature to affect the rate of drying although there is little experimental evidence.

Samples of sugar beet root were wrapped in thin aluminium foil and immersed in boiling water during 5 min. The samples were then dried at two different air temperatures (47 and 60°C) and compared to unblanched (control) samples. The results are presented in Fig. 4; blanching had no significant effect on drying rate at 60°C, but at 47°C it increased the rate. It is known that heating leads to changes in the physical properties of tissue, among them it destroys the semi-permeability of the cell membranes

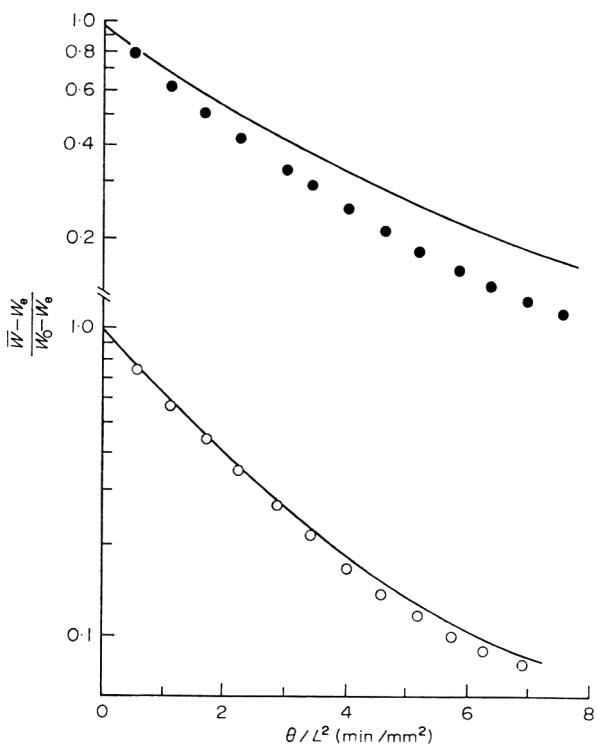

FIG. 4. Effect of blanching on the drying rate of sugar beet root. ●, 47°C; ○, 60°C; ——, controls.

and with it the osmotic properties of the cell (Gane & Wager, 1958). It could be argued that drying at elevated temperatures also modifies the permeability characteristics of the cell masking the effect of blanching, so no net effect in the drying curve was observed. Saravacos & Charm (1962) also found that blanching of potatoes had no significant effect on drying rate when compared with a control sample dried at 66°C.

Surface and centre temperatures of a sugar beet root sampled during dehydration were measured by inserting very fine wire thermocouples.

No significant temperature difference between the centre and surface was found. This is in agreement with results of Jason (1958), Saravacos & Charm (1962) and Chirife (1971) who also found little or no temperature gradient inside foodstuffs undergoing drying.

The slab temperature was plotted against time and the results are shown in Fig. 5 (lower curve). It can be seen that the temperature increases rapidly at the beginning of drying approaching the dry bulb temperature; however, the difference becomes negligible only when about 90% of the initial water was evaporated (see middle curve).

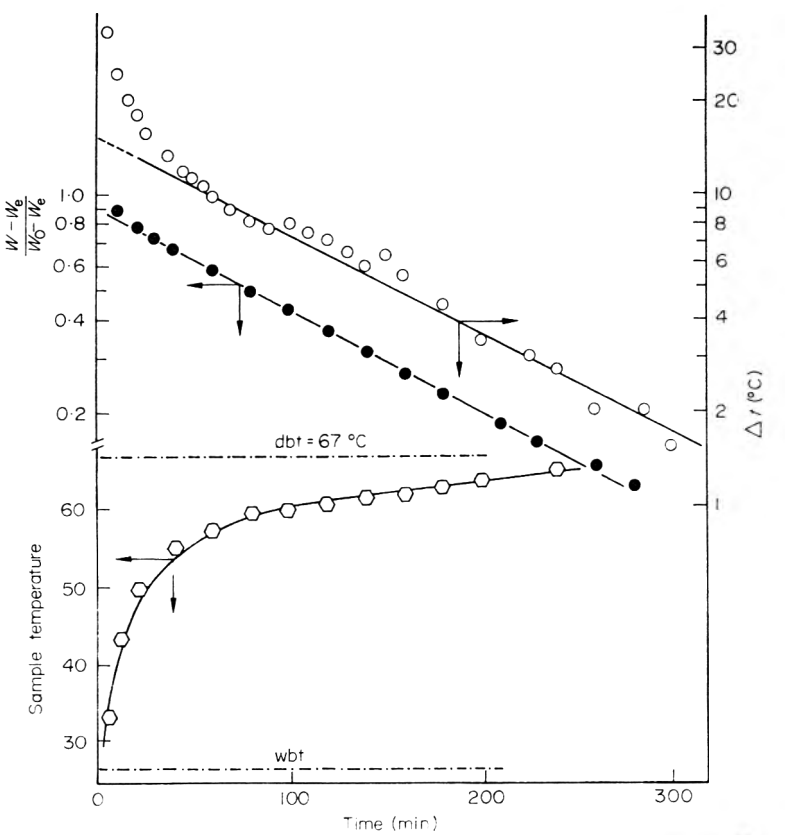

FIG. 5. Material temperature and moisture changes during dehydration. $\Delta t = \text{dbt} - t_s$.

In the same figure the temperature difference between the air and the sample is plotted (upper curve); it is seen that after an initial period log Δt versus time is also a straight line having the same slope as log $(\overline{W}-W_e)/(W_0-W_e)$ versus time. This analogy is a consequence of the heat and mass balance, which can be written—neglecting the sensible heat in comparison with the latent heat of evaporation—

$$\lambda\,(-d\overline{W}/d\theta)m = hA\Delta t. \tag{3}$$

The assumption of negligible sensible heat will be discussed in a future publication. According to Fig. 5 we have:

$$\log\,(\overline{W}-W_e)/(W_0-W_e) = -\alpha\theta \tag{4}$$

$$\log \Delta t = \log \Delta t_0 - \alpha\theta. \tag{5}$$

By substitution in equation (3) the value of the heat transfer coefficient was found to be $1{\cdot}3\times 10^{-3}$ cal/sec cm^2 °C. This is in agreement with data quoted by Saravacos & Charm (1962).

We may conclude that the sample temperature can be characterized by a single value at each instant of time and can be considered to be time dependent. Equation (5) represents the food temperature rate equation and can be used to calculate the temperature of sugar beet root during a significant part of the drying process, provided the kinetic constant of drying is known.

We also studied the ability of Fick's law, with a single constant diffusion coefficient, to predict the internal moisture distributions during drying. Sugar beet root samples were dried at two dry bulb temperatures and moisture distributions were measured at different drying times using the experimental technique previously described. The measurements were restricted to average moisture contents within the first falling rate period.

The theoretical moisture distributions were calculated using Fick's law:

$$W = W_e + 4/\pi\,(W_0-W_e)\left\{\sum_{n=0}^{\infty}\frac{1}{(2n+1)}\exp\left[-(2n+1)^2\,\pi^2\,\frac{D\theta}{L^2}\right]\sin\left[(2n+1)\,\frac{\pi x}{L}\right]\right\} \tag{6}$$

with experimental diffusion coefficients obtained from Fig. 3. Comparison of experimental and predicted moisture distributions are shown in Figs 6 and 7. These figures were plotted employing fractional dry masses as abscissa instead of thickness, to take into account the effect of shrinkage. This is permitted because Fick's law is defined in this work using moisture content in dry bases as driving force, instead of moisture concentration. A good agreement is observed which indicates that equation (6) based on Fick's law, may be used to predict the moisture distribution especially at the lower

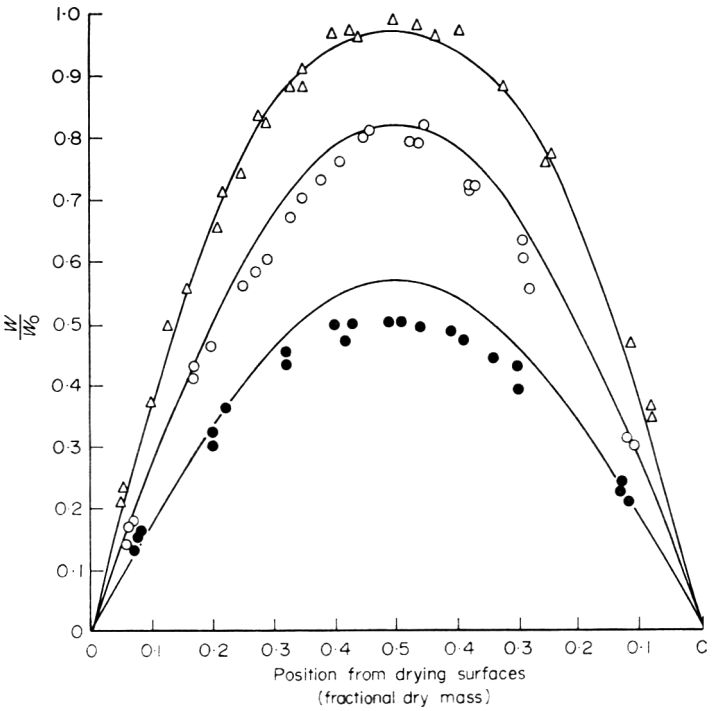

FIG. 6. Moisture distribution curves for various times: comparison of theoretical and experimental results. dbt 47°C. Experimental: △120 min; ○, 220 min; ●, 410 min. ——, Theoretical.

drying temperature. Under more severe drying conditions, i.e. 81°C the less satisfactory agreement with the theoretical equation is probably due to structural and chemical changes and the existence of internal temperature gradients, which were not observed at lower temperatures.

Conclusions

The experimental results found for sugar beet root drying showed qualitative agreement with several previous works reported in the literature on the drying behaviour of foodstuffs.

Main factors affecting the drying rate of sugar beet root slabs were temperature of drying and particle size, and to a lesser extent, velocity of airflow.

An exponential residual moisture-time relationship in the first part of drying was found. Diffusion coefficients calculated on this basis were correlated with temperature in accordance with an Arrhenius type relationship. Fick's law was used here as a mathematical tool rather than a physical model and it was shown that this simple

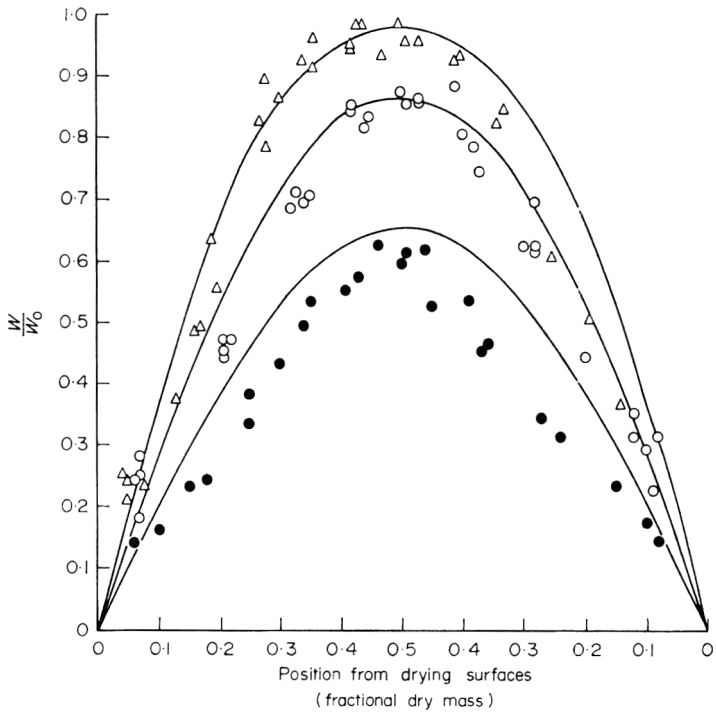

FIG. 7. Moisture distribution curves for various times: comparison of theoretical and experimental results. dbt 81°C. Experimental: △ 45 min; ○, 65 min; ●, 120 min. ——, Theoretical.

mathematical model allowed us to predict average drying times, internal moisture distributions and sample temperature with reasonable accuracy.

Acknowledgments

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Nomenclature

A, Constant (cm^2/sec).
D, Diffusion coefficient (cm^2/sec).
dbt, Dry bulb temperature (°C).

E_a, Activation energy (cal/g mol).
h, Heat transfer coefficient (cal/cm^2 sec °C).
L, Thickness of the sample (cm).
m, Dry mass (g).
R, Gas constant (1·98 cal/°K mol).
t, Temperature (°C).
T, Absolute temperature (°K).
W, Local moisture content (g water/100 g dry solid).
$\overline{W}$, Average moisture content (g water/100 g dry solid).

Greek letters

α, Drying constant (min^{-1}).
Δt, Temperature difference = dbt $- t_s$ (°C).
λ, Latent heat of vaporization (cal/g).
θ, Time.

Subscripts

0 Initial.
e, Equilibrium.
s, Sample.

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A combined experiment-computer technique for determining heating programs for batch and continuous freeze dryers

J. M. AGUILERA* AND J. M. FLINK

Summary

Two parameters, which characterize the freeze drying process, are obtained by a linear regression of variables derived from data from a limited number of freeze drying experiments. Freeze drying times and sample temperature profiles can then be simulated for other processing conditions, using computer techniques. The simulation has been used to predict the drying behaviour of skim milk samples for a continuous freeze drying process. The results of the simulation agree closely with a batch freeze drying operated so as to model a continuous process.

Introduction

Production of freeze dried food products has shown such sizeable growth in the past few years, especially in the area of instant coffee, that the process must be considered as established on an industrial scale. Further growth of the process by application of freeze drying to products of lower inherent value requires close regulation of process parameters so that freeze dryer utilization is optimized.

The ability to rapidly determine this optimum would allow use of continuous freeze dryers for short production runs (days or weeks) of freshly harvested products or contract freeze drying. The economic savings by using continuous freeze dryers has been quoted to be 30–40% over batch type units (Anon., 1972). This type savings could further fuel the interest in freeze drying of foods. Flink & Fosbøl (1972) described a number of techniques by which optimal freeze dryer heating programs could be determined; however, most of these are difficult to implement at present. Thus, in practice, the technique used is a trial and error approach. As noted by Blair (1966), 'specifying an optimum processing cycle usually required many full scale production runs, each an improvement over the proceeding'.

The development of adequate mathematical techniques would greatly improve the situation over trial and error, for computer techniques could be applied to the mathematical formulations. A few studies have been reported for the air drying of beds of

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grain products. Bakker-Arkema, Evans & Farmer (1971) simulated the multizone drying of grain and were able to study the influence of a number of process variables on sample moisture contents and temperatures. Experimental measurements closely followed the computed values.

Two works have been reported in which physical parameters that are obtained during drying are used in the mathematical expressions for further simulation. Rowe & Gunkel (1972) used a diffusional model to evaluate thin film drying of forage. Application of suitable simplifying assumptions allowed calculation of a drying coefficient which was directly proportional to the moisture diffusivity. Other unknown parameters were determined by least square fits of normalized drying equations. The drying simulation was then obtained by simultaneous solution of mass and energy balances of the system. Husain *et al.* (1972) required simplification of the complex equations for simultaneous heat and mass transfer during the falling rate period in order to apply digital computer finite difference methods to the resulting differential equations. Experimental drying data were used to evaluate the parameters for use in theoretical drying solutions. Good agreement between experiment and theory were obtained only when certain parameters were divided so that different values applied to different stages of the drying.

Mathematical approaches to the modelling of freeze drying have been presented by a number of authors, though in most cases little consideration was made to utilization of these techniques to simulation problems. Two research groups have been particularly active in developing mathematical expressions for the freeze-drying process and associated physical behaviour. Both utilize the Uniformly Retreating Ice Front (URIF) model, in which a sharp boundary is postulated to exist between the ice and the dry layer. King (1971) has presented a review of arguments for and against this model. Tests on the applicability of the URIF model showed that it was usable only up to the removal of 65–90% of the initial water (Sandall, King & Wilke, 1967) and that other difficulties related to product nonuniformity and piece size (edge effects) are possible (Margaritis & King, 1971).

In a series of literature reports, mathematical expressions have been developed for the analysis of freeze drying under various processing conditions (Dyer & Sunderland, 1967, 1968, 1971; Cho & Sunderland, 1970; Massey & Sunderland, 1972). They showed that by treating freeze drying as a quasi-steady state process the mathematical expressions could be sufficiently simplified to allow computer solutions. Through this, it was shown that convection heat transfer was negligible. For radiation heat transfer, relationships developed between radiator temperature, sample surface temperature and ice front temperature showed a two phase temperature behaviour of constant heater/variable surface followed by variable heater/constant surface. Meo & Friedly (1973) have shown this to be a condition for optimal control of freeze dryers.

Heldman & Hohner (1972) simulated mathematically the atmospheric freeze drying of beef cubes. Simplifications of the complex equations (heat and mass transfer balances)

were evaluated by numerical analysis techniques using the digital computer. Using values of product properties from the literature, they obtained acceptable results.

Flink & Fosbøl (1972) noted that mathematical approaches to simulation of freeze drying could be based on (1) complex equations with independent determinations of the various parameters or (2) simplified equations with lumped parameter determination obtained from experiment. Each has positive and negative factors, relative to speed, convenience, accuracy, etc. For developing practical heating programmes for continuous freeze drying of a particular product, we feel that the second method will be more successful.

This paper discusses the development of (1) simplified drying equations having grouped mathematical constants which are the parameters of drying; (2) obtaining of these parameters by means of a few simple experiments; (3) computer simulations of drying (batch and continuous) using the experimentally measured parameters.

Methods

Experimental

Reconstituted nonfat dry milk powder (20% w/w) with added sodium carboxymethyl cellulose (0·5%) (Dupont Chemical Co.) was used for freeze drying. An Avicel, dextrose, CMC model system was also successfully used, but will not be reported upon here (see Aguilera, 1973). After mixing, the sample was poured into an aluminium pan which was fitted with seven fine-wire thermocouples at known locations and a rubber ring on the circumference to prevent edge effects during drying (Fig. 1). Freezing was conducted by placing the sample holder on a thick aluminium plate which was immersed to a known depth in liquid nitrogen. In this way reproducible and controlled rates of freezing could be obtained. The sample was completely frozen in 1 hr. Prior to placing in the freeze dryer, the internal temperature gradients were allowed to equilibrate by holding the sample in a styrofoam support. The equilibrated frozen sample in its styrofoam holder (now used to insure one-sided heat transfer) is placed in the freeze dryer. The freeze dryer is quickly evacuated and then the heating plates are activated (come-up times of less than 5 min). The energy input to the heating plates is controlled by a thermocouple type controller which can use measurements at the heating plate or various sample locations as inputs. In these experiments, control was based on either the heater plate or sample surface thermocouples. The sample and system temperatures were measured each 6 min with a 12 point recorder. Additionally, instantaneous readings of selected locations could be made using an electronic thermometer. Chamber pressures were monitored using both Alphatron and thermocouple gauges. The sample was supported in the drying chamber on a Mettler balance, so that continuous weight measurements could be made.

The drying procedure involved utilization of a constant heater temperature until the surface reached a particular temperature value called the 'scorch' temperature; that

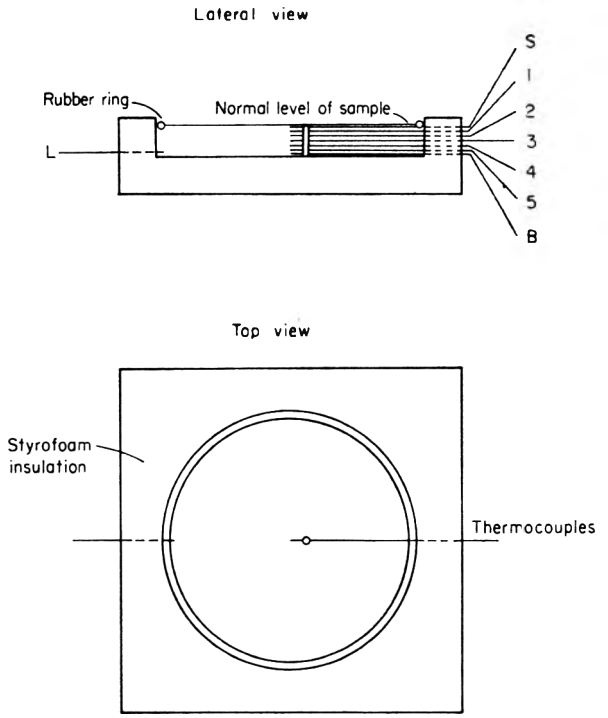

FIG. 1. Schematic of sample holder (to scale).

is, the maximum allowable temperature for retention of product quality. At this point the controller was switched to the sample surface, which was then maintained at the scorch temperature. For these experiments, a scorch temperature of 56°C was arbitrarily chosen.

The end point of drying was designated as the time when the temperature difference between the surface and bottom thermocouples was less than 6°C. Following drying, moisture contents of the dried material were measured gravimetrically using vacuum oven heating of 24 hr at 70°C. The thermocouple tips were carefully exposed and their location measured with a cathetometer.

Parameter determination

Relationships between various freeze drying variables were obtained based on the model used by Cho & Sunderland (1970), a semi-infinite slab dried by radiation from one side. Equations involving a single parameter were developed for heat transfer from the radiator to the ice interface of the sample

$$r=\Omega\ \frac{T_S-T_I}{T_H^4-T_S^4}$$

and for conduction heat transfer through the dry layer supplying the energy for sublimation.

$$T_S - T_I = \phi r \frac{dr}{dt}.$$

Thus, the parameter Ω relates external and internal heat transfer, while ϕ relates internal heat transfer with energy consumption. Neither parameter has been restricted as to its functional dependence on other system factors.

The data obtained by the experiments were analysed by digital computer using a polynomial regression subroutine. The main steps of this program are presented in Fig. 2. The maximum degree of the polynomial was chosen to be one for the determinations of Ω and ϕ, because of a desire to have a linear relationship. A cubic equation was developed for interface position *v.* time, based on times at which thermocouple readings raised from the ice front temperature. The first derivative of this equation

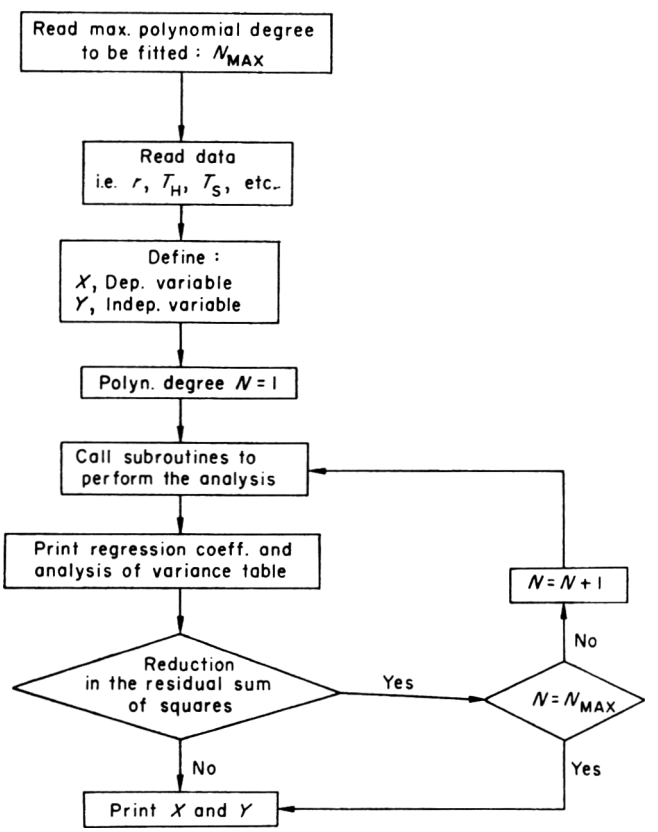

FIG. 2. Flow diagram of the computer program for parameter determination.

gave the value of dr/dt. This is then used in the determination of ϕ. Referring again to the programme flow diagram (Fig. 2) the following were the x and y variables:

	Variable	
Parameter	x	y
Ω	$\frac{T_S - T_I}{T_H^4 - T_S^4}$	r
ϕ	$r\frac{dr}{dt}$	$T_S - T_I$

Drying simulation

The basic equations presented above are coupled to appropriate boundary conditions and solved simultaneously to give drying temperatures and interface positions as a function of time. The major steps of this process are shown in Fig. 3. The calculation considers the time necessary to get the interface to the sample bottom (disappearance of ice). The equation including the Ω factor will give the surface temperature, while the ϕ containing equation gives the time to reach various interface locations.

Results

Initial experiments showed that the ice front was quite sharp and uniformly retreating into the sample. Also, the sample underwent some cracking during the drying process. The cracks were quite small and uniformly distributed.

Duplicate freeze drying experiments which were conducted at heater temperatures of 78, 100 and 128°C gave good reproducibility. Typical results are presented for a drying at 128°C (Fig. 4) in which the scorch temperature (56°C) is reached after about 3½ hr. The linearity of the temperature profiles in the dry layer (Fig. 5) shows that the quasi-steady state approach is applicable. The curves lose their linearity when the ice layer disappears. The constant ice temperature value observed allows the use of the mentioned equations. Measurements of the final moisture content at three locations showed no radial distribution of moisture. At the end of sublimation, moisture contents varied from 12–32 g H_2O/100 g solids, while at the end of drying the moisture contents were below 1 g H_2O/100 g solids.

The parameters were obtained by fitting a straight line to the data. Fig. 6 shows that excellent correlation was obtained for Ω giving values of 0·659, 0·648 and $0{\cdot}617 \times 10^8$ for 78,100 and 128°C respectively. For simulation purposes a mean value of $0{\cdot}640 \times 10^8$ °K^3 was used.

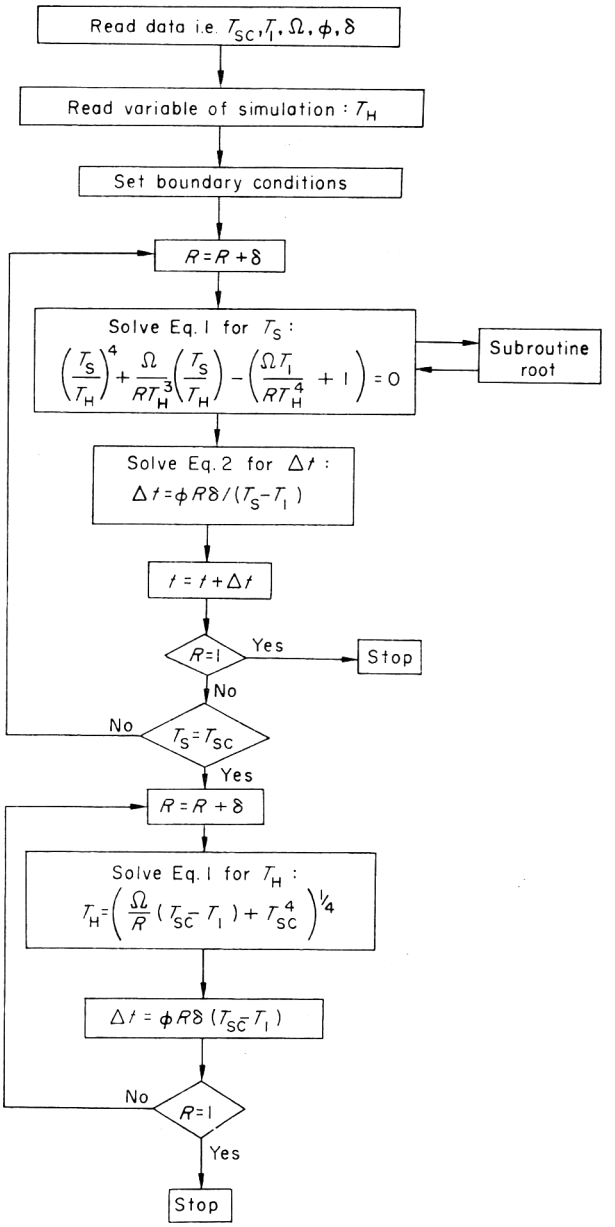

FIG. 3. Flow diagram of the computer program for freeze drying simulation.

For the ϕ parameter determination (Fig. 7) a straight line fit of all data was satisfactory only for a heater temperature of 78°C, where the entire sublimation process occurs without the surface reaching scorch conditions. In the other two cases, 100 and 128°C, two clearly distinctive zones, one before reaching scorch temperature and the

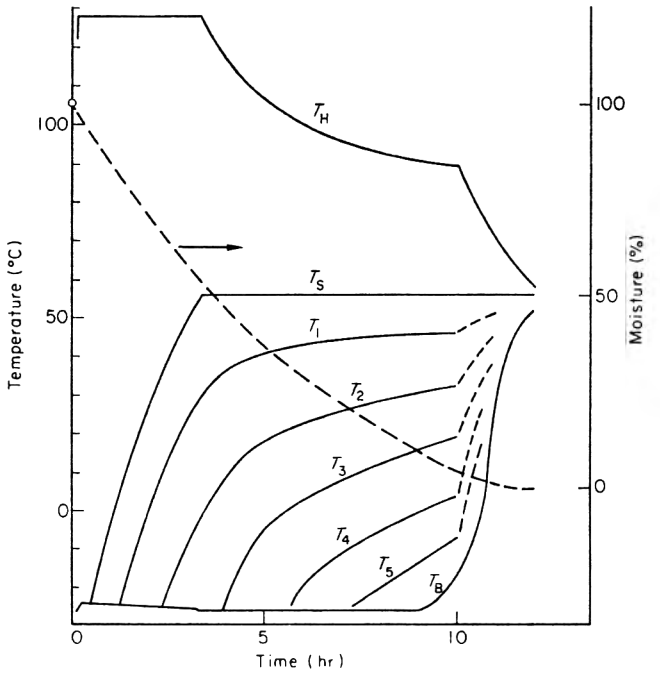

FIG. 4. Sample temperatures at seven locations and sample moisture loss when freeze drying skim milk. Initial heater temperature = 128°C; scorch temperature = 56°C.

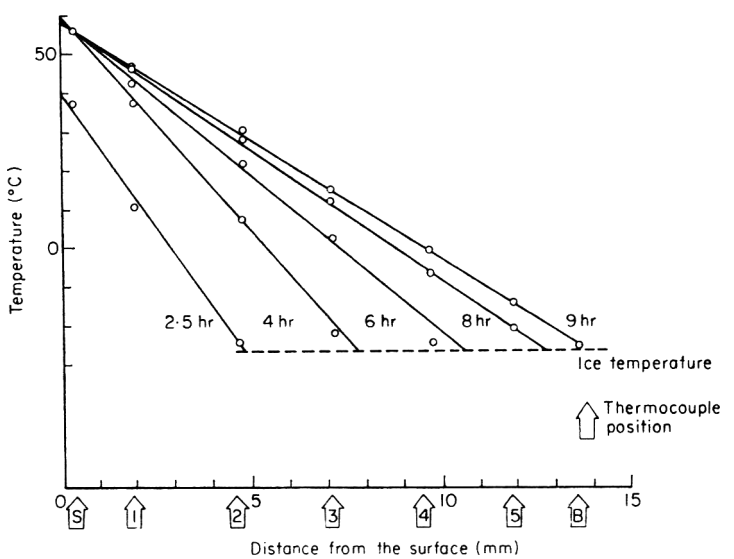

FIG. 5. Temperature profiles in freeze drying skim milk when the initial heater temperature is 128°C and the scorch temperature is 56°C.

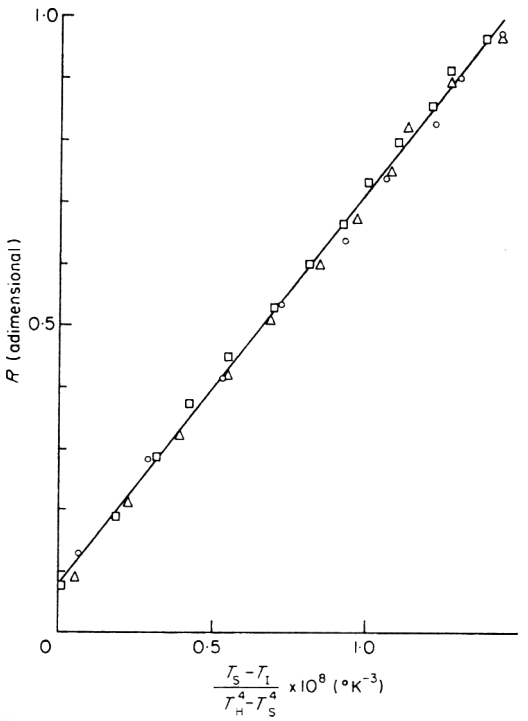

FIG. 6. Redefinition and replotting of temperature and moisture data for determination of Ω parameter. T_H(°C): □, 78; △, 100; ○, 128.

other after, were observed. The value of the slope of the least squares straight line fitting all data at each temperature was defined as ϕ mean ($\bar{\phi}$) for that temperature. Values of ϕ for the initial time (prior to attaining the scorch temperature) and for the entire process are shown in Fig. 7 for a 100°C heater temperature.

Considering the data further shows the following facts.

(1) The value of the slope of the imaginary straight line that fits the data prior to attaining the scorch temperature ($\bar{\phi}^{initial}$) represents the ϕ value for a given heater temperature with the following relation being observed:

$$\bar{\phi}^{initial}_{128} > \bar{\phi}^{initial}_{100} > \bar{\phi}^{initial}_{78}$$

(2) After attaining the scorch temperature, the temperature of the heater will be reduced. Another evaluation method for ϕ could take this into account by lowering ϕ with heater temperature (Fig. 8). It would also be possible to find a function that correlated the reduction in ϕ with the interface position.

The parameters Ω and ϕ, obtained as described above, are used in the computer simulation programme to calculate freeze drying behaviour of skim milk samples. Table 1 gives results of experiments and various simulations for three initial heater

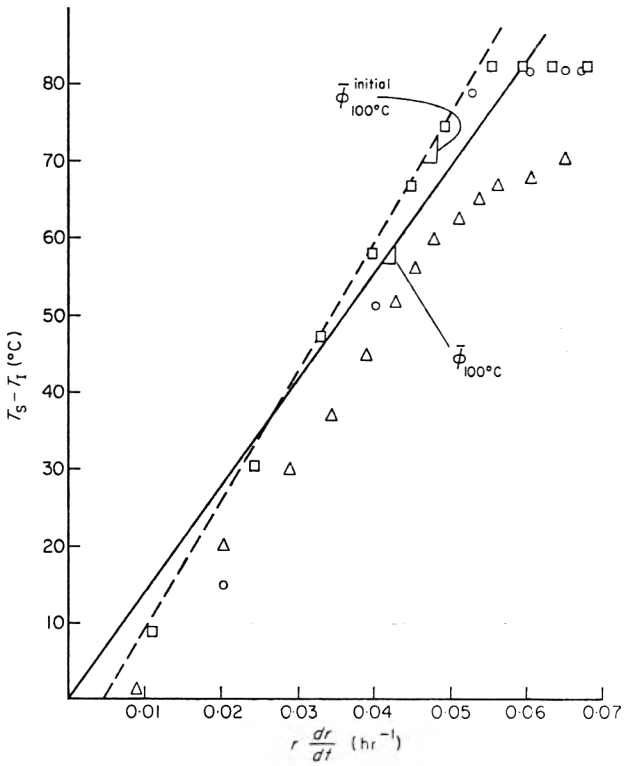

FIG. 7. Redefinition and replotting of experimental data for determination of ϕ parameters (see text). T_H(°C): △, 78; □, 100; ○, 128.

temperatures. For the purposes of simulation, freeze drying can be divided into four periods: (1) an initial lag or equilibration period, (2) a period before the sample surface reaches the scorch temperature (period or stage I), (3) a period where the surface is at the scorch temperature but ice is still present in the sample (period or stage II) and (4) the desorption period. It was observed that equilibration took approximately 20 min, and desorption about 2 hr, independent of initial heater temperature. In the case of desorption, this occurs since the initial desorption conditions (sample surface at the scorch temperature and sample bottom at the ice front temperature) give an identical temperature gradient in all cases. Table 1 gives times to reach the scorch temperature, and the total time to achieve disappearance of the ice. For each heater temperature two basic types of results are presented: (1) results of duplicate freeze drying experiments and (2) results of simulations based on the derived parameters. Since the values of the Ω parameter were independent of heater temperature, the average Ω value was used in all simulations. The variation of the ϕ parameter necessitated simulations according to three approaches, (1) self-simulation in which ϕ values for a given heater temperature are used to calculate the drying behaviour for that

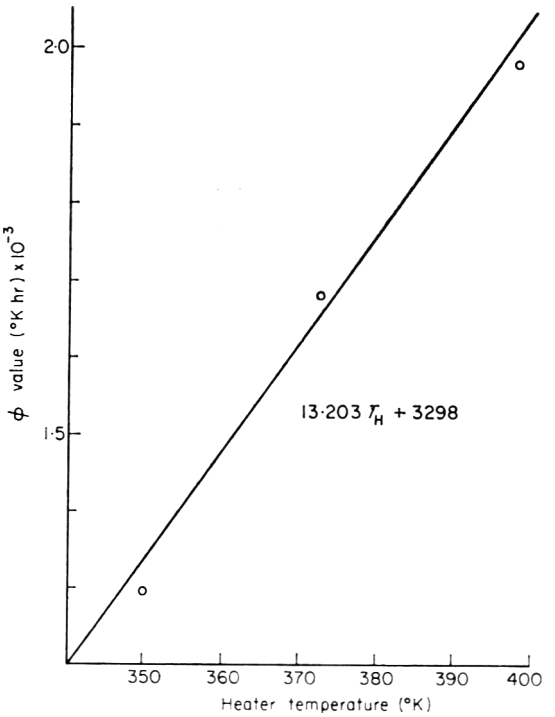

FIG. 8. Variation of ϕ parameter with heater temperature.

heater temperature, (2) cross-simulation in which ϕ values at other heater temperatures are used and (3) the case where $\phi = F(T_H)$ is used for the simulation. (Thus for example, in Table 1, for a heater temperature of 78°C, the data source *Experiment* gives the observed results, $\bar{\phi}_{78}$ gives a simulation using the ϕ obtained from a 78°C experiment, $\bar{\phi}_{100}$ and $\bar{\phi}_{128}$ gives simulations of drying at 78°C using $\bar{\phi}$ values obtained from 100 and 128°C experiments and $\phi(T_H)$ gives simulation using ϕ values as a function of calculated heater temperature.)

At a heater temperature of 78°C all predictions were below the experimental value from 5 to 50 min. The fact that $\bar{\phi}_{78}$ gave the poorest simulation could indicate that tailing effects at the end of drying, especially when the scorch temperature is not reached, could be of importance. Simulated surface temperatures at the end of sublimation were in excellent agreement with the 46°C value obtained in the experiment; differences were less than 2°C.

At a heater temperature of 100°C, predicted times were again less than the experimental value. For constant $\bar{\phi}$ simulations the best prediction corresponded to the self simulation ($\bar{\phi}_{100}$). Sizeable improvements were obtained with the use of ϕ (T_H) which predicted times within 30 min of the experimental time for periods I and for the total drying time. Again, final heater temperatures were in excellent agreement with the experiment; differences varied less than 1·5°C.

TABLE 1. Experiment and computer simulations of batch freeze drying of skim milk

Heater temp.	Data source	Time in Stage I	Interface position Stage I	Surface temp Stage I	Heater temp Stage II	Time in Stage II	Total time
78°C	Experiment	13 : 25		46·0			13 : 25*
	$\bar{\phi}_{78}$	12 : 35		46·2			12 : 35
	$\bar{\phi}_{100}$	13 : 20		46·6			13 : 20
	$\bar{\phi}_{128}$	12 : 50		47·7			12 : 50
	ϕ_{TH}	12 : 50		46·9			12 : 50
100°C	Experiment	7 : 40	0·73	56·0†	88·0	3 : 45	11 : 30
	$\bar{\phi}_{78}$	6 : 00	0·70	56·0	88·4	4 : 00	10 : 00
	$\bar{\phi}_{100}$	6 : 10	0·69	56·0	87·9	4 : 30	10 : 40
	$\bar{\phi}_{128}$	5 : 30	0·65	56·0	86·6	4 : 50	10 : 20
	ϕ_{TH}	7 : 10	0·69	56·0	88·0	4 : 50	12 : 00
128°C	Experiment	3 : 20	0·45	56·0†	88·2	6 : 15	9 : 35
	$\bar{\phi}_{78}$	2 : 10	0·38	56·0	88·9	6 : 45	8 : 55
	$\bar{\phi}_{100}$	2 : 15	0·37	56·0	88·1	7 : 15	9 : 30
	$\bar{\phi}_{128}$	2 : 05	0·36	56·0	87·0	7 : 15	9 : 20
	ϕ_{TH}	3 : 10	0·38	56·0	88·0	8 : 20	11 : 30

$\bar{\phi}_{78} = 1294 \cdot 3$; $\bar{\phi}_{100} = 1387 \cdot 1$; $\bar{\phi}_{128} = 1365 \cdot 5$; $\phi_{TH} = 13202_{TH} + 3298$.

* No stage II present, so total time is equal to time in stage I.

† By definition, at end of stage I $T_s = 56$°C.

At a heater temperature of 128°C errors of more than 1 hr in period I times, using constant ϕ, were greatly reduced when $\phi = F(T_H)$ was used. However, the total times were overestimated by almost 2 hr while good results were obtained with mean ϕ values.

In summary, mean values of ϕ tend to produce slight underestimation in total times and sizeable underestimation in period I times. On the other hand, the relation $\phi = F(T_H)$ was extremely precise in the determination of period I times, but underestimated total times at low heater temperatures and overestimated them at high heater temperature.

Basically, the theory and numerical simulation methods for continuous freeze drying are the same as those for the batch process. In continuous freeze drying, it is not a single heater temperature that characterizes the process, but the existence of several 'zones', each at a particular constant heater temperature, and where the product resides for fixed (usually equal) periods of time.

Thus, the simulation of a continuous freeze drying can be performed with the same programme as developed for batch processes. The two parameters, temperature and time can be manipulated so as to calculate minimum total drying time with acceptable sample temperatures.

In this case, however, to evaluate the potentialities of the method a predetermined three-zone heater programme was used to dry a skim milk sample and then the same experiment was simulated in the computer using Ω and ϕ values from the batch experiments. Results, presented in Fig. 9, show the good agreement between experiment and simulation.

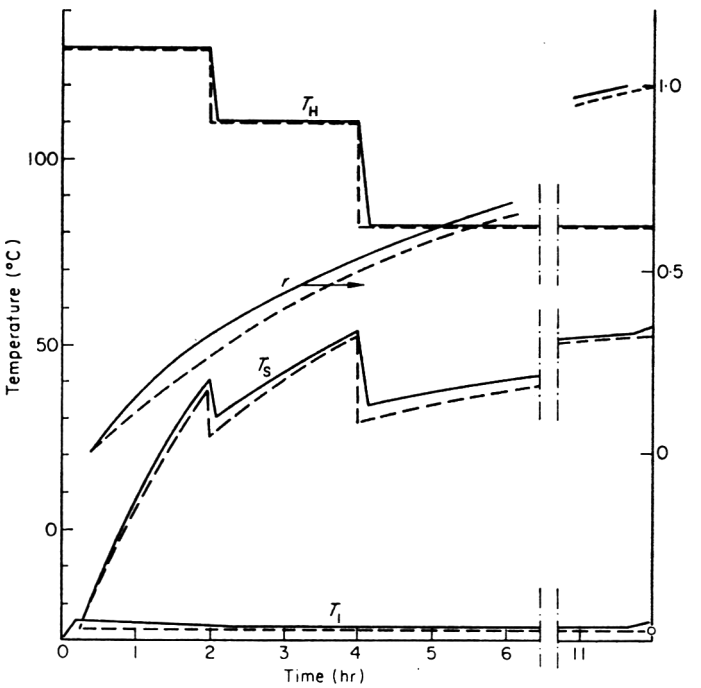

FIG. 9. A comparison of experiment with computer prediction for a simulated continuous freeze drying process (see text). ——, Experimental; – – –, simulation.

Discussion

The determination of drying parameters was made under a self-imposed constraint of simplicity; meaning in this case, the desirability of linear relationships between variables so that the slope of the straight line is numerically equal to the drying parameter.

The Ω parameter gave the desired behaviour in all cases. A general trend, noted by Aguilera (1973) shows that the linear relationship holds for various levels of heat transfer efficiency, the slope changing (i.e. Ω changing) to give revised drying times. The accuracy of the Ω parameter is reflected in the excellent agreement between experimental and calculated temperatures.

Since from simple theory Ω represents $k/\epsilon\sigma$, values of thermal conductivity can be calculated from Ω values, if a combined dryer efficiency (geometry) and heater emmisivity is equal numerically to the emissivity of the sample and is taken as 0·8 (used by

Gentzler & Schmidt, 1972). Calculated values of k vary from 0·0340 to 0·0354 kcal/m hr °K comparing well with recent values reported by Gentzler & Schmidt (1972) for freeze-dried evaporated skim milk (0·0450 kcal/m hr °K for transient determinations and 0·024 kcal/m hr °K for steady state methods).

With respect to the ϕ parameter, which is directly responsible for the time determination, some special factors which reduce the accuracy must be noted. The ϕ parameter is associated with the distribution of heat inside the sample. It is well known that there exist several sinks for heat conducted through the dried layer, the principal one being the sublimation of ice. As drying proceeds the relative importance of other heat sinks, especially the desorption of water, tend to increase as monolayer moisture values are reached. Thus, it is not surprising to find a tailing effect in the plot for ϕ determination (Fig. 7) in experiments where nonscorch conditions prevail over the entire sublimation period (such as 78°C). Another source of inaccuracy of the ϕ parameter is that ϕ is doubly dependent on the interface position ($r\,dr/dt$) whose accurate measurement is difficult due to both theoretical considerations and experimental difficulties.

The theoretical type considerations deal primarily with definition of the interface and its measurement. It has been postulated here that the interface passes a particular location (a thermocouple tip) when the temperature starts to rise above the ice front temperature. From the practical view point, the response time for descernible rise of the temperature places a degree of uncertainty on the time. Interface location can also be measured by weight loss, but here dry layer desorption will show some influence on presumed interface location.

The experimental difficulty which influences ϕ parameter determination relates to sample holder design giving a slightly uneven interface. While it has been demonstrated that the freeze drying is quasi-steady state (Fig. 5), with a constant ice front temperature (Fig. 4), the presence of a planar ice front was true for only a part of the process. Comparison of moisture loss (by weight measurements) with interface position (by thermocouple measurements) showed uniform drying for 2/3 of the thickness, followed by a nonuniform period. This difficulty is probably related to heat conduction in the sides and bottom of the sample holder. Thus an improved sample holder and definition of interface location will lead to improved values of ϕ.

In the practical case, the ϕ values obtained allowed simulation of the drying times. The small errors involved in self simulation are explicable by the use of average ϕ values, although in no case does the difference between calculated and experimental time exceed 50 min or an error of 8·0%.

Cross-simulation using average ϕ values was successful in predicting total times, but less accurate for period I times. This was due to the fact that the actual slopes in the ϕ determination plots were steeper in the first part of drying than the calculated mean value.

The slight refinements of the relationship between drying parameters (in this case ϕ)

and the measured drying variables (in this case T_H) gives a sizeable improvement in the subsequent computer drying simulations. Thus, the use of the linear function $\phi = F(T_H)$ enchanced the simulation of the period I times.

Conclusions

Computer simulations of freeze drying behaviour can be produced using simple equations and experimentally determined parameters for these drying equations. A minimum of three batch freeze drying experiments are necessary to obtain the linear relationship of the ϕ parameter.

The computer simulation has been used to predict the drying behaviour of a skim milk sample under conditions present in continuous freeze dryers, with very close agreement with experiment.

The technique of computer simulation will allow optimization of continuous freeze drying processes. In particular, it should be possible to contemplate achieving the improved economies of large continuous freeze dryers for small production runs of seasonable products, or for contract freeze drying by means of three rapid small batch freeze dryings (for parameter determination) followed by the computer simulation and optimization for the continuous freeze drying for production.

Acknowledgment

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Appendix

Symbol	Description	Units
k	Thermal conductivity	kcal/hr m °C
r	Interface position	Adimensional
t	Time	hr
T	Temperature	°K
ϵ	Emissivity	
σ	Stefan-Boltzmann constant	kcal/m² hr °K⁴
ρ	Density	g/cm³
ΔH	Heat of sublimation	kcal/kg
Ω, ϕ	Freeze drying parameters	

Subscript	Refers to
H	Heater
S	Surface
I	Ice
$^{1}, ^{2}, ^{3}, ^{4}, ^{5}$	Thermocouple position
sc	Scorch conditions

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Effects on bovine l. dorsi muscle of conventional and microwave heating

P. C. B. ROBERTS AND R. A. LAWRIE

Summary

A sequence of adjacent samples of beef l. dorsi muscle were alternately exposed either to conventional heating for periods of 0–70 min at temperatures between 45° and 90°C or to microwaves at a frequency of 2450 MHz for periods of 0–10 sec at power levels between 131 and 1050 W. Protein denaturation was assessed by measuring nitrogen distribution between sarcoplasmic and crude myofibrillar fractions and by electrophoresis; and concomitant superficial changes in free water and plasticity measured.

The attainment of a given temperature by microwave energy was associated with less detrimental change than when achieved by conventional heating; but this could be attributed merely to further progression of the same type of effects due to the time necessitated with the latter procedure and not to any qualitative difference. The findings suggest that microwave heating might permit microbial inactivation in meat products with minimum loss of organoleptic quality. It is also suggested that the observed initial resistance of myoglobin to heat denaturation and its subsequent loss of electrophoretic mobility between 75° and 88°C could be developed as an index of the temperature attained by meat products.

Introduction

Meat and poultry products are among those foodstuffs to which microwave heating has been extensively applied in the last decade (Decareau, 1970), the technique being of interest because the profile of temperature developed is the reverse of that obtained in conventional heating; and heating is faster. Thus Kierebinski (1969) found the rates of temperature rise to be five to ten times greater on the surface, and twenty times greater in the interior, of beef l. dorsi muscle cooked by microwaves at 2450 MHz than those obtained by boiling. In terms of higher rate of cooking, however, it has become clear that microwaves are likely to be of advantage economically when heat-processing

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relatively small portions of preformed, portioned or particulate meat or meat products.

Apart from economic considerations there has been interest in the relative nutritional effects of these two methods of applying heat to meats (cf. Hallmark & van Duyne, 1961); and, for a number of reasons, there is current interest in ascertaining whether or not their relative effects on the muscle proteins differ. The present work was undertaken to elucidate this point.

As measures of the more important organoleptic aspects of the protein, water-holding capacity and texture were determined: concomitant changes in the water-soluble and crude water-insoluble fractions were measured.

The compression of meat samples, supported on an absorbent surface and recording the free-water area, is an effective means of determining water-holding capacity (Grau & Hamm, 1953); and the area over which a known weight of meat can be spread in response to an applied force gives a good estimate of the texture or rigidity of the tissue (Hamm, 1956) and reflects the extent of myofibrillar coagulation (Hamm & Deatherage, 1960). A procedure, developed for measuring these two parameters simultaneously in the raw tissue (Roberts, Ritchie & Lawrie, 1972) was used in the present study.

Methods

General procedure

The entire right l. dorsi muscle was removed from the fully conditioned (6 days at 0°C) carcass of a Friesian heifer. The muscle (4637 g) was dissected in the cold into twenty-five sections of 180 g each. These were vacuum packed in 18 × 36 cm Cryovac bags, blast-frozen for 10 hr at −28°C and transferred to cold storage at −20°C to await analysis. (Since, clearly, investigation of all the samples could not be carried out simultaneously, storage at −20°C was deemed the best compromise.)

Individual samples were removed as required, the temperature raised to −1°C and weighed portions of lean muscle (10 g ± 0·05 g) transferred to tared Pyrex test tubes. These were capped with aluminium foil and held for 30 min in ice at 0°C to equilibrate.

Alternate tubed samples were treated by (i) immersion in a water-bath equipped. with a controlled temperature unit and circulating pump (Tecam TU8, Techne Ltd, Cambridge) or (ii) exposure to microwaves in an apparatus developed in collaboration with Rotax Ltd, Luton, from Rotax model No. MH/1500/50. Full details are given elsewhere (Roberts, 1972a).

Times of exposure to conventional heating were, 0, 10, 20, 30, 40, 50, 60 and 70 min; and temperatures of exposure were 45°, 50°, 55°, 60°,65°, 70°, 75°, 80°, 85° and 90°C.

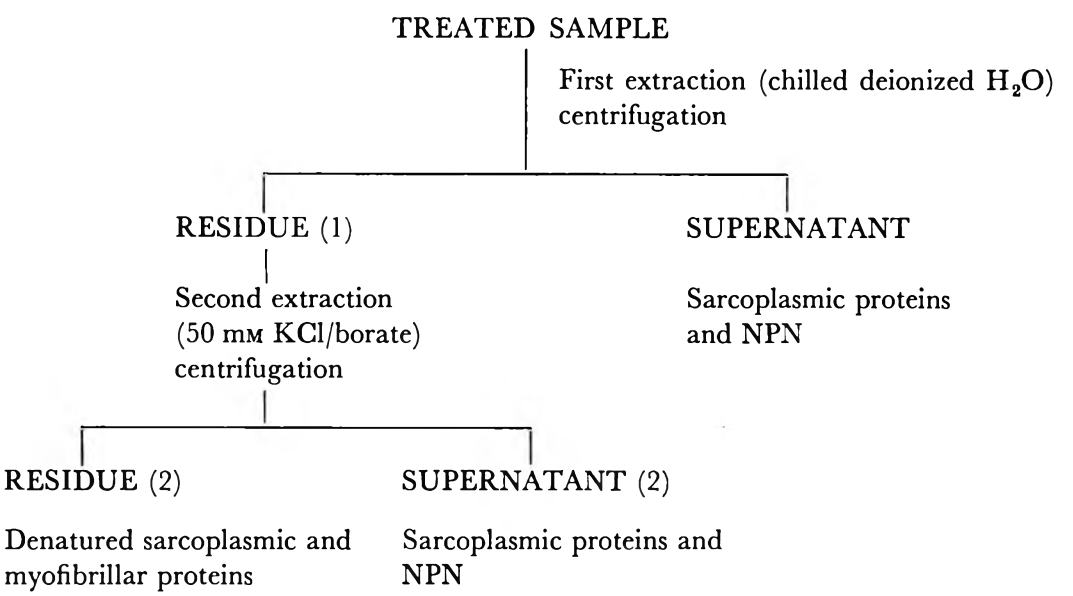
Times of exposure to microwave were 0, 2, 4, 5, 6, 7 and 10 sec; and power levels were 131, 267, 394, 525, 656, 788, 919 and 1050 W (in some cases 53 W). As it was not possible to monitor the temperature of samples exposed to microwaves, direct comparison with conventionally heated samples was based on the final temperature immediately after exposure.

All data reported below are the mean of duplicate determinations at each time × treatment combination.

Fractionation

Muscle samples, after treatment, as above, were transferred quantitatively to a 100 ml homogenizer vortex flask with 25 ml prechilled deionized water. The homogenizer was run for 20 sec at 2000 rev/min; and then for 40 sec at 14,000 rev/min. A further 5 ml of chilled deionized water was used to transfer homogenates to 50 ml centrifuge tubes. After standing for 1 hr at 2°C, these were centrifuged for 40 min at 18,700 rev/min. The supernatants were carefully decanted, filtered through cotton wool to remove fat, made up to 50 ml with distilled deionized water and retained for subsequent analysis. The extracts contained those components of the sarcoplasmic proteins which remained water soluble. The residues were extracted with 50 mM KCl/borate and yielded a further minor fraction of sarcoplasmic protein. This was not included in Fig. 5, but the water and KCl/borate extracts were pooled for electrophoretic investigation (below).

Samples of 500 mg of the residues from the KCl/borate extractions, representing crude myofibrillar protein and containing insoluble sarcoplasmic proteins (and connective tissue proteins) were weighed out for determination of plasticity index and free water. The fractionation scheme may be summarized thus:



Plasticity index and free water

The plasticity index (as a measure of toughness in the uncooked material) and free water (as a measure of water-holding capacity) were determined on the crude myofibrillar protein according to the procedure already published (Roberts *et al.*, 1972).

Electrophoresis

Electrophoresis of the sarcoplasmic extracts were carried out on thin, vertically oriented slabs of acrylamide, according to a procedure (Roberts, 1972b) modified from that of Akroyd (1968). Gels were stained with 1% w/v Naphthalene Black 10B in 7% v/v acetic acid.

Total nitrogen

This was determined after Kjeldahl digestion of 1 g samples. The ammonium ions in the digest were reacted with alkaline phenol and hypochlorite and the blue colour developed on heating measured at 625 nm (Varley, 1966). The latter two steps were automated using a Technicon MkI autoanalyser. pH was determined by glass electrode; for this purpose 2 g samples were homogenized for 60 sec in 20 ml neutralized 0·01 M iodoacetic acid.

Results

The effect, on the free water content of crude myofibrillar preparations from bovine l. dorsi muscle, of conventional heating at temperatures from 45° to 90°C for periods between 0 and 70 min are given in Table 1. Increase in free water represents a diminution of water-holding capacity in the system; and *vice versa*.

It is clear that the free water increased from *c.* 50% to *c.* 85% of the total water as the temperature was raised between 45° and 65°C. At temperatures between 65° and 90°C, there was some further increase in the free water. The *time* of heating—between 10–70 min—had no significant effect.

Corresponding data, showing the effect of microwave heating on this parameter, as

TABLE 1. The effect of time on free water content of crude myofibrillar preparations of bovine, l. dorsi muscle heated between 45° and 90°C (free water expressed as % total water)

Temperature (°C)	Heating time (min)							
	0	10	20	30	40	50	60	70
45	51	47	48	54	51	51	54	53
50	52	61	59	62	59	65	61	61
55	56	62	68	69	70	68	70	72
60	55	71	74	76	79	83	82	78
65	54	78	83	82	83	88	87	84
70	54	87	83	85	81	84	84	88
75	51	84	80	79	80	81	83	81
80	50	77	78	80	81	79	79	78
85	54	89	95	89	93	87	88	86
90	54	95	93	86	89	89	89	90

All values represent mean of duplicate treatments and determinations.

the microwave energy increased from 131 to 1050 W., are given in Table 2. There was concomitant increase in free water from 50% to 80% of the total water. With increasing time of exposure from 2 to 10 sec there was also a progressive loss of water-holding capacity as measured by the criterion of free water.

TABLE 2. The effect of time on the free water content of crude myofibrillar preparations of bovine l. dorsi muscle exposed to heating by microwaves at 2450 MHz between 131 and 1050 W (free water expressed as % total water)

Power	Exposure time (sec)							
(W)	0	2	4	5	6	7	8	10
131	54	53	57	—	53	—	57	54
262	50	55	58	—	55	—	57	64
394	55	57	61	—	62	—	67	72
525	51	57	62	—	66	—	77	79
656	52	55	67	72	73	78	82	82
788	53	57	67	74	78	77	80	82
919	51	55	82	82	84	85	87	86
1050	59	66	78	84	81	87	87	91

All values represent mean of duplicate treatments and determinations.

The corresponding effect of conventional heating on the plasticity index (as a measure of toughness: Roberts *et al.*, 1972) is shown in Table 3. There was a progressive decrease in the plasticity index (i.e. increasing toughness) from *c.* 75–95% to *c.* 40–45%, as temperatures were raised between 45° and 65°C: beyond the latter temperature, there was relatively little effect. While increasing time of heating caused decrease in plasticity at 45°C, this effect was independent of time of heating at higher temperatures. The decrease in plasticity was reciprocal with increase of free water.

Corresponding data, showing the effect of microwave heating on plasticity index, are given in Table 4. As the power of the microwaves was increased from 131 to 1050 W, there was a progressive decrease in the index (i.e. increasing toughness); and, with increasing time of exposure at a given energy level, there was a somewhat less marked decrease in plasticity.

The concomitant effects of conventional and microwave heating on the pH of bovine l. dorsi muscles are given in Tables 5 and 6 respectively. Again the greatest effect of conventional heating was between 45° and 65°C, when the pH of the system rose by *c.* 0·30 units. Between 65° and 90°C, there was some suggestion of a pH fall. No effect of *time* of heating, between 10 and 70 min was apparent.

A similar increment of 0·30 pH units was observed as the power of microwave radiation was increased from 131 to 656 W: thereafter, as the power was increased to 1050 W, there was some suggestion of fall in pH. A relatively minor effect of increasing time of exposure between 2 and 8 sec in increasing pH was also apparent.

TABLE 3. The effect of time on the plasticity index of crude myofibrillar preparations of bovine l. dorsi muscle heated between 45° and 90°C (plasticity index expressed as % mean value for control)

Temperature (°C)	Heating time (min)						
	10	20	30	40	50	60	70
45	97	78	79	82	74	80	76
50	77	72	73	69	61	67	73
55	63	62	56	54	54	60	61
60	64	58	56	56	55	52	49
65	55	49	47	44	46	43	38
70	47	43	43	43	41	39	39
75	40	42	42	42	42	39	40
80	46	45	42	41	40	40	42
85	43	41	41	42	43	43	43
90	41	42	41	43	42	41	42

All values represent mean of duplicate treatments and determinations.

TABLE 4. The effect of time on the plasticity index of crude myofibrillar preparations of bovine l. dorsi muscle exposed to heating by microwaves at 2450 MHz between 131 and 1050 W (plasticity index expressed as % mean value for control)

Power (W)	Exposure time (sec)						
	2	4	5	6	7	8	10
131	96	96	—	94	—	90	86
262	100	91	—	84	—	71	64
394	97	82	—	70	—	58	55
525	88	71	—	53	—	42	46
656	92	59	55	51	48	47	44
788	84	58	52	49	50	46	45
919	75	42	44	44	43	47	42
1050	72	49	48	46	44	44	42

All values represent mean of duplicate treatments and determinations.

The approximate temperatures attained during exposure to microwaves of different power and for different periods, are given in Table 7. The values have been used to compare directly the effects on the above parameters of conventional heating for 20 min with microwave heating, for less than 1 min in Figs 1–5. (The 20 min treatment was chosen because it represented the minimum treatment period required to ensure equilibrium of the centre temperature with that of the heating medium.)

It is clear from Fig. 1 that, whereas most of the increase in free water in crude myofibrillar preparations, occurs with conventional heating as the temperature is

TABLE 5. The effect of time on the pH of bovine l. dorsi muscle heated between 45° and 90°C

Temperature (°C)	Heating time (min)							
	0	10	20	30	40	50	60	70
45	5·52	5·57	5·60	5·63	5·63	5·64	5·63	5·67
50	5·57	5·68	5·70	5·79	5·74	5·74	5·74	5·76
55	5·47	5·66	5·65	5·66	5·68	5·71	5·70	5·70
60	5·56	5·78	5·82	5·82	5·79	5·80	5·81	5·83
65	5·61	5·84	5·84	5·84	5·93	5·93	5·90	5·92
70	5·61	5·96	6·10	5·93	5·94	5·91	5·91	5·96
75	5·58	5·97	5·94	5·95	5·95	5·91	5·87	5·87
80	5·59	5·97	5·95	5·95	5·97	5·94	5·91	5·91
85	5·51	5·86	5·88	5·86	5·87	5·87	5·88	5·86
90	5·56	5·77	5·78	5·79	5·81	5·83	5·83	5·83

All values represent the mean of duplicate treatments.

TABLE 6. The effect of time on the pH of bovine l. dorsi muscle exposed to heating by microwaves at 2450 MHz between 131 and 1050 W

Power (W)	Exposure time (sec)							
	0	2	4	5	6	7	8	10
131	5·59	5·58	5·55	—	5·54	—	5·62	5·62
262	5·48	5·49	5·44	—	5·49	—	5·56	5·61
394	5·53	5·59	5·64	—	5·72	—	5·77	5·77
525	5·61	5·62	5·68	—	5·81	—	5·90	5·94
656	5·66	5·81	5·89	5·92	5·93	5·91	5·91	5·93
788	5·63	5·62	5·79	5·86	5·87	5·90	5·92	5·96
919	5·58	5·65	5·85	5·85	5·87	5·89	5·89	5·88
1050	5·55	5·65	5·80	5·83	5·84	5·84	5·80	5·81

All values represent the mean of duplicate treatments.

TABLE 7. Effect of time on centre temperature of bovine l. dorsi after exposure to microwaves of 2450 MHz at various power levels

Power (W)	Exposure time (sec)				
	2	4	6	8	10
131	12	24	34	42	45
262	28	50	60	70	77
394	40	62	72	80	90
525	60	78	86	94	96
919	65	84	94	—	102

raised from 45° to 70°C, there is no such increase with microwave heating until temperatures rise above 70°C. Loss in plasticity showed an approximately reciprocal trend (Fig. 2), the plasticity index not appreciably decreasing in the samples heated by microwaves until temperatures exceeded 65°C, whereas, once again with the conventionally heated samples, the plasticity index decreased progressively between 45° and 70°C.

The data on pH (Fig. 3), notwithstanding the scatter of the data, reflect the same pattern; but there is some evidence for a fall in pH at temperatures above 80°C.

The relative distribution of the muscle proteins accords quite well with the evidence for heat-induced damage. The percentage of insoluble protein in the muscle (i.e. the crude myofibrillar fraction which also includes stroma denatured sarcoplasmic proteins, Fig. 4) increases sharply with conventional heating over 20 min at temperatures of heating between 50° and 70°C; but there is some suggestion of a subsequent decrease at temperatures above 80°C. The values for insoluble protein increase much more gradually with increasing temperature in the microwave samples and even at 100°C have not attained those of the conventionally heated samples at 65°C. On the other hand the corresponding percentage of water-soluble sarcoplasmic protein falls relatively more sharply with temperatures between 45° and 65°C, achieved by conventional heating: with microwave heating there is little loss of the water solubility of sarcoplasmic proteins before 65°C.

In Plate 1 photographs of the electrophoretic patterns obtained for sarcoplasmic proteins after conventional heating for 0–70 min at (a) 45°C, (b) 65°C (c) 75°C and (d) 85°C, are presented. It is clear that after heating at 65°C for 20 min or more, most of the sarcoplasmic proteins fail to appear on the gel. Virtually the only stained band still prominent at 65°C is myoglobin. Although this still persists in electrophoretograms of extracts from sarcoplasmic proteins heated to 75°C (Plate 1c) it is no longer visible after heating to 85°C for even 10 min (Plate 1d).

The electrophoretic patterns reflect the marked loss in solubility of sarcoplasmic proteins which occurs with conventional heating, for 20 min or more, between 45° and 65°C (Fig. 5): the subsequent slow loss in solubility above 65°C is largely accounted for by the denaturation of myoglobin between 75° and 85°C.

Plate 2 depicts electrophoretic patterns of sarcoplasmic proteins obtained from meat exposed to microwaves at 2450 MHz and 788 W for 0–10 sec. The corresponding nominal temperatures measured after these treatments are also given. These suggest that myoglobin survives higher temperatures when the exposure time is of the order of seconds.

Discussion

It is apparent, first of all, that loss of water-holding capacity, and hardening of texture, accompany denaturation and insolubilization of the two major groups of protein in

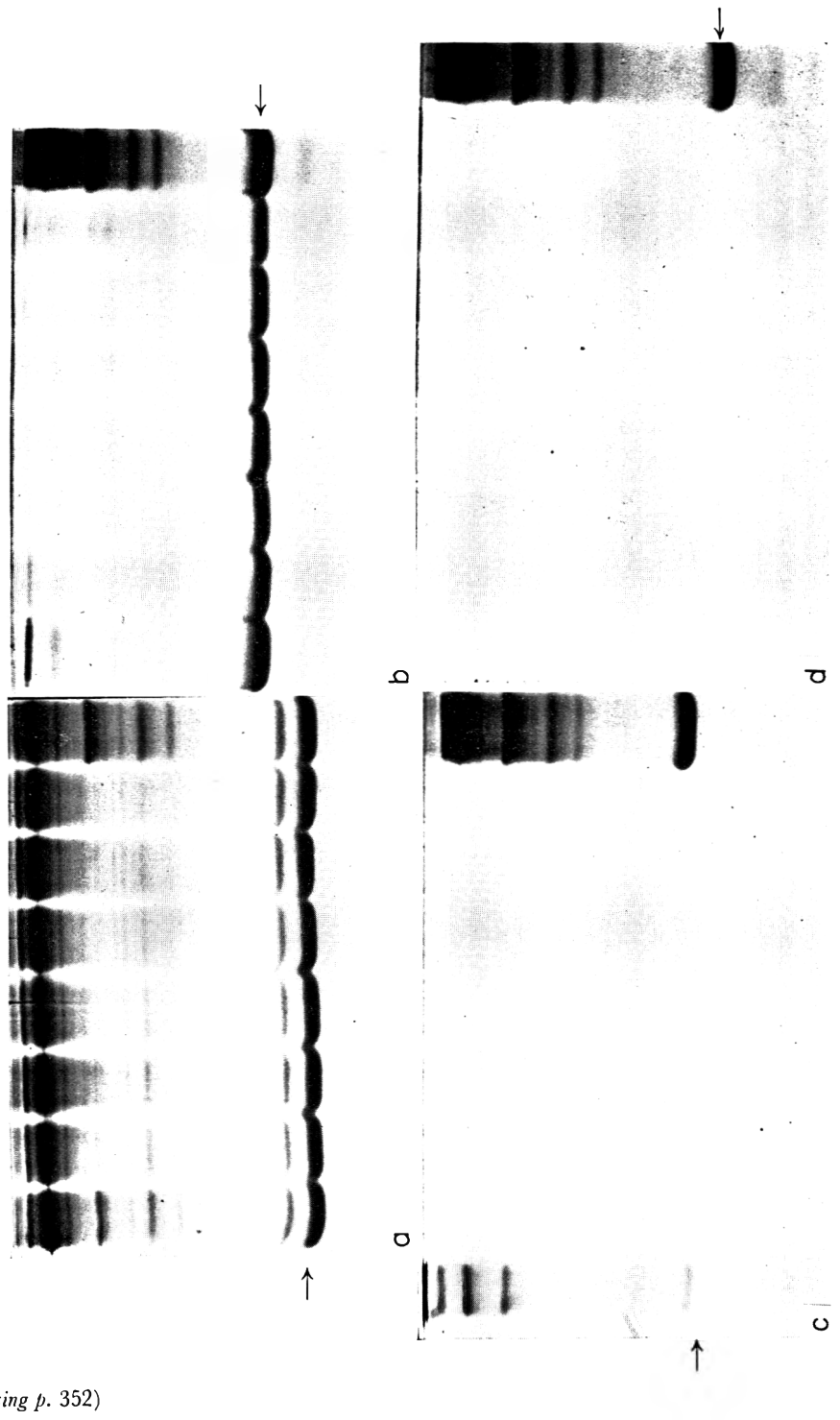


PLATE 1. The separation by vertical, flat-bed acrylamide gel electrophoresis of sarcoplasmic proteins after conventional heating of bovine l. dorsi muscle at various temperatures for 10–70 min. (a) 45°C, (b) 65°C, (c) 75°C, (d) 85°C. Slots (L to R): 10, 20, 30, 40, 50, 60, 70 0 min heating. Arrows indicate myoglobin.

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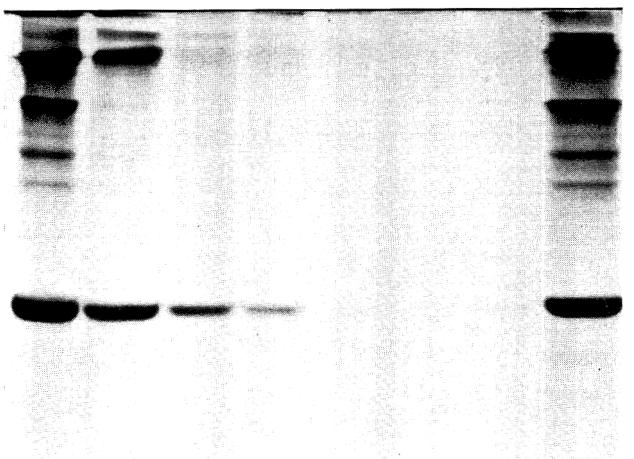

PLATE 2. The separation by vertical, flat bed acrylamide gel electrophoresis, of sarcoplasmic proteins after exposure of bovine l. dorsi muscle to 2450 MHz microwaves at 788 W. Slots (L to R): 2, 4, 5, 6, 7, 8, 10 and 0 sec: corresponding nominal temperature, 61°, 75°, 81°, 91°, 92°, 97°, 101°, 18°C respectively.

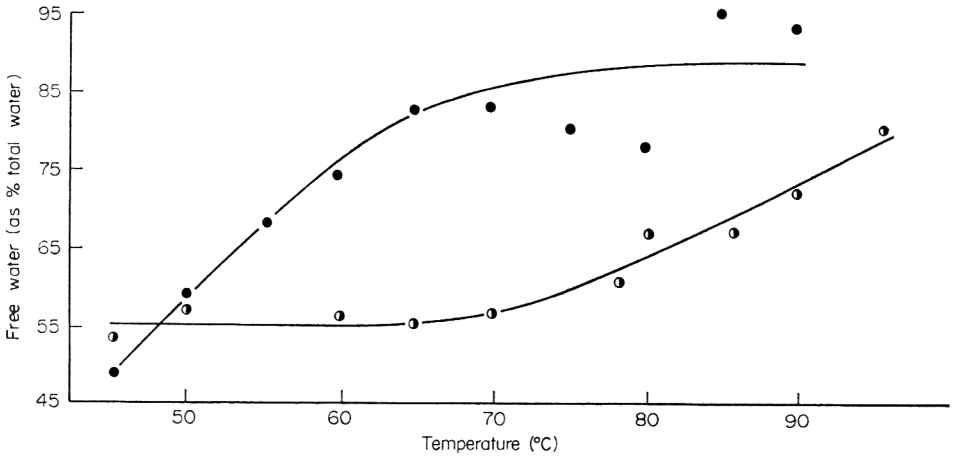

FIG. 1. Comparative effects of temperature on water-holding capacity of crude myofibrillar fractions from bovine l. dorsi muscle induced by conventional heating for 20 min (●) and heating by microwaves at 2450 MHz for less than 1 min (◑). Free water expressed as percentage of total muscle water.

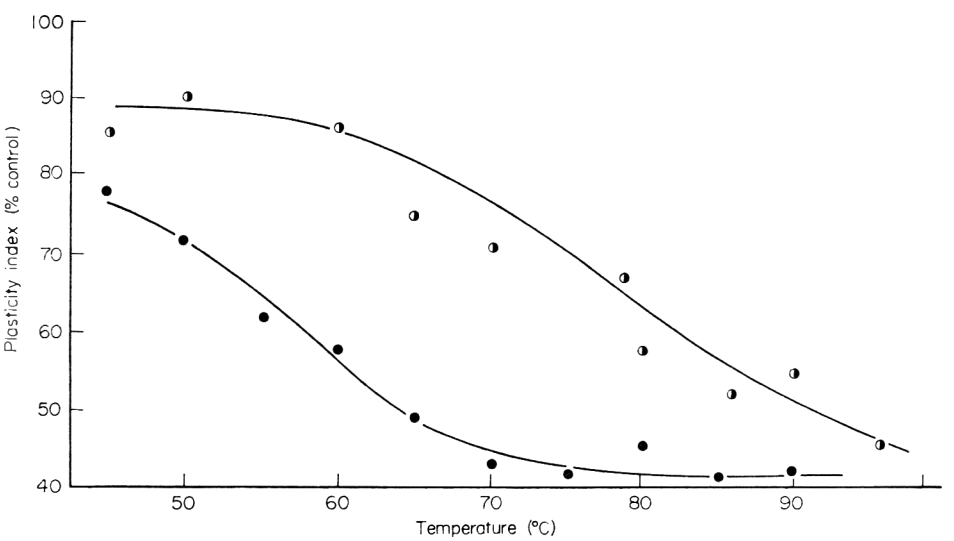

FIG. 2. Comparative effects of temperature on plasticity index of crude myofibrillar fraction from bovine l. dorsi muscle induced by conventional heating for 20 min (●) and heating by microwaves at 2450 MHz for less than 1 min (◑). Plasticity index expressed as percentage of value in unheated control.

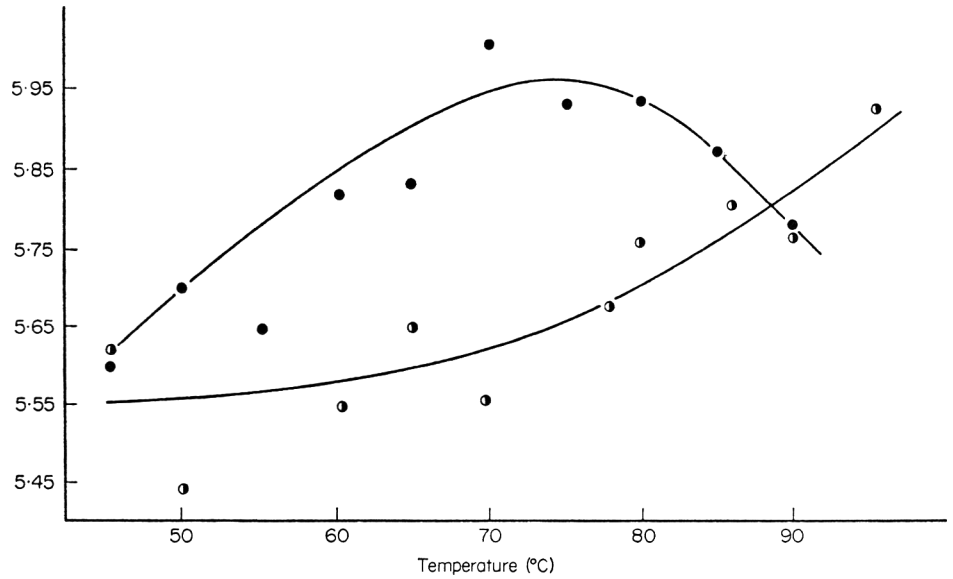

FIG. 3. Comparative effects of temperature on pH of l. dorsi muscle induced by conventional heating for 20 min (●) and heating by microwaves at 2450 MHz for less than 1 min (◑).

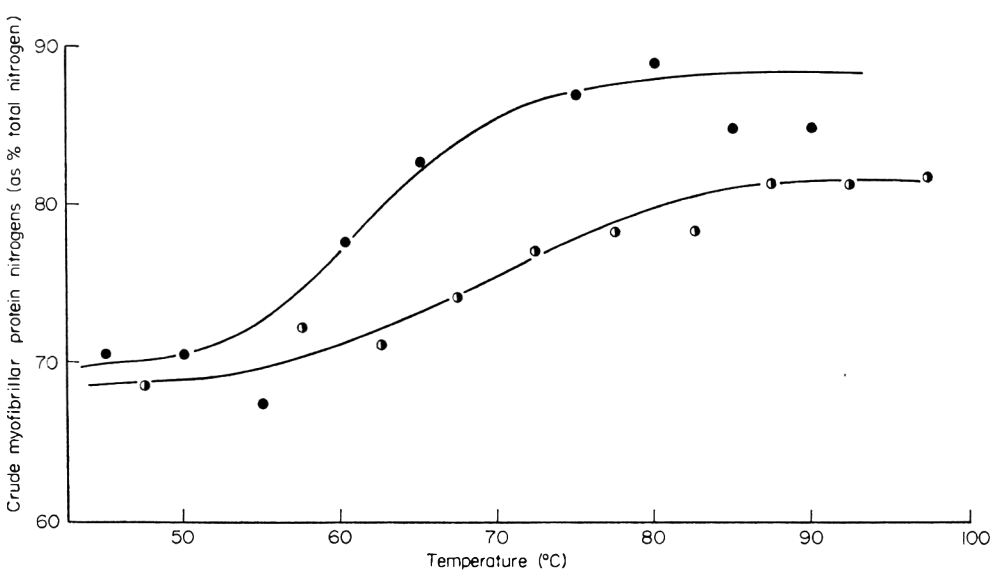

FIG. 4. Comparative effects of temperature on relative percentage of crude myofibrillar protein fraction from bovine l. dorsi muscle induced by conventional heating for 20 min (●) and heating by microwaves at 2450 MHz for less than 1 min (◑). Crude myofibrillar protein nitrogen expressed as percentage of total muscle nitrogen.

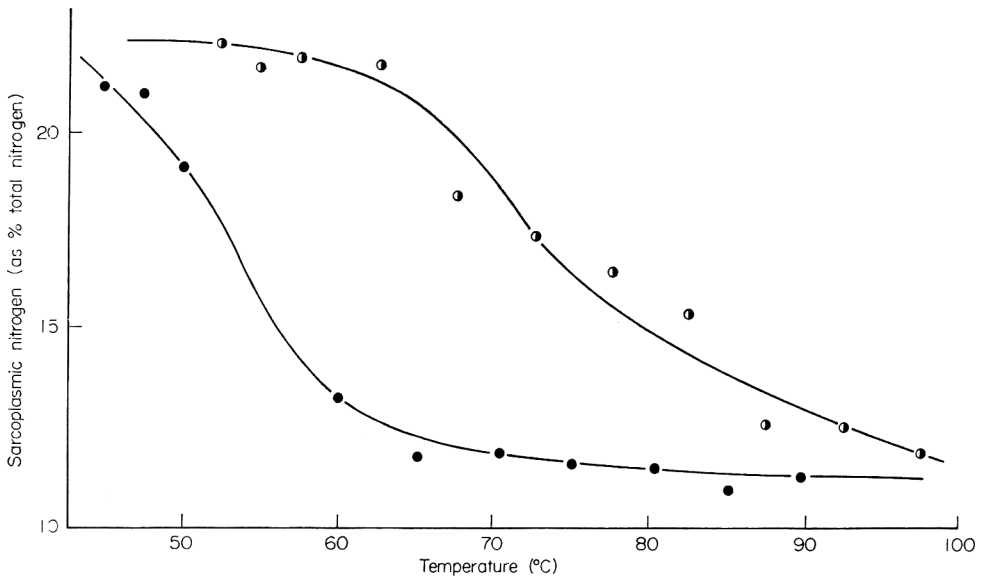

FIG. 5. Comparative effects of temperature on relative percentage of water soluble sarcoplasmic protein fraction from bovine l. dorsi muscle induced by conventional heating for 20 min (●) and heating by microwaves at 2450 MHz for less than 1 min (◑). Sarcoplasmic protein nitrogen expressed as percentage of total muscle nitrogen.

muscle—water-soluble (sarcoplasmic) and water-insoluble (crude myofibrillar)—whether induced by conventional or microwave heating. This applies over the entire range of times, temperatures and energy levels studied; and is not unexpected.

On the other hand, it is evident that the attainment of a given temperature by microwave heating is associated with a markedly greater retention of the original properties of the proteins than by conventional heating. This represents no fundamental difference in action, however. Clearly, with conventional heating, where the heat is generated outside the muscle and has to penetrate within, it takes longer for a given temperature to be developed in the interior. The total time/temperature combination sustained by the proteins is thus greater—and has a correspondingly greater denaturing effect—than with microwave heating. With the latter, heat is generated swiftly throughout the tissue, and preferentially at the centre, wherever the microwaves encounter water (or other reactive dipoles). As with conventional heating, increased time of microwave exposure leads to increased damage (Tables 2, 4 and 6).

Nevertheless, the markedly increased time involved in attaining higher temperatures in the range studied with conventional heating may well induce some conversion of collagen to gelatin. This would tend to diminish the overall proportion of insoluble protein in the system; and may account for the slight fall in the percentage of crude myofibrillar protein detected at temperatures above 80°–85°C with conventional heating (Fig. 4). It may also account for the tendency for the pH of such samples to fall

at temperatures above 80°C (Fig. 3); whereas those of the microwave samples are concomitantly increasing (presumably reflecting changes in the myofibrillar and sarcoplasmic proteins).

Another indication of possible differences in the effects of microwave and conventional heating at higher temperatures of the system is shown by their effect on myoglobin (Figs 6, 7). Whereas, after conventional heating, myoglobin denatures between 75° and 85°C, it retains its original electrophoretic mobility at nominal temperatures which are about 10° higher when these are achieved by microwave energy. Once again, however, this difference can be attributed to the lower degree of overall damage sustained by proteins when heat is applied in high temperature/short time procedures.

Similar considerations of heat transfer may explain the observations of Bernofsky, Fox & Schweigert (1959): they found that myoglobin could withstand higher temperatures in solution than *in situ* in meat.

The possibility that myoglobin denaturation could be employed as an index of whether or not temperatures of 75°–85°C had been attained in a meat product clearly arises from the present findings; but it is likewise evident that the interpretation of such an index would require a knowledge of the mode of heating employed.

By analogy with HTST and UHT processes with milk, microwave heating would appear to offer advantages where micro-organisms in meat were to be inactivated with minimum loss of the attributes of eating quality. Not all micro-organisms, however, would be so affected before a significant degree of cooking had occurred (Roberts, 1970).

Acknowledgment

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Post mortem changes and spoilage in rock lobster muscle

I. Biochemical changes and rigor mortis in *Jasus novae-hollandiae*

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Summary

Glycolysis in rock lobster comprising the oblique extensor, oblique flexor and enveloping muscles of *Jasus novae-hollandiae* was studied under anaerobic conditions at 20°, 15° and 0°C. After bisecting the muscles along the medio-saggital plane one section was treated by dipping for 4 min in 3% sodium chloride solution containing 15 ppm oxytetracycline (treated). The other (untreated) was immersed in sodium chloride solution containing no antibiotic. In the treated muscles lactate production showed a linear relation to the fall in pH over a range of 7·2–6·0, a fall in one unit of pH corresponding to 118 μmol of lactate per g of tissue. In spite of a relatively high production of lactate the ultimate pH did not fall below 6·0 because of a high buffering capacity of the muscle.

Breakdown of phosphoraginine and ATP and accumulation of lactate and inorganic phosphorus at 20° and 15°C followed patterns similar to those observed in mammalian and poultry muscle but at 0°C these processes were slowed down to a much greater extent. There was no accumulation of glucose 6-phosphate.

Concentrations of arginine rose as a result of phosphoarginine breakdown in both treated and untreated samples. However, in the untreated samples at 20° and 15°C, arginine concentrations started to fall 24 hr post mortem probably due to bacterial attack; the fall being more rapid at 20°C.

When mounted in a specially designed rigorometer at 20° and 15°C the muscle strips started to shorten concurrently with only a slight drop in ATP concentrations. The extent of shortening did not exceed 10% of the original and in some cases the contraction was immediately followed by a slow lengthening.

The findings of this study are discussed in relation to glycolysis and rigor mortis in mammalian and fish muscles.

The following abbreviations are used in this paper: ATP, adenosine 5′-triphosphate; ADP, adenosine 5′-di-phosphate; PEP, phosphoenolpyruvate; NAD^+ and NADH, oxidized and reduced forms of nictotinamide-adenine dinucleotide; Q_{10}, increase in rate constant for a 10-degree Celsius change in temperature.

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Introduction

Post mortem physical and biochemical changes in muscle have a profound effect on the edible quality of meat from mammals, fish, and poultry and extensive experimental data and reviews are available on the general phenomenon of rigor mortis and associated biochemical changes in the muscles of these animals (Bendall, 1960; de Fremery & Pool, 1960; Amlacher, 1961; Partmann, 1965; Newbold, 1966; Tarr, 1966; Marion, 1967). However comparable studies on crustacean muscle have been few and fragmentary, and mainly confined to nucleotide breakdown (Tarr & Comer, 1965; Arai, 1966a, b; Dingle, Hines & Fraser, 1968; Porter, 1968; Groninger & Brandt, 1969; Stone, 1970). The principal reason for the emphasis on this aspect is that some breakdown products of nucleotides have been thought to enhance the edible quality of marine foods while others cause deterioration (Kuninaka, Kibi & Sakaguchi, 1964; Kuninaka, 1966; Burt & Simmonds, 1969; Spinelli, Pelroy & Miyauchi, 1969).

International trade in frozen and processed forms of crustacea has expanded considerably in recent years and the trend is likely to continue. The way rock lobster is processed and marketed at present requires rapid cooling, freezing, prolonged periods of storage and transport of the products over long distances. A better understanding of post mortem changes and their relationship to eating quality might help in establishing the best processing procedure.

Onset of rigor mortis in rock lobster or prawn cannot be detected by physical examination because no stiffening is apparent, and it must, therefore, be defined through biochemical criteria. A satisfactory way of finding the onset is to determine when the glycolytic metabolites fall below a critical level and when isolated muscle strips begin to shorten. This paper describes biochemical and rigorometer studies on isolated sections of rock lobster muscle.

Materials and methods

Animals and muscle samples

Adult rock lobsters were transported alive by air from Hobart, Tasmania, and maintained at the CSIRO Food Research Laboratory, North Ryde, in glass tanks containing aerated sea water at 15°C. When required the animals were killed by excising the cephalothorax from the abdomen. After freeing the abdomen from the exoskeleton and gut, the left and right oblique extensor, oblique flexor and enveloping muscles were bisected along the mediosaggital plane. One section (treated) was dipped for 4 min in 3% sodium chloride solution containing 15 ppm oxytetracycline and the other (untreated) was dipped for the same period in 3% sodium chloride solution. Both the treated and untreated sections were then transferred to petri dishes and placed in a desiccator over water through which a continuous stream of nitrogen was bubbled.

The muscle tissues were stored in this way at three experimental temperatures 20°, 15° and 0°C, and samples were removed periodically for analysis.

Enzymes

Adenosine triphosphate: arginine phosphotransferase (EC 2.7.3.3, arginine kinase) was prepared from rock lobster muscle by a slight modification of the method of Uhr, Marcus & Morrison (1966). Enzymatically active fractions from the eluate of the first DEAE cellulose column were pooled and the enzyme precipitated by the addition of solid ammonium sulphate. The precipitate was dissolved in N-ethyl-morpholine-HCl buffer (pH 8·0) and dialysed. The preparation was tested for myokinase activity and was found to be essentially free of myokinase at 100 times the concentration normally used for testing arginine kinase activity. All other enzymes and biochemicals were purchased from the Sigma Chemical Co., U.S.A.

Preparation of extracts

One g of muscle was homogenized for two periods of 30 sec each in 20 ml 0·6 N-perchloric acid in a Bühler homogenizer. The homogenate was filtered immediately into 5 ml 1M-K_2CO_3, and the pH adjusted to 6·5 using a Radiometer TTTlc titrator. The extract was refrigerated overnight to precipitate potassium perchlorate, then filtered and stored at 1°C after adding a drop of 20% (w/v) sodium azide.

Enzymatic methods

Glucose 6-phosphate, ATP and phosphoarginine were measured sequentially in muscle extracts by using the enzymes glucose 6-phosphate dehydrogenase (EC 1.1.1.49), hexokinase (EC 2.7.1.1) and arginine kinase as described by Lamprecht & Trautschold (1963).

ADP and arginine were determined by the following reactions:

$$\text{Arginine} + \text{ATP} \xrightleftharpoons{\text{arginine kinase}} \text{phosphoarginine} + \text{ADP}$$

$$\text{ADP} + \text{phosphoenolpyruvate} \xrightarrow{\text{PEP kinase}} \text{ATP} + \text{pyruvate}$$

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightleftharpoons{\text{lactic dehydrogenase}} \text{lactate} + \text{NAD}^+$$

Conditions were selected according to Adam (1963) to push the equilibrium far to the right so as to reduce all the pyruvate formed.

Lactate was determined using the method described by Newbold & Scopes (1967).

The enzymatic methods were adapted wherever convenient for use on the Technicon Auto Analyser.

Chemical methods

For glycogen estimation muscle samples were digested according to the method of Good, Kramer & Somogyi (1933) and the colour developed by the anthrone reagent of Carroll, Longley & Roe (1956). Since variations in manual mixing of sample and reagent were observed to effect the final colour, the method was automated.

Arginine in muscle extracts was estimated by the method of Rosenberg, Ennor & Morrison (1956). This chemical method gave values not significantly different from those obtained by the enzymatic method but it was found to be more convenient.

Inorganic phosphorus (Pi) was estimated by the method of Knight & Woody (1958). The chemical methods mentioned above were adapted for use in the Technicon Auto Analyser with minor modifications.

Measurement of pH

Two g of muscle tissue was homogenized in 20 ml 5 mM-sodium iodoacetate solution and the pH measured on a Radiometer Model TTTlc with a scale expander (Copenhagen, Denmark).

Rigor measurements

The rigorometer designed by Bate-Smith & Bendall (1949) to measure post mortem shortening and loss of elasticity in mammalian muscle was found to be unsatisfactory because of the unusual viscoelastic properties of rock lobster muscle. Clips used to hold a strip of muscle cut through the fibres and the loads of 10 g and 2 g applied during the 'load on' and 'load off' cycles respectively stretched the muscle continuously. It was apparent that a machine with greatly increased sensitivity and with comparative freedom from friction was required. For this purpose a sensitive two-pan air-damped balance was modified (Fig. 1) by removing the heavier series of weights and substituting a linear motion transducer (A) supported by a clamp (B) fixed to the balance column. The core (C) of the transducer, narrowed to give an annular clearance of 0·033 cm,

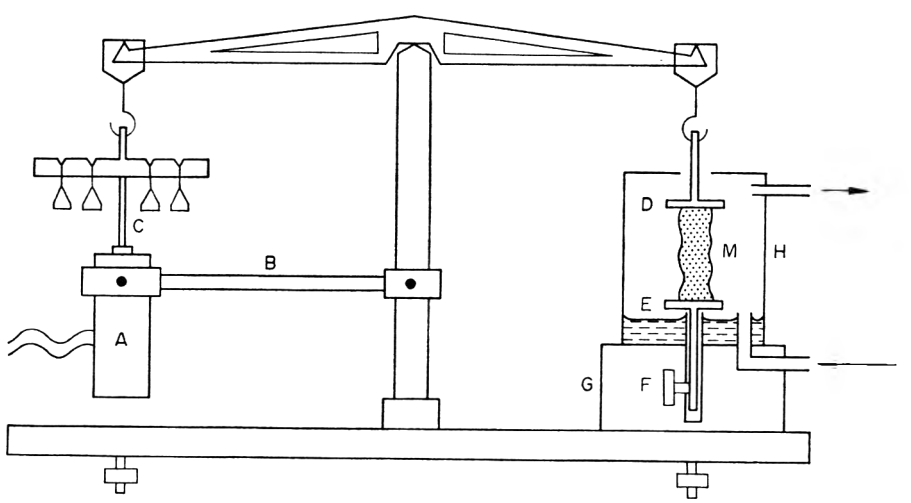

FIG. 1. Diagrammatic sketch of the rigorometer. A, Linear motion transducer; B, clamp; C, core of the transducer; D and E, upper and lower stainless steel plates; M, muscle; F, clamp; G, fixed bridge; H, glass cover.

was suspended from a hook on the left pan supports. From the hook on the right pan supports a stainless steel plate (D) was suspended. One end of the muscle strip (M) (3·5–4 cm long × 0·5–0·7 cm thick) was glued to plate D using a cyano-acrylate adhesive (Eastman 910) and the other end glued to a bottom plate (E). Only 10 sec contact was required to stick the muscle to the plates. The stem of plate (E) could be clamped with a screw (F) to the bridge (G) over the right pan and fixed to the base of the balance. Before clamping E, the weight of the muscle was balanced by adding weights to the left hand pan. Changes in the length of the muscle could now be transmitted through the transducer to a Siemens 6-channel recorder. The muscle and mountings were enclosed in a glass chamber (H) into which humidified oxygen-free nitrogen was passed. The whole equipment was placed in a constant temperature room. Before each experiment a micrometer screw gauge was used to work out a relationship between the distance moved by the core of the transducer and any change in the readings of the recorder.

Buttkus (1963) and Burt *et al.* (1970) have used apparatus similar in design to measure isometric rigor tensions in the muscles of ling cod (*Ophiodon elongatus*) and cod (*Gadus morhua* L.).

Results and discussion

Initial values

When rock lobsters were removed from the sea water they showed signs of stress by intermittent tail flipping. Approximately 20 min elapsed between the time the rock lobster was killed and the time (designated as zero time) the muscle samples were weighed and homogenized in perchloric acid. Under these conditions some breakdown of glycogen and phosphoarginine was unavoidable. The mean values for metabolites and their standard deviations at zero time are given in Table 1. ATP values in rock lobster are higher than those reported for king crab (*Paralithodes camtschatica*), crab (*Erimacrus isenbeekii*), prawn (*Pandalus hypsinotus* and *Penaeus aztecus*), (Porter, 1968; Arai & Saito, 1961; Flick, 1969), scallop (*Pecten* sp.), (Arai & Saito, 1961; Hiltz & Dyer, 1970); and higher also than those reported for carp (*Cyprinus carpio* L.), abalone (*Haliotis discus hannai*), and squid (*Ommastrephes sloani pacificus:* Steenstrup), (Arai & Saito, 1961), and for the blowfly (*Phormia regina*), (Sacktor & Hurlbut, 1966). They approach those reported for some vertebrates e.g. chicken, rabbit (de Fremery & Pool, 1960) and in some instances beef (Bodwell, Pearson & Spooner, 1965), and are much higher than those observed for whale, horse, pig, and rat muscle (Long, 1961).

Values for phosphoarginine in rock lobster muscle (Table 1) are much higher than the corresponding values for this metabolite or creatine phosphate reported for vertebrates and some other crustaceans (Long, 1961; Flick, 1969). In some muscle samples from resting cod (*Gadus morrhua*) creatine phosphate values approaching those of phosphoarginine in rock lobster have been reported (Fraser *et al.*, 1966). In muscle extracts obtained from tails frozen in liquid air immediately after removal from the live

TABLE 1. Constituents of rock lobster muscle measured at 20 min post mortem

Constituent	No. of samples analysed	Concentration μmol/g fresh wt*
Phosphoarginine	26	26·5±1·3
ATP	26	8·79±0·2
Glucose 6-phosphate	22	2·64±0·2
Arginine	22	36·8±1·7
ADP	16	1·05±0·16
Lactate	23	15·5±1·5
Glycogen (as glucose)	12	36·4±6·5
Inorganic phosphate	16	43·6±2·2
Total soluble phosphate	20	129·0±2·0
pH (units)	20	7·13±0·03

* Nos prefixed by ± show standard deviation.

rock lobster (*Jasus verreauxi*), a phosphoarginine/arginine ratio of 63 : 37 was obtained by Marcus & Morrison (1964) and a similar ratio was observed in scampi (*Nephrops norvegicus*) by Robertson (1961). The mean ratio of 42 : 58 calculated from the data in Table 1 indicates that some breakdown of phosphoarginine occurred.

Total acid soluble P in rock lobster muscle was almost twice the concentration observed in mammals and cod (Long, 1961; Fraser *et al.*, 1966).

Effect of temperature on lactate production and pH

Production of lactate and changes in pH in the treated muscle samples maintained at 0°, 15° and 20°C are shown in Fig. 2. At higher temperatures, not only was the rate of lactate production faster but also the total amount produced was greater than at the lower temperatures. At 0°C only about 60% of the glycogen present initially (Table 1) appeared as lactate while at 15° and 20°C the values were close to 90%. This indicates the more active operation of alternate pathways of glycogen breakdown at 0°C resulting in the production of compounds other than lactate in rock lobster muscle. The existence of such pathways has been reported by Burt (1966) and Burt & Stroud (1966) in cod muscle. In the treated muscle samples bacterial activity concerned with the production of volatile bases was greatly retarded and their production very much reduced. The pH at the three temperatures in these samples was related to lactate as shown in the following regression equations:

$$0° \quad pH = 6{\cdot}81 - 0{\cdot}00846\ (L - 60{\cdot}3)$$
$$15° \quad pH = 6{\cdot}61 - 0{\cdot}00846\ (L - 62{\cdot}5)$$
$$20° \quad pH = 6{\cdot}31 - 0{\cdot}00846\ (L - 80{\cdot}1)$$

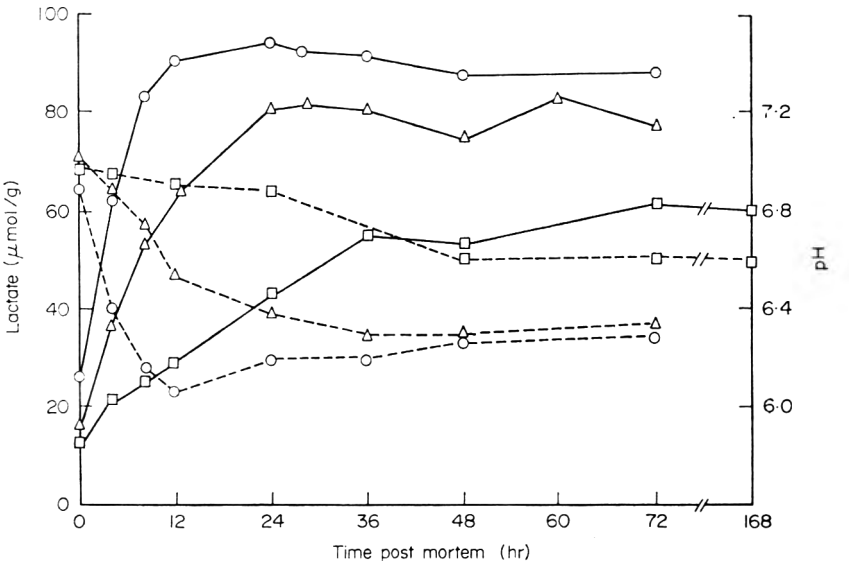

FIG. 2. Time course of changes in concentrations of lactate in treated muscle (———) and in pH in treated muscle (– – –) kept at 0°C (□), 15°C (△), and 20°C (○). Each point represents the mean value of four to eight muscles.

Where L = lactate in μmol/g tissue. Differences between regression coefficients for pH and lactate were not significant among the three temperatures, the mean value being $0{\cdot}00846 \pm 0{\cdot}0008$ for $n = 57$ d.f. This indicates that 118 μmol of lactate/g tissue resulted in a fall of 1 pH unit in the pH range of 7·2–6·0. For mammalian and fish muscles the corresponding values for lactate range from 60–65 μmol/g (Bate-Smith & Bendall, 1956; Newbold & Scopes, 1967; Fraser *et al.*, 1967). The high buffering capacity of the rock lobster muscle presumably brought about by high levels of amino acids and inorganic phosphate accounts for the relatively small drop in pH in relation to lactate production.

In the untreated muscle samples the rate and amount of lactate produced did not differ from the treated samples but bacterial growth accompanied by the production of volatile bases especially during the latter stages of the experimental period resulted in a rise in pH after an initial fall.

In mammalian and fish muscles with high glycogen contents glycolysis stops when an ultimate pH of about 5·5 is reached even though some residual glycogen may be still left in the muscle (Newbold & Lee, 1965; Partmann, 1965). In the rock lobster muscle even after complete disappearance of glycogen it was observed the pH seldom fell below 6·0.

The rate of glycolysis as measured by lactate production and fall in pH was much slower at 0° in rock lobster muscle than at 15° and 20°C (Fig. 2). In beef muscle it has

been shown that in the range from 5° to 37°C the pH fell more slowly the lower the temperature, but at 1°C the rate of pH fall was faster during the first 10 hr of glycolysis than at 5°C and was similar to the rate at 15°C (Cassens & Newbold, 1967; Newbold & Scopes, 1967).

Phosphoarginine, ATP and glucose 6-phosphate

Post mortem changes in the concentration of these three metabolites in both treated and untreated samples were similar and pooled results are shown in Figs 3 and 4. Breakdown of phosphoarginine was very rapid at 20°C but considerably slower at lower temperatures. It took 6, 12 and 72 hr at 20°, 15° and 0°C respectively for phosphoarginine to drop to a concentration of approximately 1 μmol/g. For ATP to decrease to a concentration of 0·5 μmol/g the time required at these three temperatures was 12, 32 and 168 hr respectively. Compared with its behaviour at 15°C the behaviour of rock lobster muscle at 0°C with respect to phosphoarginine and ATP breakdown is quite different from that of beef and poultry muscles. In the latter two the rates of creatine phosphate and ATP breakdown were lowest at 5°–10°C. However, cod muscle resembles rock lobster in this respect (Partmann, 1965).

As glycolysis proceeded, glucose 6-phosphate concentration rose to a peak reached after 4, 8 and 24 hr at 20°, 15° and 0°C respectively (Fig. 4). After reaching their peaks glucose 6-phosphate concentrations showed a fall more or less parallel to the fall in concentrations of ATP at the three temperatures. In beef muscle the concentration

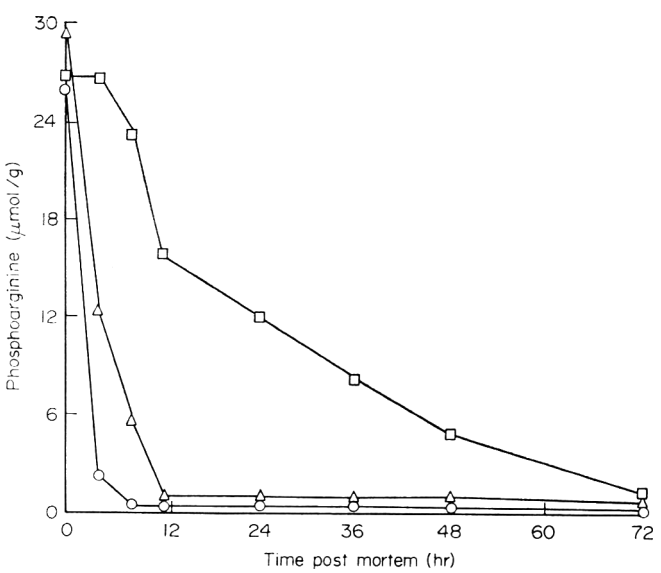

FIG. 3. Time course of changes in the concentrations of phosphoarginine in both the treated and untreated muscle samples at 0°C (□), 15°C (△), and 20°C (○). Each point represents the mean value of four to eight muscles.

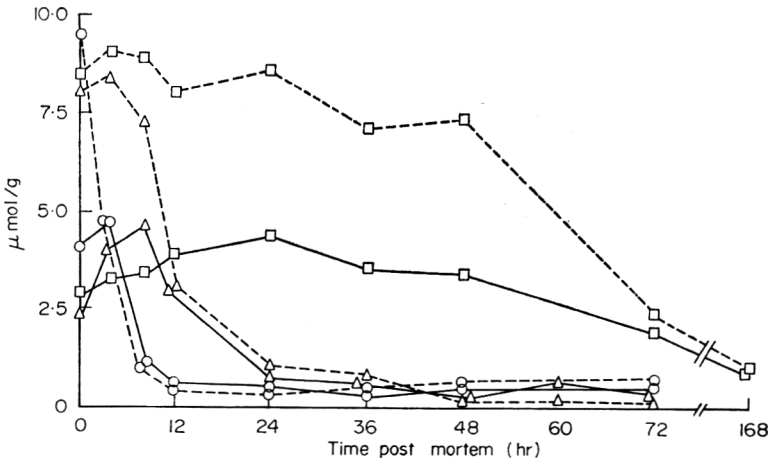

FIG. 4. Time course of changes in the concentrations of ATP (– – –) and glucose 6-phosphate (———) in both the treated and untreated samples at 0°C (□), 15°C (△), and 20°C (○). Each point represents mean value of four to eight muscles.

of hexose 6-phosphate at 1° and 5°C showed a gradual increase reaching a maximum of up to 9 μmol/g when the ATP concentration had fallen to a low value (Newbold & Scopes, 1967). These differences could be explained by slight variations in the rates of synthesis and breakdown of glucose 6-phosphate in these two muscles.

Inorganic phosphorus and arginine

Changes in both treated and untreated muscle samples in Pi concentration with storage time at the three temperatures are depicted in Fig. 5. Increase in Pi concentrations reflect the breakdown of phosphorylated metabolites, mainly phosphoarginine and ATP (Figs 3 and 4). At 0°C the increase in Pi concentration occurred at a much slower rate than that observed at 15° and 20°C. The final concentration was higher at 20° than at 0°C indicating that at the end of the experimental period at the latter temperature more metabolites remained unhydrolysed.

Increase in arginine concentration (Fig. 6) at the three temperatures in the initial stages is related to the breakdown of phosphoarginine both in treated and untreated samples. However, after the breakdown of phosphoarginine and ATP in the untreated samples arginine concentration started to fall as a result of bacterial breakdown. The fall in concentration was more rapid the higher the temperature. In the treated samples the fall in concentration was very small and occurred only at 20° and 15°C towards the end of the experimental period.

Rigorometer studies

Changes in a 35 mm strip of muscle placed in the rigorometer at 15°C are shown in Fig. 7. Concentrations of ATP and phosphoarginine determined at the same time

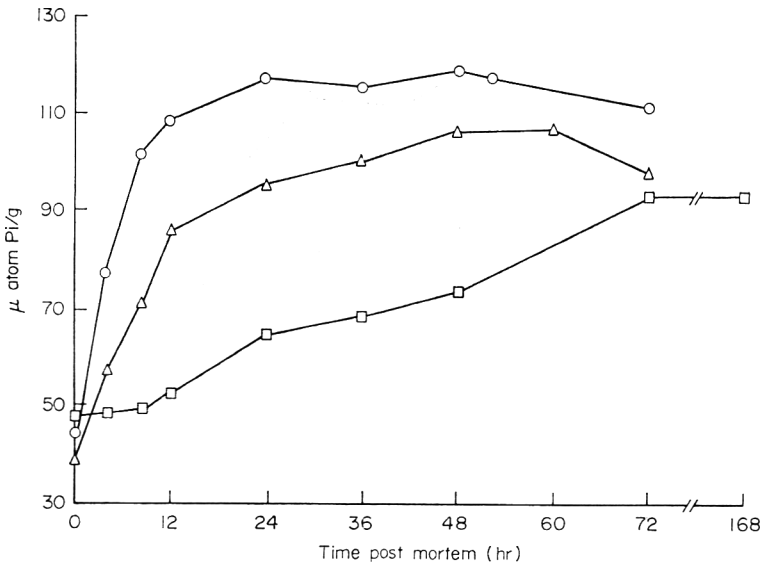

FIG. 5. Time course of changes in the concentrations of Pi in both the treated and untreated muscle samples at 0°C (□), 15°C (△), and 20°C (○). Each point represents mean value of four to eight muscles.

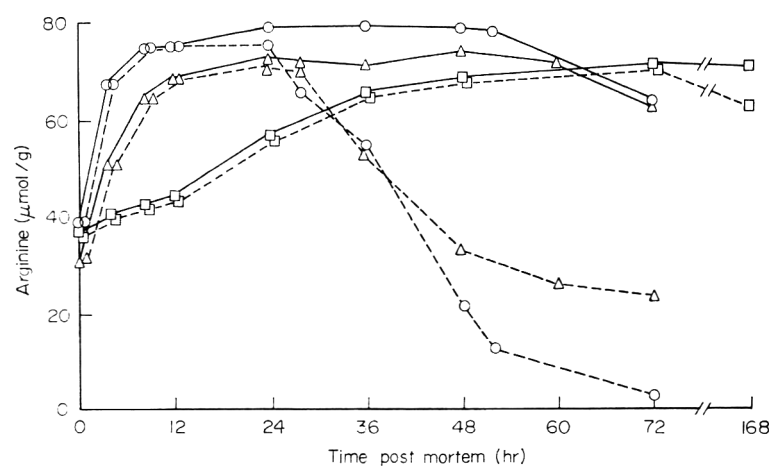

FIG. 6. Time course of changes in the concentrations of arginine in the untreated (– – –) and the treated (———) muscle samples at 0°C (□), 15°C (△), and 20°C (○). Each point represents the mean value of two to four muscles.

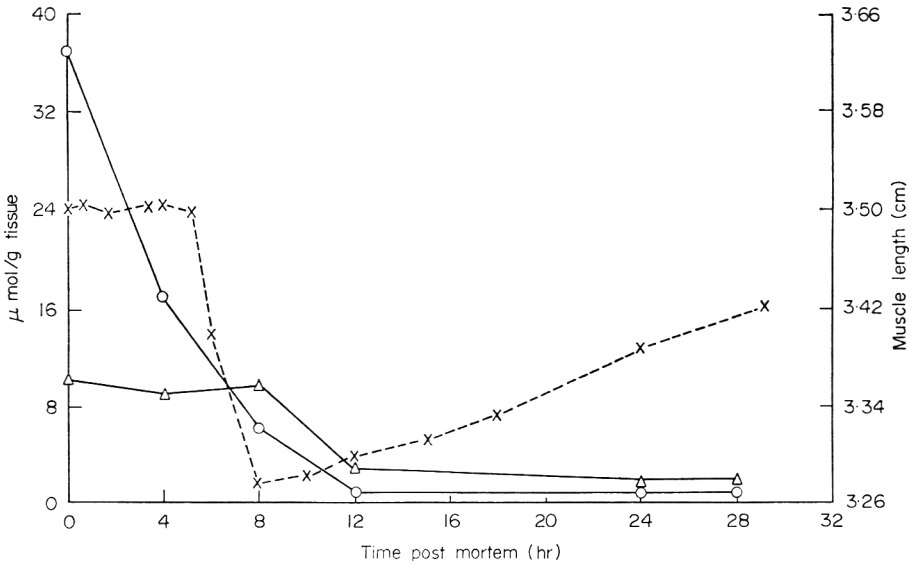

FIG. 7. Time course of changes in muscle length (×), and concentrations of phosphoarginine (○), and ATP (△), in paired whole muscles from the same rock lobster kept at 15°C. One muscle was mounted in the rigorometer and the other was used for the determination of phosphoarginine and ATP.

intervals in a similar paired muscle from the same animal are also shown. Shortening of the muscle started when no decrease in the initial ATP concentration had occurred but the phosphoarginine concentration had fallen to about 38% of the initial level. Shortening was complete by approximately 8 hr post mortem and amounted to 7% of the initial length. The muscle started lengthening after this period. In some other muscle strips lengthening did not occur and they remained in a shortened condition till the end of the experimental period. Behaviour of the treated and untreated muscles was similar in this respect and it was unlikely that bacterial breakdown of muscle fibres caused this lengthening. The time of onset and time of commencement of resolution of rigor at the three temperatures studied are given in Table 2.

The pattern of post mortem glycolytic changes in rock lobster with respect to lactate production, fall in pH, breakdown of phosphorylated intermediates and accumulation of Pi are generally similar to that observed in most mammalian and fish muscles. However, there are specific differences which result from the different physico-chemical nature of rock lobster muscle. The rigor in this muscle does not manifest itself to the same extent as in mammalian muscle with regard to extent of shortening and the force developed (Sidhu & Montgomery, 1967). Shortening is usually followed immediately by lengthening and this may explain why post mortem stiffening is not observed in rock lobsters.

TABLE 2. Times of onset of rigor and the commencement of resolution of rigor

Temperature	Hr post mortem		No. of observations
	Onset	Commencement of resolution	
20°C	3·7	10·5	5
15°C	4·9	8·6	3
0°C	Very small shortening occurred over a long period		

The rate of development of rigor in warm-blooded animals and fish varies greatly from one species to another, from one individual to another within the same species and also from one muscle type to another within the same animal. Ambient temperature affects the rates of glycolysis and rigor development differently in different animal species. The Q_{10} values between 0° and 20°C for ATP breakdown were 2·52, 2·64, 1·66 and 1·44 for carp muscle, white muscle of rainbow trout, beef muscle, and white breast muscle of hen respectively (Partmann, 1961). In rabbit muscle Q_{10} values for glycolysis, creatine phosphate and ATP breakdown range from 1·55 to 1·63 (Bendall, 1951; Bate-Smith & Bendall, 1956). For rock lobster muscle Q_{10} values for lactate production, ATP and phosphoarginine breakdown in the present investigation range from 2·8 to 3·4 in the temperature range 0°–20°C. This indicates that rapid cooling to 0°C would be more effective in slowing glycolytic changes in rock lobster muscle than muscles from other animals mentioned above.

Contrary to earlier indications it seems that ATP concentrations vary markedly in muscles from fish, pork and beef entering rigor and factors other than ATP alone are responsible for bringing about post mortem stiffening (Newbold, 1966; Jones *et al.*, 1965). In rock lobster ATP concentrations did not register any appreciable fall at 15°C and were falling at 20°C when isolated muscle strips started shortening. However, when the shortening was complete phosphoarginine levels had fallen very low (Fig. 7). This fits with the observations made by Newbold (1966) on the fact that creatine phosphate virtually disappears from various muscles when post mortem shortening is complete.

Increase in the rate of lactate production and breakdown of ATP and creatine phosphate below 15°C in beef and lamb muscles has been ascribed to the activation of contractile ATP-hydrolase of the myofibrils (Newbold, 1966; Bendall, 1971) due to the inefficient working of the calcium pump at lower temperatures. Presumably low temperatures do not affect rock lobster and cod muscles in the same way as they affect beef and lamb muscles. Whether or not there are basic differences in the working of the calcium pump at lower temperatures between cold-blooded and warm-blooded animals remains to be resolved. The significance of biochemical changes observed during this investigation in relation to spoilage is discussed in the second paper of this series.

Acknowledgments

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Post mortem changes and spoilage in rock lobster muscle

II. Role of amino acids in bacterial spoilage and production of volatile bases in the muscle of *Jasus novae-hollandiae*

G. S. SIDHU, W. A. MONTGOMERY AND M. A. BROWN

Summary

In an attempt to elucidate the pattern of spoilage in rock lobster (*Jasus novae-hollandiae*), samples of abdominal musculature consisting of flexor, extensor and enveloping muscles were subjected to two treatments; half the musculature, after bisecting along the mediosaggital plane, was immersed for 4 min in 3% sodium chloride solution (untreated) and the other half in 3% sodium chloride containing 15 ppm oxytetracycline (treated). After being drained, the muscles from different lobsters were stored under nitrogen, some at 20°C or 15°C for 72 hr, and some at 0°C for 216 hr.

In the untreated samples stored at 20°C or 15°C the concentration of total volatile bases (TVB) and trimethylamine (TMA) rose sharply after 24 hr storage. In both the untreated and treated samples at 0°C for up to 168 hr post mortem and in treated samples at 15°C for up to 56 hr post mortem TVB concentrations remained well below 30 mg N/100 g muscle. The TMA concentrations did not rise above 5 mg N/100 g muscle during the entire experimental period in samples held at 0°C.

The increase in total bacterial counts correlated well with the rise in the concentrations of TVB and TMA in the untreated samples.

Changes in the concentrations of free amino acids showed that bacterial breakdown of arginine into ornithine and ammonia accounted for a very high proportion of ammonia present in the TVB.

The significance of these observations to rock lobster spoilage is discussed.

Introduction

A wide spectrum of physical and biochemical changes occur from the time a rock lobster is removed from sea water till the time it is classified as spoiled. These changes are brought about mostly by the combined action of enzymes present endogenously in the muscle and those produced by bacteria contaminating the flesh. The nature of

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bacterial spoilage and its relationship with post mortem biochemical changes has not been studied in any detail in rock lobster muscle. Papers published, however, on general spoilage of other decapod crustaceans such as different species of prawns and scampi (*Nephrops norvegicus*) showed that flesh from these crustaceans spoiled at a faster rate than that from cod and other teleosts under similar conditions (Fieger & Novak, 1961; Vyncke, 1968; Walker, Cann & Shewan, 1970). The high rate of spoilage has been ascribed to a higher proportion of α-amino acids in the non-protein nitrogen (NPN) fraction of crustacean muscle (Simidu, 1961). For example, fresh rock lobster muscle contains 60–80 μmol/g free arginine and/or phosphoarginine (Sidhu, Montgomery & Brown, 1974) whereas cod muscle contains hardly any detectable arginine (Shewan & Jones, 1957). In rock lobster muscle stored at 20°C and 15°C, arginine showed an initial rise due to breakdown of phosphoarginine but it started to fall sharply 24 hr post mortem. This suggested that arginine, because of its susceptibility to enzymatic cleavage (Barker, 1961; Ramaley & Bernlohr, 1966) could form an important substrate for bacterial growth and might constitute the main source of total volatile bases (TVB) in the form of ammonia.

Attempts have been made to use changes in the concentration of amino acids in fish during storage as indices of spoilage (Ranke, 1955; Shewan & Jones, 1957; Cohen & Peters, 1962; Bramstedt, 1961). In prawn (*Penaeus aztecus*) and blue crab (*Callinectes sapidus*) a picric acid turbidity method, presumably based on changes in free amino acids and non-protein nitrogen (NPN) substances, was found to be a reliable spoilage index (Kurtzman & Snyder, 1960a, b; Bethea & Ambrose, 1962). Loss of α-amino NPN from prawns (*Metapenaeus dobsonii* and *Penaeus indicus*) has been associated with deterioration of organoleptic quality (Nair & Bose, 1965; Pillai, Nair & Choudhury, 1965). However, information on the role of individual free amino acids in bacterial spoilage of crustacean muscle is lacking. This paper describes results of a study undertaken on rock lobster muscle to determine the relationship between spoilage at different temperatures with changes in the free amino acids.

Materials and methods

Rock lobster and preparation of material

Methods of keeping rock lobsters and preparation of muscle samples have been described in Part I of this paper (Sidhu *et al.*, 1974). In the present studies, abdominal muscles were bisected along the mediosaggital plane, and one section of each was dipped in 15 ppm of oxytetracycline in 3% sodium chloride solution at room temperature (treated) for 4 min while the other was dipped in 3% sodium chloride solution for the same period (untreated). After being drained, both treated and untreated sections were transferred to desiccators in an atmosphere of nitrogen saturated with water vapour and stored in rooms maintained at 20°, 15°, and 0°C. Samples were taken at intervals for analysis.

Amino acid analysis

Perchloric extracts for amino acid analyses were prepared by the method previously described (Sidhu *et al.*, 1974). To obtain good separation of ornithine from the other basic amino acids on a Beckman 120 C analyser, a 12·5 cm column packed with PA35 Beckman resin was used with 0·35 M citrate buffer adjusted to a pH of 4·5. Unidentified peptides in perchloric acid extracts interfered with the separation of acidic and neutral amino acids in the 4 hr programme of the Beckman Analyser. To remove the peptides an aliquot of the extract was hydrolysed with 2 ml 6 N HCl in a sealed, evacuated tube for 20 hr at 110°C and the hydrolysate was subjected to amino acid analysis.

TVB and trimethylamine (TMA)

Total volatile bases and TMA were estimated by the method of Montgomery (1960) after extracting 5 g tissue samples in trichloroacetic acid (TCA). Ammonia was determined either by difference between the values obtained for TVB and TMA or along with basic amino acids in the Beckman Analyser.

Urea

Urea in the neutralized TCA extracts was converted into ammonia with urease obtained from Sigma Chemical Co., U.S.A., and the ammonia thus produced was estimated by a modified procedure of Conway (1962).

Bacterial counts

For total viable bacterial counts 5–10 g tissue was accurately weighed, homogenized in 200 ml sterile saline using aseptic techniques and after serial dilutions were made, pour plates were prepared in nutrient agar medium. Plates were incubated at 37°, 30°, and 25°C for 2–4 days and colonies counted.

Results and discussion

TVB and TMA

Production of TVB and TMA at the three temperatures i.e. 20°, 15° and 0°C in treated and untreated muscle samples is depicted in Figs 1 and 2. During the first 24 hr there was little change in the level of TVB in the untreated muscle samples maintained at 20° and 15°C but after this period TVB rose sharply, the level reached being much higher at 20°C at 72 hr post mortem than at other temperatures. In the treated samples at all the three temperatures and in untreated samples at 0°C the production of TVB remained low during the entire experimental period. Even after 168 hr (7 days) TVB were below 30 mg N/100 g muscle in untreated samples maintained at 0°C. This level has been considered the upper limit above which some fisheries products are considered as spoiled and unfit for human consumption, though agreement on this is not universal.

The production of TMA followed a pattern similar to that of TVB. In untreated

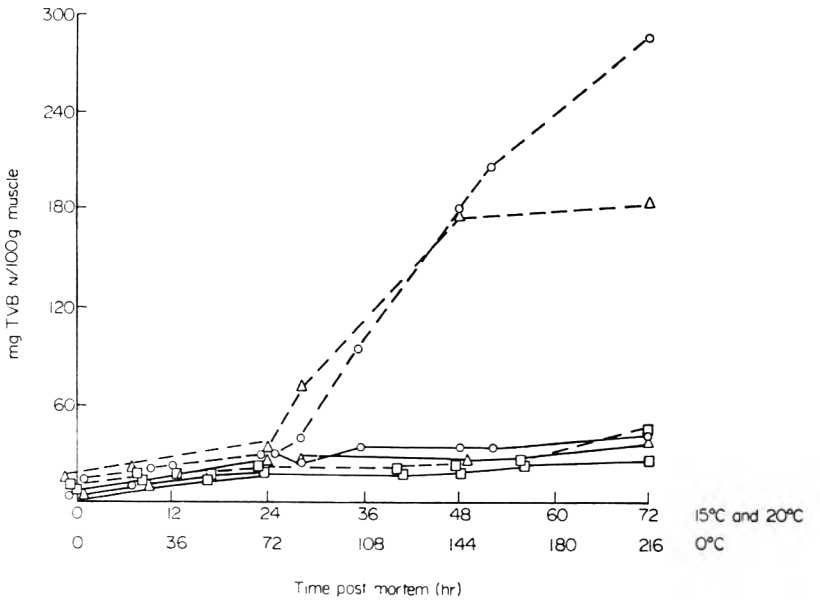

FIG. 1. Time course of changes in concentrations of TMA in treated (———) and untreated (– – –) muscle samples stored at 20°C (○), 15°C (△), and 0°C (□). Each value represents a mean of duplicate determinations made on muscles from three lobsters.

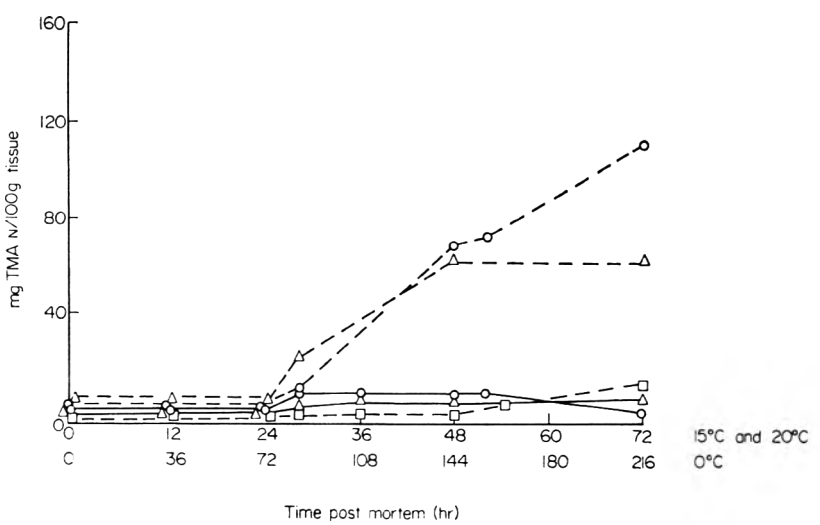

FIG. 2. Time course of changes in concentrations of TVB in treated (———) and untreated (– – –) muscle samples stored at 20°C (○), 15°C (△), and 0°C (□). Each value represents a mean of duplicate determinations made on muscles from three lobsters.

samples at 20° and 15°C the TMA production rose rapidly 24 hr post mortem and at 48 hr it constituted about 36% of the TVB nitrogen. In treated samples at 20° and 15°C TMA production remained below 5 mg N/100 g muscle for periods of up to 72 hr post mortem. In untreated samples at 0°C TMA could only be detected 168 hr (7 days) post mortem and not at all in the treated samples at the same temperature.

Total bacterial counts

Total bacterial counts on treated and untreated samples kept at 20° and 15°C are shown in Fig. 3. In untreated samples the counts correlate well with the production of

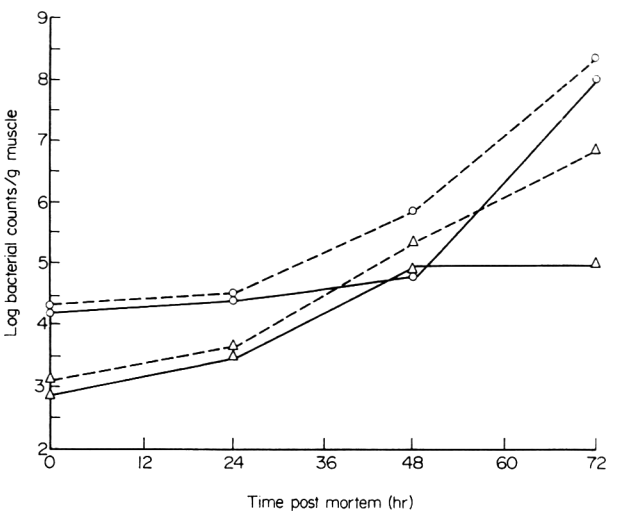

FIG. 3. Time course of changes in bacterial counts in treated (———) and untreated (– – –) muscle samples stored at 20°C (○), and 15°C (△). Each value is an average of determinations on muscle samples from three lobsters.

TVB and TMA observed in Figs 1 and 2. However, in treated samples kept at 20°C, though the total bacterial counts at the end of the experimental period were high, the corresponding production of TVB and TMA was comparatively low. This indicates that the types of bacteria which multiplied on these samples were mainly those which produced little TVB and TMA. In both treated and untreated samples kept at 0°C, total bacterial counts at the end of 9 days did not exceed 1×10^4. Castell & Greenough (1957), using washed cell suspensions, showed that oxytetracycline did not itself inhibit the bacterial reduction of trimethylamine oxide to TMA. Obviously reduction in TVB and TMA production in treated samples was brought about by a selective reduction in the numbers of bacteria producing these compounds. These observations are at variance with those made on prawns (*Pandalus* sp.) treated by dipping for 2 min in 5%

brine containing 2 ppm chlorotetracycline. Little difference either in the extension of storage life or production of TVB was observed between treated and untreated prawns (Farber, 1954). The dipping solution used by the author did not come into contact with bacteria in the gut of the whole prawns, and the low concentration of antibiotic used was presumably ineffective against surface bacteria.

Amino acids

Table 1 shows changes in the concentrations of 'free' amino acids, ammonia, and urea in treated and untreated samples stored at 20°C for 4 and 48 hr post mortem. These

TABLE 1. Amino acid analysis of extracts from rock lobster muscle stored at 20°C. Each value is an average of determinations made on extracts from four lobsters

Amino acids (μmol/g)	Treated		Untreated	
	4 hr	48 hr	4 hr	48 hr
(A) Neutral and acidic				
Taurine	3·4	4·3	5·2	3·0
Aspartic acid	5·5	8·1	7·3	5·9
Threonine	3·6	5·5	3·6	5·4
Serine	6·5	6·1	6·8	4·7
Glutamic acid	21·2	24·6	17·7	25·0
Proline	13·9	14·9	13·0	18·1
Glycine	86·7	88·1	96·0	95·0
Alanine	11·2	18·9	8·9	21·5
Half cystine	0·2	0·2	0·4	0·3
Valine	6·8	8·7	6·8	8·7
Methionine	Trace	Trace	Trace	Trace
Isoleucine	3·6	4·2	2·6	4·6
Leucine	4·1	6·2	2·9	7·0
Tyrosine	0·3	0·2	Trace	0·7
Phenylalanine	Trace	0·4	0·9	Trace
Total	167·0	190·4	172·1	199·9
(B) Basic				
Ornithine	0·6	0·6	0·5	51·0
Lysine	3·6	4·0	2·6	4·5
Histidine	2·6	3·4	3·2	3·7
Arginine	62·1	64·2	61·8	14·2
Total	68·9	72·2	68·1	73·4
Total A and B	235·9	262·6	240·2	272·3
(C) Ammonia	16·5	27·2	12·3	108·0
Urea	1·1	0·9	0·6	0·9

time intervals were selected on the basis of observations reported earlier (Sidhu *et al.*, 1974). By the end of 4 hr post mortem most of the phosphoarginine has broken down to arginine in rock lobster muscle maintained at 20°C and by the end of 48 hr post mortem most of the arginine had disappeared from untreated samples as a consequence of bacterial multiplication causing spoilage.

The total concentrations of amino acids increased by 11·2 and 13·8% in treated and untreated samples respectively during the period of observation. Changes in the concentrations of individual neutral and acidic amino acids in both the treated and untreated samples were slight and similar. There were small increases in the concentrations of threonine, glutamic acid, alanine, isoleucine and leucine from 4 hr to 48 hr post mortem. Major changes, however, occurred in some of the basic amino acids and in ammonia in the untreated samples. The arginine concentration fell from 61·8 μmol to 14·2 μmol/g tissue and ornithine concentration rose from 0·5 to 51·0 μmol/g tissue in the untreated samples during a period of 4–48 hr post mortem. As there was little accumulation of urea or citrulline it was obvious that arginine had quantitatively broken down to ornithine. Breakdown of 47·6 μmol of arginine would have produced 95·2 μmol of ammonia and an increase of 95·7 μmol/g tissue was observed. In the treated samples there was no breakdown of arginine and consequently no accumulation of ornithine. However, ammonia increased by 10·7 μmol/g which apparently resulted from the breakdown of adenine nucleotides and some other amino acids. If these sources contributed the same amount of ammonia in the untreated samples also a total accumulation of 105·9 μmol/g would be expected instead of the 95·7 μmol/g as observed. It is probable that some of the ammonia, being volatile escaped with the nitrogen which was continuously passed over the muscle samples to keep the atmosphere anaerobic; ammonia would certainly become more volatile with the rise in muscle pH. A small amount no doubt was incorporated into the bacterial cells.

Compared with changes in the levels of arginine and ornithine, changes in the lysine and histidine concentrations in both the treated and untreated muscle samples were slight. This contrasts with an increase of at least five-fold in lysine, observed in spoiling cod muscle (Shewan & Jones, 1957).

Arginine constitutes about 27% on a molecular basis and 43% on a nitrogen basis of the total free amino acids present in rock lobster muscle. The total amino acids in turn constitute about 50% of the total NPN in the muscle. These values are similar to those reported for scampi (Robertson, 1961). Observations made by Walker, Cann & Shewan (1970) showed that the bacterial spoilage of scampi differed in two main aspects from that of cod and haddock. Firstly, the predominant types of bacteria changed from coryneforms to *Achromobacter* as spoilage proceeded, and the values of TVB and TMA on the tenth day of storage on ice were much higher in scampi than in cod and haddock under similar conditions. A similar change in the types of bacteria appearing during spoilage of Mexican Gulf prawns was noticed by Campbell & Williams (1952). Arginine and glycine are the predominant amino acids present in the

flesh of different species of prawns so far analysed quantitatively for free amino acids (Simpson, Allen & Awapara, 1959; Konosu, Akiyama & Mori, 1968; Rangaswamy, Rao & Lahiry, 1970). It seems that a common pattern of bacterial spoilage in scampi and some species of prawns is determined mainly by a relative abundance of arginine and high ultimate pH of flesh.

It is probable that a similar pattern exists in the rock lobster.

Ammonia is mainly derived from bacterial breakdown of arginine in spoiling rock lobster flesh (Table 1). The suppression of both ammonia and TMA production in the treated samples suggests that either the same species of bacteria is involved both in the breakdown of arginine to ammonia and reduction of trimethylamine oxide, or the growth of different species involved in these two series of reactions is inhibited to the same extent by oxytetracycline. In prawns, indications are that different species of bacteria may be involved. In white shrimp (*Penaeus setiferus*) inoculated with pure cultures of different species of bacteria, ornithine and ammonia were produced from arginine by non-fluorescent *Pseudomonas* sp. and *Bacillus* sp., while ammonia and TMA were produced by coryneform bacteria (Cobb & Vanderzant, 1971).

Since 1 mol of ornithine accumulates for every 2 mol of ammonia produced it might be possible to use ornithine as an index of bacterial spoilage of decapod crustaceans with high initial levels of arginine. A photometric method for the estimation of proline and ornithine had been described by Chinard (1952). Since proline does not change appreciably (Table 1) during spoilage and since interference from other amino acids is negligible, this method could be used to estimate changes in ornithine concentrations during storage.

It has been reported (Miyake & Tanaka, 1971) that when sections of muscle from beef, shark, whale and squid were dipped in 1% arginine solution or smeared with solid arginine powder, there was a great reduction in cooking losses and marked improvement in tenderness and other organoleptic properties. Rock lobster muscle contains 1·3–1·5% arginine and its loss due to bacterial attack not only produces undesirable odours but also means a probable loss of delicate flavour and other desirable properties.

Post mortem glycolytic studies reported earlier (Sidhu *et al.*, 1974) showed that breakdown of phosphoarginine and ATP was greatly reduced when the temperature of rock lobster muscle was lowered from 15° to 0°C. Consequently, as long as the energy supply of the muscle cell in the form of ATP is not exhausted, the cell can maintain its integrity and retain amino acids and other solutes in its internal environment, thus denying easy access of nutrients to spoilage bacteria. But once the integrity of cell membranes breaks down and solutes leak out, suitable conditions are created for the spoilage bacteria to multiply. The present study shows a greater interdependence of glycolytic changes and subsequent bacterial spoilage; it also indicates that cooling to 0°C is more effective in checking spoilage than observed in some species of teleost fish.

According to export regulations in Australia, rock lobsters (*Jasus* spp.) must be alive

before processing. Adherence to these regulations produces a pack of top quality. However, several species of tropical lobsters, (e.g. *Panulirus versicolor*) are difficult to capture alive as they seldom enter lobster pots, and are usually taken by spearing. Our studies indicate that if the rock lobsters are headed, gutted and the tails cooled immediately and kept out of contact with water, these could be kept in good condition for up to a week. On the basis of studies conducted in New Zealand, Thomas (1969) has recommended limiting storage of iced rock lobsters to 5 days.

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Spoilage and spoilage indicators in queen scallops (*Chlamys opercularis*)

I. Held in ice

A. B. THOMSON, H. K. DAVIS, J. C. EARLY AND J. R. BURT

Summary

A number of bacterial, organoleptic and chemical evaluations were made on iced queen scallops in shell over 14 days total. The spoilage flora, qualitative and quantitative, is described. Organoleptic descriptions are given along with determinations of trimethylamine, total volatile bases, volatile reducing substances, glycogen, hypoxanthine, ribose fractions and optical density ratios at 248 nm before and after treatment with ion-exchange resin. Hypoxanthine showed the most promise as a spoilage indicator while total volatile bases and optical density ratio showed some promise but to a lesser extent.

Introduction

The British commercial fishery for queen scallops, for human consumption, is a fairly recent one. Until the 1960's it was considered only as a seasonal source of income for a few boats around the coast. Continuous fishing during the summer months has greatly increased the landings and hence the commercial importance. With these increased landings came the need for a greater understanding of the spoilage pattern and for the development of a spoilage indicator that would be applicable throughout the storage life of queen scallops.

Work has not previously been done on the deterioration of queen scallops although some workers have investigated changes in the closely related, but larger, species of calico scallops (*Pecten gibbus*) (Waters, 1964), sea scallops (*Placopecten magellanicus*) (Hiltz & Dyer, 1970, 1971, 1973) and weathervane scallops (*Platinopecten caurinus*) (Groninger & Brandt, 1970). Of the possible spoilage indicators examined in some of these other species it would appear that only hypoxanthine (Hiltz & Dyer, 1970, 1973) could be used throughout the storage life in ice, although octopine (Hiltz & Dyer, 1971) and glucose 6-phosphate (Hiltz & Dyer, 1973) gave good correlations with spoilage over the first 6–8 days in ice. Waters (1964) found that with the possible tests he

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examined, he could detect spoilage only beyond the point of inedibility but could not distinguish between various stages of deterioration up to that point.

The general pattern of nucleotide breakdown in molluscs has been studied by Arai (1966) and found to differ from fish in that the degradation to hypoxanthine does not go via inosine monophosphate. For one species of scallops, *Pecten yessoensis*, he gave the principal pathway of nucleotide breakdown pattern to be Adenosine 5′-triphosphate→ Adenosine 5′-diphosphate→Adenosine 5′-monophosphate→Adenosine→Inosine→ Hypoxanthine, with the dephosphorylation of adenosine 5′-monophosphate being the rate limiting step.

A little work has been published on the initial and spoilage floras of molluscs (e.g. Colwell & Liston, 1960 for oysters) but so far nothing on queen scallops. Most of the published bacteriological studies of molluscs have concentrated on organisms of public health significance.

Materials

The queen scallops used in this experiment were caught by the station's research vessel, 'Sir William Hardy', off the Cock of Arran on the West Coast of Scotland in the month of June. All samples measured 5–7 cm across the shell.

Enough shellfish were washed and shucked immediately to give approximately 60 g of adductor muscle, from which the 'zero control' extracts were prepared. The remaining queen scallops were washed with sea water and stowed in ice between layers of polythene (to prevent leaching).

On the second day after catching, all the material was transferred from the boat and taken by road to the laboratory for further storage and sampling.

Methods

Organoleptic assessments

These were made by up to four at a time of the people actively concerned with work of this type. All were trained members of a white fish taste panel but had limited experience with scallops or queen scallops. Descriptions were given of odours of raw meats and odours, flavours and texture of the meats after cooking for 20 min in a steam oven.

Bacteriology

Sufficient numbers of shucked meats for from two to four 10 g samples were extracted aseptically at each assessment and these were then rinsed in approximately 100 ml of sterile normal strength Ringers solution to remove any mud, sand or other matter adhering to the meats after shucking. From each batch, 10 g of meat was then weighed into a sterile flask and homogenized with 90 ml sterile normal strength Ringers solution.

Bacterial counts were estimated by inoculation of serial duplicate decimal dilutions into roll tubes containing a 2% (in sea water) nutrient agar (Spencer, 1961) and incubation at 20°C for 5 days. The same serial dilutions were used for the preparation of pour plates from which colonies were picked in a random sequence for subsequent classification using primarily the screening sequence described by Shewan, Hobbs & Hodgkiss (1960).

Chemical analysis

The following extracts were made at each sampling time, in duplicate from the third day onwards.

(1) Fifty grams of shucked meats were homogenized with 100 ml of 0·6 N perchloric acid for 1 min and the homogenate filtered. A sample of the filtrate was kept for trimethylamine determination (Murray & Burt, 1965) and determination of total volatile bases using a Hoskins apparatus followed by titration of the collected distillate.

(2) A portion (60 ml) of the above filtrate was neutralized with potassium hydroxide and the total volume adjusted to 100 ml with distilled water. This extract was used for total ribose, barium soluble-alcohol soluble ribose (Meijbaum, 1939) and hypoxanthine (an adaptation of the method of Jones *et al.* 1964 for use with a BTL analmatic analyser) determinations and for optical density measurements before and after treatment with ion-exchange resin (Jones & Murray, 1964).

(3) Two 1 g portions of muscle were each digested with 10 ml of 30% potassium hydroxide containing 10% saturated sodium sulphate. The final volumes were adjusted to 25 ml with the same solution of alkali. Glycogen was precipitated from these extracts according to the procedure of Handel (1965) and determined by the method of Fairbairn (1953).

(4) Approximately 50 g shucked meats were pressed in order to obtain the juice for determining the concentration of volatile reducing substances present by the method of Farber & Ferro (1956).

Results

Organoleptic

Cooked flavour descriptions showed four stages during decay. The first (0–3 days) is characterized by a strong sweetness after which a less sweet character was observed (4–5 days). A definite sourness was detected between 6 and 8 days and after 8 days the samples were regarded as too foul to taste.

Cooked odours showed three stages of decay, sweet (0–5 days), sour (6–8 days) and faecal (after 8 days).

Raw odours showed no well defined stages but marked spoilage was detectable after 8 days by the strong faecal odours.

Texture remained chewy and gelatinous throughout the edible storage period.

Bacteriology

Bacterial counts increased from an initial 10^3/g in the fresh samples to 10^5/g after 8 days (Fig. 1). Of the initial flora, the *Moraxella/Acinetobacter* group comprised 22%, *Pseudomonas* 16%, Gram positive cocci 22%, Coryneforms 17% and Flavobacterium/*Cytophaga* 12%. The *Moraxella/Acinetobacter* group shows an increasing predominance during spoilage (Table 1) reaching over 90% of the total organisms present after 6 days.

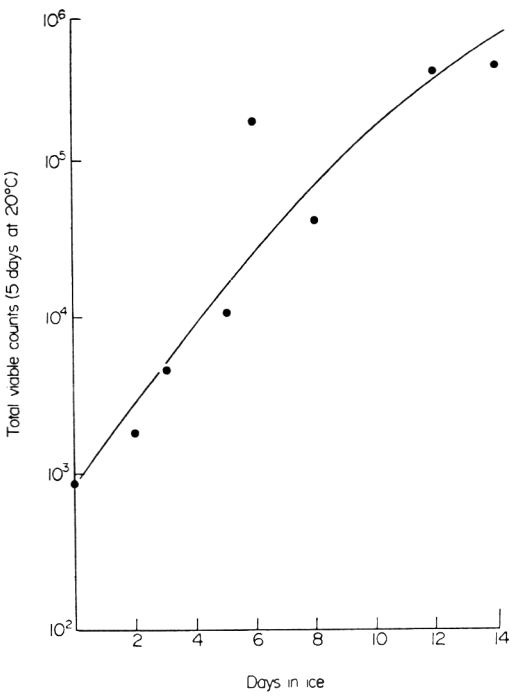

FIG. 1. Changes in total viable counts (5 days at 20°C) during iced storage of whole queen scallops.

Chemical

(1) Glycogen. Values ranged from 3·4% in the fresh meats to 0·2% after 14 days in ice but with a wide scatter of results in between. Even duplicate extracts gave a four-fold variation in concentration.

(2) Volatile reducing substances ranged from 1 to 33 micro equivalents per 5 ml press juice with no relation to days held in ice.

(3) Trimethylamine. Values ranged from 3·7 to 8·5 mgN/100 g but gave no correlation with days held in ice. In some instances, as a result of chemical interference, the extracts could not be analysed. Even after dilution they appeared to be too concentrated to read.

TABLE 1. Distribution of different bacterial groups during iced storage of whole queen scallops

Days in ice	No. of isolates	*Pseudomonas*	*Moraxella/ Acinetobacter*	*Flavobacterium/ Cytophaga*	*Coryneforms*	Gram positive cocci	*Entero-bacteraceae*	Unclassified	No growth
0	101	16	22	12	17	22	2	3	7
2	106	16	52	10	22	3	0	2	1
3	100	20	54	9	10	3	0	3	1
5	100	22	76	0	0	0	0	0	2
6	101	3	91	2	1	3	0	0	1
8	101	8	91	0	2	0	0	0	0
12	102	6	87	1	1	1	0	0	6
14	102	8	80	0	0	0	0	0	14

No growth refers to primary isolates not viable on sub-culture.

(4) Total ribose values tended to decrease from just over 300 mg/100 g in the fresh meats to just below 200 mg/100 g after 14 days in ice. However, duplicate extracts did not correspond at all well while fluctuations from day to day were marked.

(5) Barium soluble-alcohol soluble ribose. Values rose from 18 mg/100 g in fresh meats to 60 mg/100 g after 3 days in ice, staying at this value for another 4 days before falling slightly.

(6) Ribose ratio. The ratio of barium soluble-alcohol soluble ribose to total ribose, expressed as a percentage, rises sharply from 6 to 23 between 1 and 2 days in ice and remains fairly steady thereafter.

(7) Total volatile bases. Values increase from 12 to 20 mgN/100 g over the first 8 days in ice and jump sharply to 40 mg/100 g thereafter (Fig. 2a).

(8) Hypoxanthine concentrations increase from zero to 2·70 μm/g during the first 10 days in ice (Fig. 2b) and level off thereafter.

(9) Optical density ratio. The ratio of optical density of extract at 248 nm after resin treatment to that before resin treatment shows a sharp increase during the first 3 days in ice (Fig. 2c) and a much shallower rise from then up to 14 days in ice.

Discussion

The organoleptic results show that the limit of edibility of iced in shell queen scallops is reached some 8–10 days after capture provided the shellfish are kept thoroughly iced during the whole time. The fact that broad bands of quality can be determined by tasters could have some potential in certain grading schemes where detailed knowledge of 'quality' is not too important. The strong sweetness detected during the first three days could be due in part to the sugar and sugar phosphates which would arise from the breakdown of glycogen which is initially present at very high concentrations. Some sweetness is still detectable up to six days in ice, when a slight sourness is obtained. No difference was found in the texture during storage, unlike Groninger & Brandt (1970), who found a toughening of the texture during storage in ice of shucked meats of weathervane scallops.

As may be seen from Fig. 1 the total viable counts rise sharply, after a relatively short lag, over the first 5 days in ice. Comparisons with other marine products are difficult because of anatomical differences and variations in sampling technique, but these results are similar to, if lower than, those reported for Norway lobster (Walker, Cann & Shewan, 1970) and white fish (Shewan, 1961).

The initial bacterial flora is comprised of the same groups of genera usually reported in marine fish and shellfish. The predominance of *Moraxella/Acinetobacter* then and more particularly during spoilage has occasionally been observed before (Walker *et al.*, 1970) though *Pseudomonas*, or a mixture of *Pseudomonas* and *Moraxella/Acinetobacter*, more usually predominate.

Of the various possible chemical tests for quality of iced in shell queen scallops

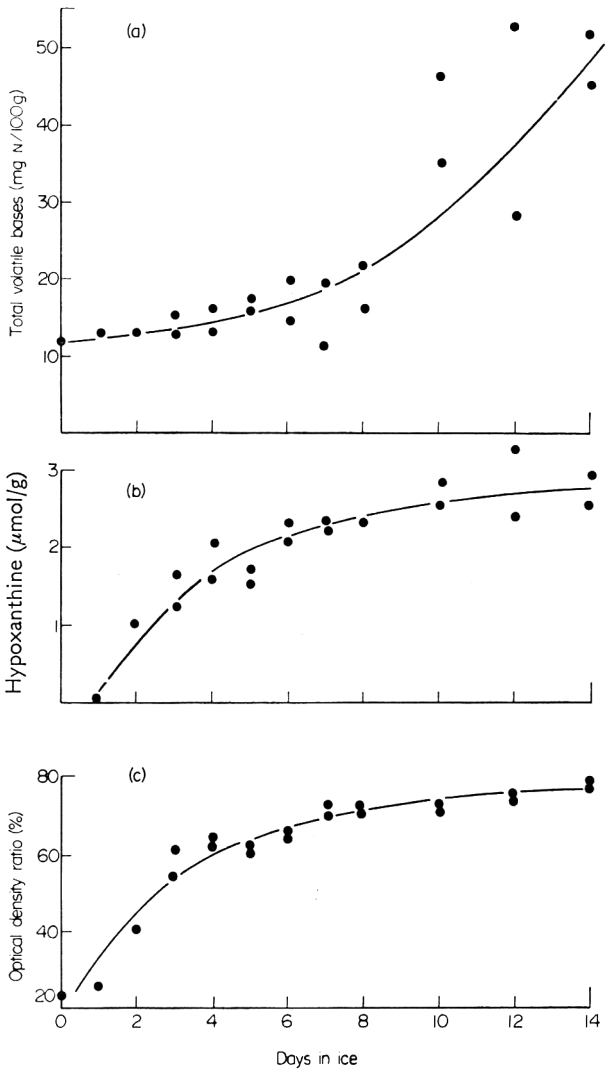

FIG. 2. Changes in the muscle concentration of (a) total volatile bases and (b) hypoxanthine and in (c) optical density ratio of muscle extracts before and after resin treatment, during iced storage of whole queen scallops. Each point is the mean of duplicate analysis on an extract.

examined, glycogen, volatile reducing substances, trimethylamine, total ribose and barium soluble-alcohol soluble ribose values showed no consistent trend when plotted against days in ice thus ruling out their possible use as quality tests.

The large variation in glycogen values obtained, even between duplicate extracts, could be due to the fact that only 1 g samples were taken i.e. only part of one meat. A

larger sample of more meats might give more representative and consistent results. The original values of approximately 3·4% were in agreement with those found by Groninger & Brandt (1970) for weathervane scallops.

Trimethylamine values estimated using an auto analyser were fairly constant, where values could be obtained, over 14 days in ice, but because of the difficulty encountered with certain of the extracts using this technique, the extracts were analysed by two further methods using picrate (Murray & Gibson, 1972) and steam distillation. Even with these other methods, very poor duplication (between duplicate extracts) was encountered. Castell (1970) estimated trimethylamine in fresh and frozen scallops using the picric acid test and said that the results he obtained 'defy interpretation'. Lartigue, Novak & Fieger (1960) found they could not estimate trimethylamine in oyster muscle by the picrate method due to interference of a substance 'S', but they got reproducible results using steam distillation. None of the methods used in this study gave good reproducibility and the determination of trimethylamine must therefore be eliminated as a possible test for freshness of iced queen scallops using any of the three methods described.

The shallowness of the increase of total volatile bases with storage time would limit the usefulness of this parameter as a quality indicator for queen scallops, but it could be used to distinguish between edible and inedible specimens. The values obtained and the trend observed are roughly similar to those reported by Tanikawa, Akiba & Yamashita (1962) for abalone (*Haliotis discus hannai*) meats stored at 5°C.

The ribose ratio increased to approximately 25% over the first 3 days in ice and then remained constant up to 14 days in ice. This is very low compared with the values of 90% found in iced cod (Shewan & Jones, 1957) after only 6 days in ice. This test could not give an indication of quality of iced queen scallops. Total ribose values were approximately twice those reported for iced cod (Shewan & Jones, 1957). Barium soluble-alcohol soluble ribose values increased at the same rate over the first 4 days and then levelled off at approximately half the value obtained for iced cod. The rate of dephosphorylation would therefore appear to be very much slower than that for iced cod, which is in agreement with the findings of Hiltz & Dyer (1970) and Arai (1966) who said that the dephosphorylation of adenosine 5′-monophosphate was the rate limiting step in the nucleotide breakdown pathway for molluscs.

The optical density ratio at 248 nm after resin treatment increases throughout the storage time in ice (Fig. 2c), but the shallowness of the increase between the third and fourteenth days would limit it's usefulness as a quality index. In iced cod, the ribose ratio and the optical density ratio at 248 nm follow the same pattern of increase throughout iced storage (Jones & Murray, 1964) with the ribose ratio being slightly slower. A comparison of these two ratios for iced queen scallops show the optical density ratio at 248 nm to be approximately three times the ribose ratio. This is presumably due to some extent to the formation of ribose phosphate in the degradation of inosine to hypoxanthine. The initial optical density ratio of 24% is very high compared with iced

cod (Jones & Murray, 1964) and is probably due to some non-nucleotidic material present in the muscle. This high optical density after resin treatment could be due to homarine which has been shown to occur in sea scallops (Hiltz & Dyer, 1970). The presence of this material would therefore give rise to higher percentages throughout the iced storage, which are not representative of the amount of dephosphorylation taking place.

Hypoxanthine concentration gives a good indication of quality over the first 10 days in ice (Fig. 2b) and thus covers the edible life of queen scallops. This result is similar to that reported for sea scallops by Hiltz & Dyer (1970) and places the queen scallop amongst the increasing number of species for which hypoxanthine concentration can act as a good index of freshness.

Conclusion

By the use of sensory judgements, the edible storage life of 8 days can be divided into three stages of spoilage and therefore three possible grades of quality can be distinguished.

Dephosphorylation of nucleotides in the adductor muscle of iced queen scallops appears to be very slow compared with iced cod. Measurement of the rate of dephosphorylation by the optical density ratio at 248 nm is not very accurate because some unknown ultra-violet absorbing material is present at death and it is not known whether or not it is still present or is decomposing on holding in ice. Measurement by the ribose ratio technique is even less satisfactory because from the results obtained it would appear that ribose phosphate is produced and although the nucleotide is dephosphorylated, it is not recorded as such in the result.

Of the chemical tests examined, only hypoxanthine would appear to give a reasonable indication of quality of iced in shell queen scallops throughout their edible storage life.

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Technical note: Determination of moisture profiles from temperature measurements during freeze drying

JOSE M. AGUILERA AND JAMES M. FLINK

Introduction

It has been shown that computerized simulation of freeze drying can be used to predict product temperatures and moisture contents (or water activities) as a function of time and geometric location (Aguilera, 1973; Aguilera & Flink, 1974). Since destruction rates of various important nutrients (as well as activation energies) are a function of temperature and moisture content, knowledge of temperature and moisture profiles inside a piece of food during processing is extremely important for predicting the extent of quality deterioration for particular process conditions. This is necessary for determining optimal processing policies (Labuza, 1972). In work done in our laboratories, literature data for non-enzymatic browning of potato dices was combined with computer-simulated product temperatures and moisture profiles corresponding to common commercial conditions. The results, which gave the distribution of brown colour, demonstrated the value of this type of technique to better define quality changes in processing (Aguilera & Chirife, 1973).

For freeze drying, many examples of temperature profiles are available from the literature. Contrary to this is the limited number of moisture content profiles available. Meffert (1965) determined moisture contents in layers of swede, finding basically the characteristic two zone phenomena, dried layer and ice core. Also, clearly distinguishable was the existence of a moisture gradient through the dried layer that varied from around 3·0 g H_2O/100 g solids near the outer surface to 33·0 g H_2O/100 g solids near the interface when the ice core had receded half-way into a 3·6 cm sample dried from both sides. In addition to the two above mentioned zones, Bralsford (1967) postulated the existence of a transition zone between them, which he labelled the 'diffusion zone'. He felt that at higher chamber pressures, appreciable quantities of liquid water were present in the ice core so that some liquid diffusion into the dried layer resulted. Broadening of this zone was postulated to be due to solute migration in which more and more ice melted as the zone receded into the ice core. In support of this hypothesis, he presented the fact that at the very end of sublimation, samples that were removed and cut open presented a soft wet area, while those sectioned at the beginning of the process had a quite solid ice core.

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Hatcher, Lyons & Sunderland (1971) measured moisture distributions in beef samples with a gamma ray attenuation technique. Their measurements showed that there was almost a complete removal of moisture in the dry layer after the passage of the phase change region meaning that any transition zone was thinner than the sensitivity of their technique ($\simeq 5$ mm). Almost linear temperature gradients were found in the dried layer during sublimation. Similar temperature profiles in beef were obtained by Brajnikov *et al.* (1969), but with respect to moisture they postulated the existence of a 'zone of sublimation'.

Recently, based on the higher desorption temperatures for bound water as compared with the sublimation temperature for frozen water, Gentzler & Schmidt (1973) postulated a relationship between bound water content (W_B) and temperature difference (ΔT_B) between any position in the dry layer (desorption temperature) and the temperature of the ice (sublimation temperature) that can be expressed mathematically as

$$W_B = e^{(a\Delta T_B + b)} \tag{1}$$

with a and b as constants dependent on each particular product. Gentzler & Schmidt (1973) presented data for skim milk. The same type of relationship can be obtained from data by Oetjen (1973) who worked with granulated coffee of 45% solids content (Fig. 1).

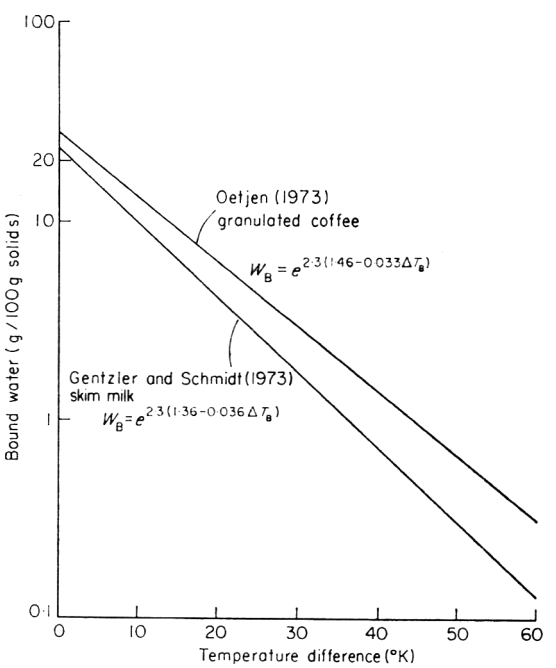

FIG. 1. Local moisture contents in the dry layer during freeze drying as a function difference of local temperature and ice front temperature.

The work reported upon here was undertaken to demonstrate the possibility of combining the bound water content and temperature difference data of Gentzler & Schmidt (1973) with dry layer temperature profiles obtained during freeze drying (Aguilera & Flink, 1974) to yield moisture profiles in the dry layer at various times in the drying process. These calculated moisture contents could then be included with temperature data in an integrated calculation of product deterioration using the temperature and moisture dependent kinetics of the degradative reactions.

Experimental

Samples consisted of a mixture of 20% non-fat dried milk, 0·5% sodium CMC and 79·5% water. The sample holder was a circular aluminum pan of 21·2 cm diameter provided with seven 30 gauge copper-constantan thermocouples. The sample thickness was about 14 mm. A 12 point temperature recorder continuously registered the temperature at those locations. After being frozen with liquid N_2, the sample was placed in a styrofoam-insulated support and placed in a pilot plant-size freeze drier. The heat input was initially controlled by a heater plate thermocouple in order to maintain constant heater temperature. When the surface thermocouple reached the maximum allowable product temperature, control of the heat input was switched to the sample surface thermocouple. Following freeze drying, determinations were made of moisture content, sample thickness and thermocouple positions.

Results and discussion

Temperature gradients in the dry layer for a heater temperature of 128°C are presented in Fig. 2. Important to note is the constancy of the ice temperature (T_I) and the linearity of the temperature gradient throughout the sublimation process. By recording the surface temperature (T_S) and following the interface position (r) by thermocouple measurements, the temperature at any point (x) in the dried layer can be easily determined using the relation:

$$T_x = T_S - \frac{x}{r}(T_S - T_I). \tag{2}$$

Another interesting fact is the 'rotation' of the gradient that occurs about the constant surface temperature point, producing decreasing values for the temperature gradient as drying proceeds. Since the moisture content remaining after the passage of the ice front is directly related to the difference between local temperature and the ice front temperature as described previously, higher moisture contents are to be expected at a given distance from the ice front as drying proceeds. This is illustrated in Fig. 3 by combining equation (1) for the case of skim milk (Fig. 1) and the temperature gradients of Fig. 2. An explanation for the observations by Bralsford (1967) and Brajnikov *et al.*

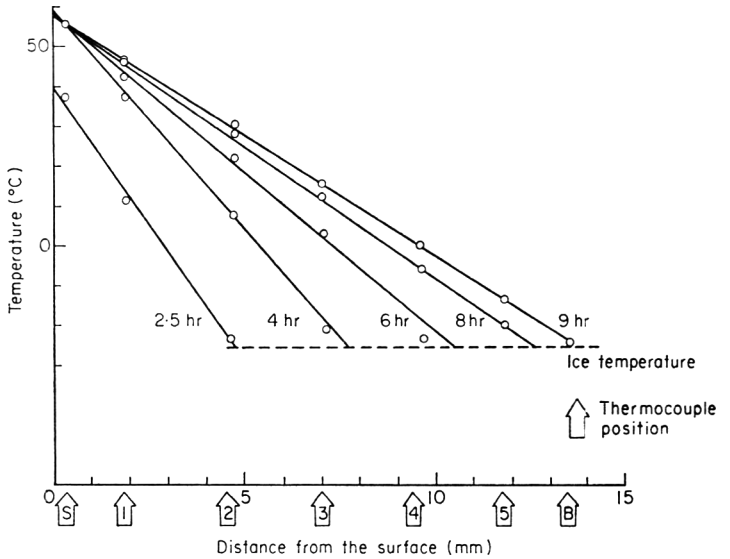

FIG. 2. Dry layer temperature gradients during sublimation period of freeze drying of skim milk (heater temperature = 128°C).

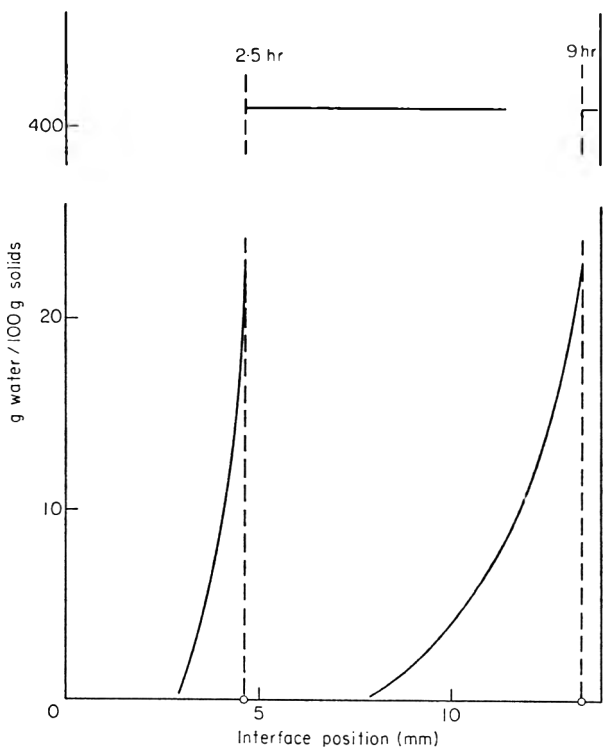

FIG. 3. Predicted moisture profiles during freeze drying of skim milk (heater temperature = 128°C).

(1969) of a soft wet broadening zone between the ice zone and the dried layer is obtainable from Fig. 3 since intermediate levels of moisture build up continuously against the ice front.

The present method, by developing moisture profiles, incorporates an important factor for optimization policies for quality retention in freeze drying. Our previous work (Aguilera & Flink, 1974) had considered only temperature effects on drying behaviour. Since quasi-steady state conditions prevail during freeze drying, a simple computer solution can combine temperature and moisture content dependencies during drying with kinetic data on product deterioration to determine a temperature-time heating program which minimizes deteriorative changes.

Conditions for quality retention are normally presented in terms of the maximum allowable product temperature (commonly called scorch temperature) which is usually considered to have a constant value. Actually, the degree of product degradation will depend on temperature, moisture content and time; hence, the 'scorch temperature', which may be defined as the temperature at which certain observable deteriorative processes acquire a predetermined critical value, will be a function of moisture content and time. Work with skim milk (to be reported later) shows the influences of moisture content (as well as temperature and time) and thus the need for developing sample moisture profiles.

Acknowledgments

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Technical note: A hydrogen sulphide detector

KNUD ØLGAARD

Introduction

In experiments on the keeping quality of vacuum-packed meats organoleptic examination of the odour is commonly used to determine whether putrefaction has taken place.

This technique has several drawbacks. The determination of an off-odour is very subjective, especially if many samples are to be examined. Furthermore, if the sample is to be examined several times, it is necessary to open the pack at suitable intervals, which undoubtedly will influence the microbial activity.

A more objective technique to determine *when* and *where* putrefaction of the meat occurs without opening the pack is desirable.

Description

The H_2S detector (HSD) consists of a vacuum sealed plastic bag (approx. 14×20 cm), which contains a sheet of Cellophane (approx. 12×16 cm–40 μm thick, not lacquered) soaked with 10% lead acetate solution. One side of the bag is transparent, while the other side is semitransparent white.

The semitransparent white side must be polyethylene, as it must have a relatively high permeability for H_2S. The white colour must be dense enough to form a distinct background so that blackening of the Cellophane (lead acetate) cannot be confused with muscle tissue below, but must be transparent enough for the individual muscles to be distinguished from one another and from other tissues. Initially, handmade bags were used with the two sides sealed together, but polyethylene bags, coated semitransparent white on one side, are now made by local manufacturers.

The HSD's must not dry out before use. Consequently, they must be stored under vacuum in laminated plastic bags. To ensure sterility of the HSD's these bags may be irradiated in a cyclotron with, e.g. 1 Mrad. On irradiation the HSD turns grey, but this disappears on storage at room temperature for approximately 1 week or on storage at 37°C for a few days.

Use of the detector

The HSD is placed on the meat to be examined. It is of the utmost importance that the semitransparent white side of the bag is in contact with the meat. If the HSD is turned

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with the transparent sheet against the meat, the white colour will cover the blackening of the Cellophane.

The sample with the HSD is then vacuum packed in a laminated bag, incubated, and inspected every day or every second day. Formation of H_2S shows on the HSD as black spots. These may be distinct or diffuse. Distinct blackening usually derives from bacterial growth on the surface of the meat, while diffuse blackening derives from tissues below the surface.

Usually the blackening grows bigger and more intense from day to day, and with heavy H_2S formation the detector may turn quite black. From time to time, however, the spots do not grow during further incubation, presumedly because H_2S formation has stopped.

If the extent of the blackening is drawn on the pack at each inspection using a fibre tip pen, and the colour of the pen is changed from day to day, it is possible at the end of the incubation period to tell exactly when and where the H_2S formation has occurred and how heavy it has been. Furthermore, it is possible—after having removed the HSD —to examine the actual part of the sample chemically and microbiologically and compare this with other parts of the sample, where no blackening of the HSD has occurred.

At the end of the incubation period there is usually a good correlation between off-odour and blackening. However, samples have been found with off-odour, but no blackening and with blackening and no off-odour. The reason why an off-odour is obtained without blackening is probably that H_2S is not always present in the metabolic products of the micro-organisms. Experience has shown, for example, that this can be influenced by curing ingredients.

The conditions under which blackening is found without off-odour have not finally been determined. Some results appear to show that this occurs if H_2S formation has started but has stopped again. Consequently, incubation should always finish with a determination of the smell even if HSD's have been used.

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Book Reviews

Aspects of Meat Inspection. By H. THORNTON.
London: Baillière Tindall, 1974. Pp. vi + 176. £3.00.

In this very readable and entertaining little book, Horace Thornton has brought together a selection of his lectures on meat hygiene. In ten brief chapters he deals with those aspects of animal and meat handling, and meat inspection, which control the quality and the safety of meat reaching the consumer. The subject matter ranges from pre-slaughter stress, to stunning and sticking procedures; from antibiotics in animal feeding stuffs, to the mechanisms and consequences of rigor mortis; from trichinosis in Eskimoes, to the occurrence and relevance (to certain African tribes) of *T. spiralis* in warthogs, hyenas and jackals. The book draws on the experiences of the author in visits which he has made to diverse regions of the globe during a career that covers over half a century in the field of meat hygiene.

By virtue of the anecdotal style of writing, this volume ranks as a lightweight extension to the same author's standard treatise, the *Textbook of Meat Inspection.*

The transportation of pigs is discussed, and there is a useful summary of the factors which contribute to aggressiveness, bruising and deaths during the journey to the abattoir. Reference is made to the use of drugs, such as Azaperone, to control this problem, which has increased in recent years, particularly in Holland and Denmark. It is also suggested that tranquillizers may be used to improve meat quality by eliminating pre-slaughter stress in the abattoir. The efficacy of drugs used for this purpose is, however, dubious, except perhaps with the notoriously stress susceptible breeds, since pre-slaughter stress is often of minor significance compared with the major shock suffered by the animal at stunning (by the electrolethaler or other means) and subsequently at sticking and death.

Insufficient emphasis is laid on the necessity for adopting the correct procedures in stunning and slaughtering, since these play a major role controlling blood splash and in determining meat quality. Thus, in dealing with electrical stunning, the vital parameters of frequency, voltage and amperage of the stun current are not mentioned, and the 'prescribed period' for applying the current remains undefined. Single handed tongs, such as those described, suffer from the disadvantage that the pressure of the electrodes across the animal's head is not readily controllable by the operator, but depends upon the size and configuration of the individual animal's skull.

There is some confusion with regard to the practice of withholding feed in the abattoir prior to slaughter. Starvation can lead to increased permeability of the intestinal walls to the gut microflora, which may consequently penetrate into the blood and tissues, causing ham taints and infecting the meat with Salmonella. A similar invasion of the tissues by gut microflora can occur, however, as a result of feeding the animal before slaughter! A study of the physiological effects of feeding and of starvation, in

relation to this migration of the gut microflora and impaired microbiological condition of the meat, is clearly overdue.

Attention is drawn to the sugar feeding of pigs prior to slaughter, to obtain a lower pH in the meat, which therefore exhibits an improved storage life. Liver weight is also usefully increased and colour of this organ tends to deepen. Further it is stated that the cured bacon will be of better quality, but the appropriate references are not given. (References are regrettably lacking throughout the book—only one or two are quoted.) It should also be remarked that this practice of feeding results in enhanced 'drip'—a distinct disadvantage in these days of supermarket retailing of prepacked fresh meat.

Recherché facts and comment abound. The volume of blood yielded by the adult elephant, the astonishing tensile strength of the Achilles tendon of a racehorse, the flavour of roasted human flesh, these may all be ascertained within these pages. One also learns that the Nostril Fly (*Oestrus ovis*) in regions where sheep are scarce, may deposit its eggs on human beings in desperation [*sic*]!

The book is adequately illustrated with simple diagrams and embellished by plates prepared from the author's photographs; the latter in particular add emphatic point to the text.

This is a book which may be read with both pleasure and profit, and not only by the meat inspectors and public health workers to whom it is principally directed. Meat scientists, food technologists, veterinarians, biologists and all those who breed, manage, handle, slaughter—and eat—our meat animals, will find much that is very relevant to their interests.

P. W. RATCLIFF

Tomato Paste and Other Products. By PETER GOOSE and RAYMOND BINSTED. London: Food Trade Press Ltd, 1973. 2nd edition. Pp. viii + 269. £6.30.

The first edition, published in 1964, was soon recognized as a valuable reference book by buyers and producers of tomato paste.

The second edition not only maintains the high standard, but has widened the scope by including a chapter on the cultivation of tomatoes for processing. It has been recognized that developing countries have an interest in tomatoes as a cash crop for tomato products and this is also taken into account in the description of techniques and requirements for cultivating tomatoes.

The second chapter has a comprehensive review of methods of manufacture and equipment used for tomato paste production. It is a pity that more emphasis is not given to the importance of back-up services for equipment, particularly where instrumentation is involved, and this applies especially in the case of developing countries. This chapter together with Chapter 3 on other tomato products, bring the reader up to date with recent advances in the tomato industry. Together with a further chapter on plant cleaning they contain a number of sound practical observations and useful

references. The importance of the correct installation of equipment and floor conditions in relation to plant cleaning could have merited more prominence.

The check list under the inspection of tomato paste factories is worthy of consultation not only by buyers but also producers of tomato paste. A good deal of space on the quality evaluation of tomato paste is devoted to quality and analytical methods for laboratories. As some of these are of minor importance relative to the space accorded them the reviewer would have preferred to see them in the form of an appendix.

The concluding chapters cover standards of identity and quality specifications and tomatoes, tomato paste and their international movements.

The book is well produced, with numerous illustrations and diagrams and a bibliography and glossary are included. It can be recommended as an up-to-date guide for the production of tomato paste and tomato products.

D. P. SLATER

Nutrition and Dietetic Foods. By ARNOLD E. BENDER.
Aylesbury: Leonard Hill, 1973. 2nd edition. Pp. xi + 298. £4.25.

The first edition of this book was published in 1967 under the title 'Dietetic Foods' and the layout, with marginal headings in heavy type, gave the impression of a dictionary to be dipped into for isolated factual information. The change of title and format for the second edition has transformed the publication into a comprehensive and very readable textbook on human nutrition. Examples of recent developments include additional information about nitrates and sodium chloride in infant foods and a section on diet margarines. Other modifications have been the inclusion of the joule for the measurement of energy—given in brackets after the calorie value—and the use of cm^3 for ml.

This revised edition has been prompted by the FAO/WHO Standards Programme Codex Committee preparing standards for 'Foods for Special Dietary Uses'. The first ten chapters deal with conditions requiring special dietary control, e.g. inborn errors of metabolism and diseases needing a low sodium intake; the following nine chapters cover the essential nutrients and include an additional chapter on the biochemistry of metabolism written by Dr David A. Bender. This arrangement has the advantage of immediately gaining the reader's attention in the fascinating topic of modifying the diet to suit the physiological status of the patient. The disadvantage is that reference is often made to information in chapters ahead which is more difficult to find than referring back to sections previously read; for example, protein-rich preparations are discussed in Chapter 8 and fundamental protein nutrition in Chapter 13.

Professor Bender defines dietetic foods as those foods which differ in their composition from ordinary foods and are intended for a limited, defined section of the community. Thus, therefore, are foods which meet the special nutritional needs of healthy persons such as infants, and foods formulated for persons suffering from physiological disorders. In connection with low-energy foods, the point is made that products in which the

sugar has been replaced by non-caloric sweeteners can be useful as a slimming aid *and* for diabetics, but foods containing substances such as sorbitol which can be tolerated by diabetics would not be suitable for weight-reduction. Such a clear explanation should help to ease the persistent confusion between 'dietetic' and 'diabetic'.

This book has comparatively few printer's errors and spelling mistakes; 'performed vitamin A' is ubiquitous in many publications, but 'Batchelor's scurvy is seen in old men living on prepacked and precooked foods' must surely be listed as a classic by food technologists! All the same, if a food technologist were restricted to one book dealing with human nutrition in prosperous countries, this revised edition would prove very valuable.

K. Mary Clegg

Molecular Structure and Function of Food Carbohydrate.
London: Applied Science Publishers Ltd, 1973. Pp. xi + 308. £10.00.

This book contains the texts of the papers presented at a Symposium held at the National College of Food Technology on 10–11 April, 1973. Many aspects of the chemical, physical and physiological effects of the common carbohydrates found in food are reviewed. The book suffers as all collections of papers must, from a lack of continuity from paper to paper. This could have been improved by a more skilful grouping of the papers which are arranged in a more or less random manner. However, the short reviews together with the references provided, form a useful source of up-to-date information for the food scientist/technologist, medical student or nutritionalist.

Several papers are mainly concerned with chemical aspects such as the thermal degradation of carbohydrates, non-enzymic browning reactions, the production of glucose syrup from starch or the formation of halogen derivatives of sucrose.

Others concern the physical properties of gelling agents and the use of reverse osmosis to remove lactose from milk and whey.

A small group of papers concentrates on the basic knowledge on starch structures and the changes which take place in starch, during the processing of rice and yam flour and also during the storage of potato tubers.

The most important aspect of the symposium is probably the blending together of papers concerning the structural and physiological aspects of sugars. Papers are included concerning the basic sugar transport systems, carbohydrate intolerance, the role of the indigestible carbohydrate, the metabolism of fructose and the biological specificity of vitamin C. Further papers are concerned with the sweetness of sugars, carbohydrates and human performance and the legendary properties of honey. These papers may be combined to give a useful review of the current knowledge of the nutritional aspects of sugars.

The book is well produced with excellent printing and diagrams. It is recommended to students of food science and to practising food scientists/technologists who wish to refresh their knowledge of carbohydrates.

R. L. E. Guy

Soft Drink Manufacture: Food Technology Review No. 8. By M. T. GILLIES. New Jersey: Noyes Data Corporation, 1973. Pp. x + 336. $36.

This book reviews US patents since 1960, relating to the manufacture of soft drinks. Its prime achievement is to highlight the significant differences between soft drink technology in the USA and in Europe especially the United Kingdom. The reader is left feeling that the USA has a pseudo-sophisticated industry with a penchant for additives with complex chemical names.

The formulation techniques, especially in the field of low calories and powdered beverages, do not appear to be widely used in products emanating from the USA, and the majority would not find use in the UK, as they rely on additives which are not currently permitted by UK legislation. Many of the sub-processes referred to would appear inordinately expensive in relation to the cost of the final product.

The biggest drawback of this type of publication is that the information contained therein can be several years old. Assuming that the UK soft drink technologist believes he can learn from his counterpart in the USA, he will already subscribe to a patent alerting service and be specific in his search for information whilst it is new and relevant. Mr Gillies has, however, succeeded in presenting patent information free from the usual legalistic jargon, so for those who have a need for a retrospective transcript of patents, at least they are in a fairly readable form.

D. M. GRESSWELL

Controlling and Analyzing Costs in Food Service Operations. By J. KEISER and E. KALLIO.
New York: John Wiley & Sons, 1974. Pp. xii + 291. £6.35.

Any attempt to deal with the subject of financial control within the Food Science field should be welcomed, even if phrased in culture bound American language and accountancy conventions.

It is therefore regrettable that the authors of this particular book have limited its appeal to lecturers, by producing a text that is generally unsuitable for students or practitioners—despite claiming that they wrote it for these markets.

Firstly, criticism may be levelled at the order of the contents. While acknowledging the vital role of cost identification and control, it would seem to be more realistic to commence with financial objectives such as profit and return on capital invested, and then to deal with constraints of cost. After all, these objectives are of universal interest, even if set at zero profit and zero return, as in the non-profit seeking sector of public services.

The second major area of criticism relates to the fact that the authors deal with several complex topics, such as 'Electronic Data Processing', capital investment and make or buy decisions. Their treatment is fair but of the kind that might normally be found halfway into a text dealing solely with the topic—as with the financial subjects

or, alternatively in a fairly trivial manner, as with the computer topics. In fact they attempt to do too much in too short a space.

In conclusion, the book should find a home in the libraries of academics, as it provides useful teaching material, but it is unlikely to become a standard text.

A. C. M. YOUNG

Books recieved

Catering Science and Technology. By MAGNUS PYKE.
London: John Murray Ltd, 1974. Pp. xi + 237. £3.50.

An elementary introduction for students.

Establishing a Manufacturing Plant in Europe.
New Jersey: Noyes Data Corporation, 1974. Pp. x + 373. $36.

The book aims at providing information on investment and plant location in twenty-two European countries.

Food Acid Manufacture. Recent Developments. By A. A. LAWRENCE.
New Jersey: Noyes Data Corporation, 1974. Pp. xi + 303. $36.

Descriptive information derived from the US patent literature on the manufacture of food acids.

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

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

SI UNITS

gram	g	Joule	J
kilogram	kg = 10^3 g	Newton	N
milligram	mg = 10^{-3} g	Watt	W
metre	m	Centigrade	°C
millimetre	mm = 10^{-3} m	hour	hr
micrometre	μm = 10^{-6} m	minute	min
nanometre	nm = 10^{-9} m	second	sec
litre	l = 10^{-3} m^2		

NON SI UNITS

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

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

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