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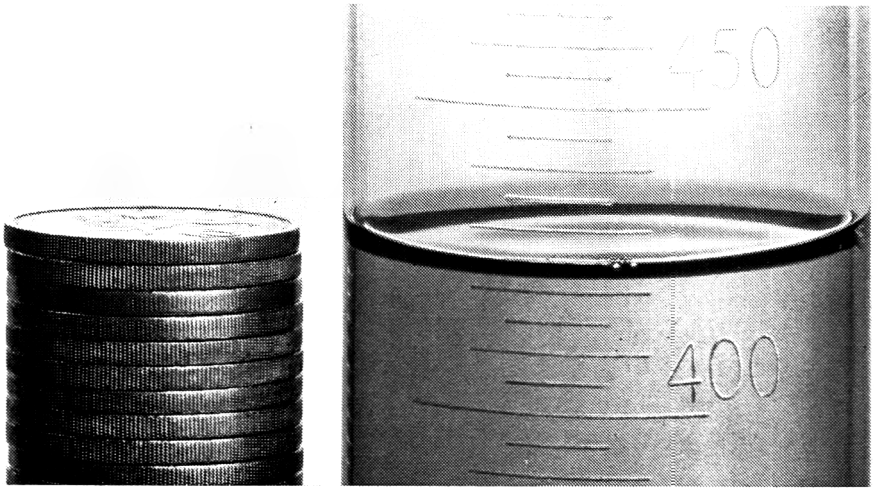

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The effect of cycled-pressure on drying conditions during freeze drying

P. F. GREENFIELD* AND J. D. MELLOR

Summary

Cyclic-pressure freeze drying has been found to improve the drying rate compared to the constant pressure process. This is due principally to the increased thermal conductivity of the semi-dried region which results from the cyclic operation. The pressure oscillation causes unsteady temperature and mass flux conditions within the material being dried. During the high pressure portion of the cycle, very little sublimation takes place and the temperatures within the material are raised. When the pressure is reduced the temperatures are reduced as sublimation occurs. The temperature oscillations were found to have the same period as the pressure cycles, but to lag behind because of the heat capacity of the material. Additionally, it was found that the cycling produced high intermittent loads on both the heating source and the vapour condenser.

Introduction

The heat and mass transfer limitations that exist during constant-pressure freeze drying have long been recognized. At the usual operating pressures (< 3 Torr) the effective thermal conductivity of the dry porous layer, through which the heat must be transferred to reach the ice interface, is so low as to become the rate limiting factor. However, it is not possible to overcome this problem by operating continuously at a very much higher pressure (e.g. 10–20 Torr). Although the thermal conductivity is improved at such pressures the vapour flow rate is now reduced to such an extent that the melting point will be exceeded.

A method has been in use for some time (Mellor, 1967) in which the operating pressure in the vacuum chamber is cycled between upper and lower limits. This has resulted in faster drying at acceptable temperature levels. Typical operation consists of a high pressure limit of 10–20 Torr held for 30–80 sec and a low pressure limit of 0·2–0·5 Torr which is reached after 100–120 sec pumping.

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The gas normally used to raise the pressure in the chamber is air. However, an additional improvement in drying rate results if helium is used, because of the higher thermal conductivity of helium gas. For such a system to be economically viable, it is essential to recycle the helium.

The fluctuating pressure of cyclic-pressure freeze drying produces unsteady drying conditions within the material, with the temperature, pressure, and vapour flux varying periodically. This is in contrast to the constant pressure process, where a quasi-steady state is considered to exist. The following sections report on this variation. The results were obtained both by theoretical analysis and by experimental observation (Greenfield, 1972).

Materials and methods

The drying chamber was made from mild steel, and is cylindrical in shape, being 2 ft in diameter and 4 ft 6 in in length (Fig. 1). The length of the chamber is divided

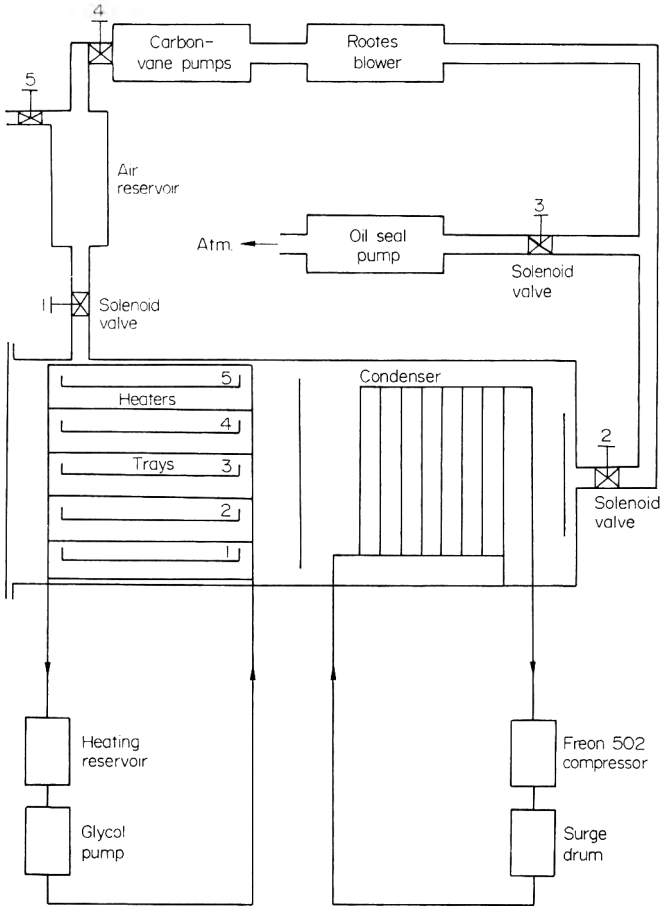

FIG. 1. Schematic diagram of cyclic-pressure freeze drying unit.

almost evenly between the heating section and the condensing section, with the latter area of the chamber being externally insulated to prevent ice buildup. A total of five trays of dimensions 60 × 35 cm can be loaded at the front by pushing them into guides between the heaters.

Radiant heating is provided by platens, interleaved horizontally between the tray rests. These platens were made from mild steel, to which tubing, carrying the heating fluid is welded in a serpentine pattern. Glycol is the heating fluid, being recirculated by a centrifugal pump, and externally heated in a central reservoir.

At the other end of the chamber is the condensing unit, which is a Freon 502 flooded-type refrigerant evaporator, equipped with a surge drum above the drying chamber. Aluminium baffles, fitted in the space between the heating end and the refrigerated end, are arranged to direct the flow of ice vapour evenly onto the condensing surface, and at the same time, minimize the incidence of radiant heat from the heating platens onto the cold surfaces. Sufficient space is left so as not to impede the vapour flow from the product surface to the condenser. The refrigeration plant, associated with the ice condenser, involves a twin cylinder Freon compressor.

Through the back-end cover, which is welded in position, a large bore pipe carries the incondensible gases to the vacuum pumps. This consists of a dual system. For pumping down from atmospheric pressure and for constant pressure freeze drying, an oil-sealed rotary pump of capacity 600 litre/min is used. For recycling vapour, the oil seal is not satisfactory, and so, a system consisting of a Roots blower, backed by two carbon-vane pumps, is employed. During the high pressure period of cyclic pressure freeze drying, a solenoid valve (1 in Fig. 1) allows vapour to escape into the chamber from a reservoir maintained at some higher pressure. For the low pressure period, valve 1 is closed and valve 2 opened to allow the gases to leave the drying chamber and to return via the pumping system to the reservoir. The vacuum solenoid valves are controlled so that they operate for predetermined time intervals or predetermined pressure levels.

Two control panels are provided, one to control the operation of the drying unit, and the other to control the selection and frequency of measurement of output variables.

Pressure, temperature, and weight readings were the output variables. The pressure was measured at various points within the chamber by means of Pirani vacuum gauges. Copper-constantan thermocouples were used to measure the temperatures within the material being dried, as well as the heating platen temperatures and the condenser temperatures. Tray 1 was balanced on a fulcrum and the resulting torque measured. The change in output indicated the amount of water that had sublimed. All outputs were in the form of e.m.f.'s and were sampled at specified intervals by a Dynamco datalogger. The relevant sampling information and input voltages were collated on paper tape and then edited and processed on a CDC 3200/3600 computer system.

Freeze-drying experiments were carried out on samples of sucrose solutions (5, 10,

15, 20%), gelatin gels (10%), and yoghurt suspensions (*c.* 10%). All samples were spread on trays to a depth of approximately $\frac{1}{2}$ in, frozen overnight at $-20°C$, and freeze dried for periods ranging from 10 to 30 hr.

Results and discussion

During the high pressure period of the cycles, the rate at which heat is transmitted through the porous region is increased because of the higher thermal conductivity. Although there is some sensible heat added by the incoming gas, this was found to be negligible. As well, there is negligible sublimation occurring, and so, the whole slab is acting as a heat reservoir. Both experimental and theoretical results suggest that the temperatures throughout the slab increase to a value which depends on the distance from the heat source. Figure 2 shows a typical temperature profile through the slab in this period, where the lowest temperature is at the centre-line.

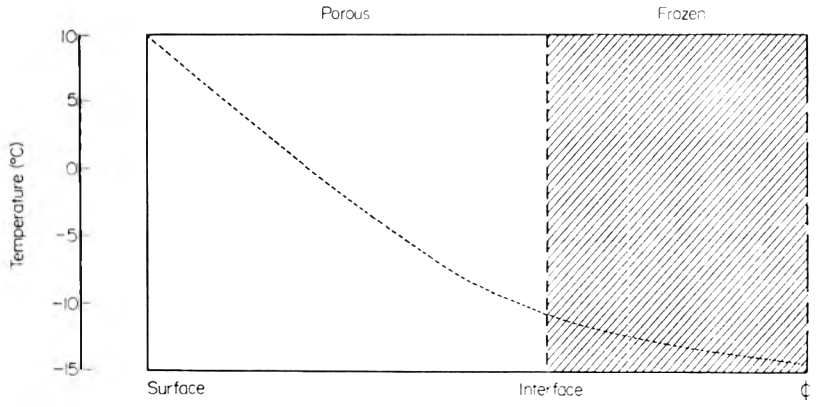

FIG. 2. Temperature profile during high pressure period.

As the pressure falls, the secondary gas (air or helium) is removed from the pores of the material. There is little water removal while the total pressure is still above the saturated vapour of the ice; therefore, the temperatures continue to rise.

Once the external pressure approaches the saturated vapour pressure of the ice, however, sublimation of the ice phase begins at the interface. Sensible heat is withdrawn from the region adjacent to the interface to provide the necessary heat of sublimation. This drop in temperature means that heat will be conducted to this region from both the porous and frozen sections. The interface becomes the coldest point in the slab, and the slab itself is acting as a heat source (Fig. 3). Of course, heat is still being radiated to the surface, but, with the drop in pressure, the reduced thermal conductivity more than offsets the increased temperature difference across the porous region. Figures 4 and 5

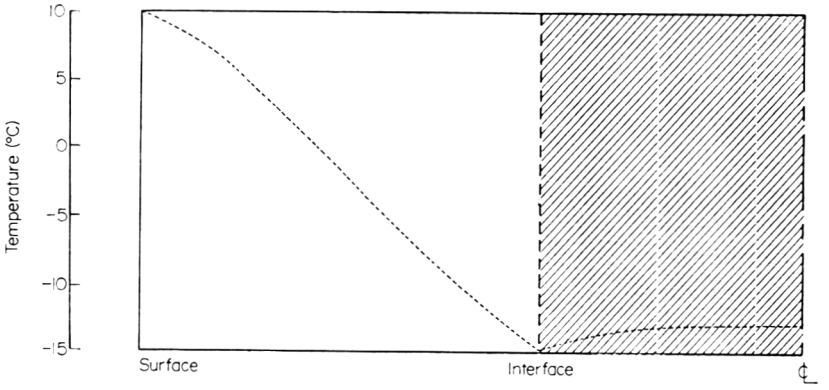

FIG. 3. Temperature profile during low pressure period.

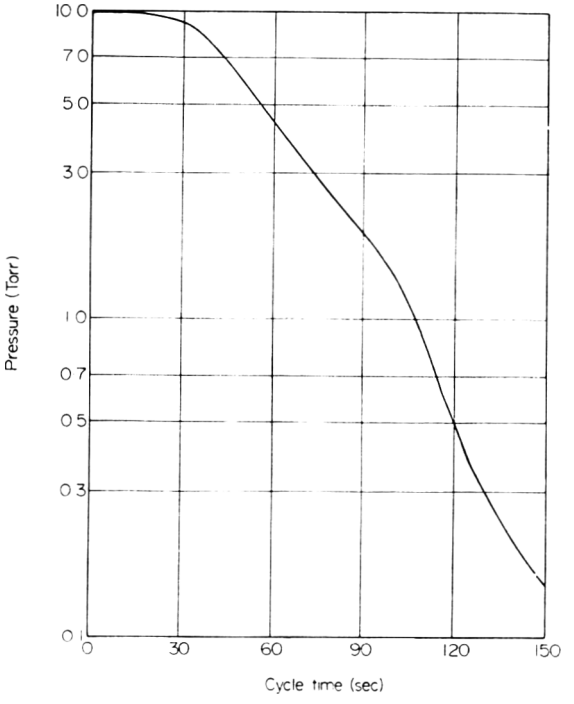

FIG. 4. Variation of pressure in freeze drying chamber.

show how some of these variables change during a pressure cycle. A plot of the pressure at the surface of the material is provided as a reference. It is noticeable that the temperature variation is of the same period as the pressure oscillation but lags behind because of the heat capacity of the material. The amplitude of the variation is greatest in the porous region near the surface of the material.

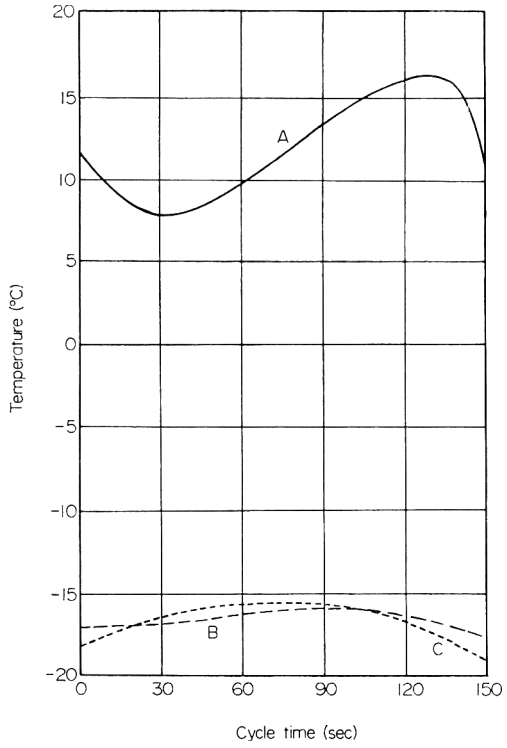

FIG. 5. Variation of temperature in product during one pressure cycle. A, Temperature at surface; B, temperature at centre-line; C, temperature at interface.

The net effect of the pressure cycling is to produce a mean thermal conductivity per cycle which is higher than the thermal conductivity experienced during constant pressure drying. This allows greater heat transfer to the ice interface and, therefore, greater potential for ice sublimation. The mean thermal conductivity per pressure cycle is given by:

$$\bar{\lambda} = \frac{\int_0^{t_{HI}} \lambda_{HI} dt + \int_0^{t_{LOW}} \lambda dt}{t_{CYC}}. \tag{1}$$

Since the pressure during the high pressure time is constant, the thermal conductivity λ_{HI} may also be regarded as such. During the low pressure period, the thermal conductivity of the porous region is considered as a function of the solid phase component:

$$\lambda = \lambda_s + \lambda_g$$

$$= \lambda_s + \frac{\lambda_{go}}{1 + C/P}. \tag{2}$$

The second term in equation 2 represents a semi-empirical relationship for the gas phase contribution determined by Harper (1962). Since the pressure variation in the vacuum chamber was found to approach an exponential relationship during the low pressure portion of the cycle, equation 1 may be integrated to give:

$$\bar{\lambda} = \frac{\lambda_{HI} t_{HI} + \lambda_s t_{LOW} + \frac{\lambda_{go}}{h} \ln \left[\frac{P_{HI} + C}{P_{HI} \exp(-h t_{LOW}) + C} \right]}{t_{CYC}}. \quad (3)$$

In this equation, h is a parameter which determines the rate of pressure variation in the vacuum chamber. Cyclic-air freeze drying of beef with a high pressure of 20 Torr for 80 sec and a low pressure time of 120 sec in a chamber where $h = 0{\cdot}037$ sec^{-1} yields a mean thermal conductivity $\bar{\lambda}$ of *c.* 0·05 Kcal/m°Ch ($\lambda_{HI} = 0{\cdot}057$ Kcal/m°Ch, $\lambda_{go} = 0{\cdot}023$ Kcal/m°Ch, $C = 1{\cdot}5$ Torr). To achieve this thermal conductivity with constant pressure freeze drying, an average pressure of 8 Torr would be necessary. Vapour transfer would become limiting with continuous operation at such pressures, and the incipient melting point be exceeded.

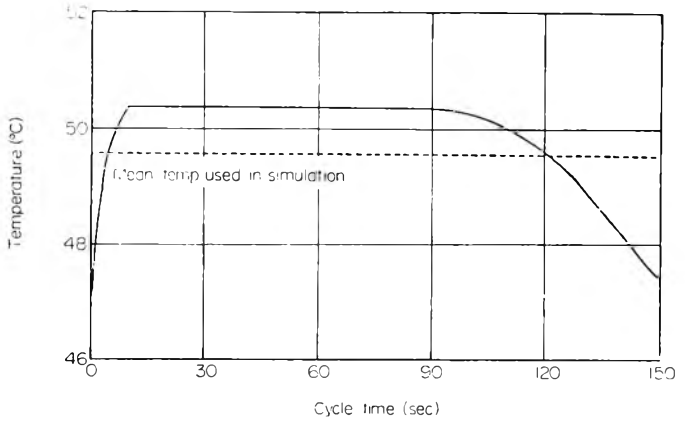

FIG. 6. Variation of heater temperature during one pressure cycle.

King (1971) objects to cyclic-pressure freeze drying on the grounds that an identical effect could be achieved by running at a higher mean pressure (e.g. 8–50 Torr), and hence, there is no need to invest the extra capital to obtain cyclic operation. However, it is doubtful whether operation could proceed at the above pressures without special precautions to ensure good vapour flow, e.g. directed gas streams across the surface of the drying product. Without this, a large number of materials would not dry satisfactorily at the high operating temperatures such conditions would imply. Certainly, no local freeze-drying units can operate successfully at these pressures. With nitrogen

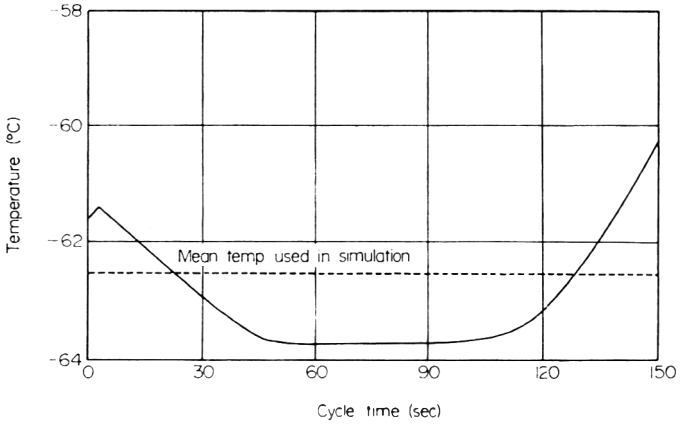

FIG. 7. Variation of condenser temperature during one pressure cycle.

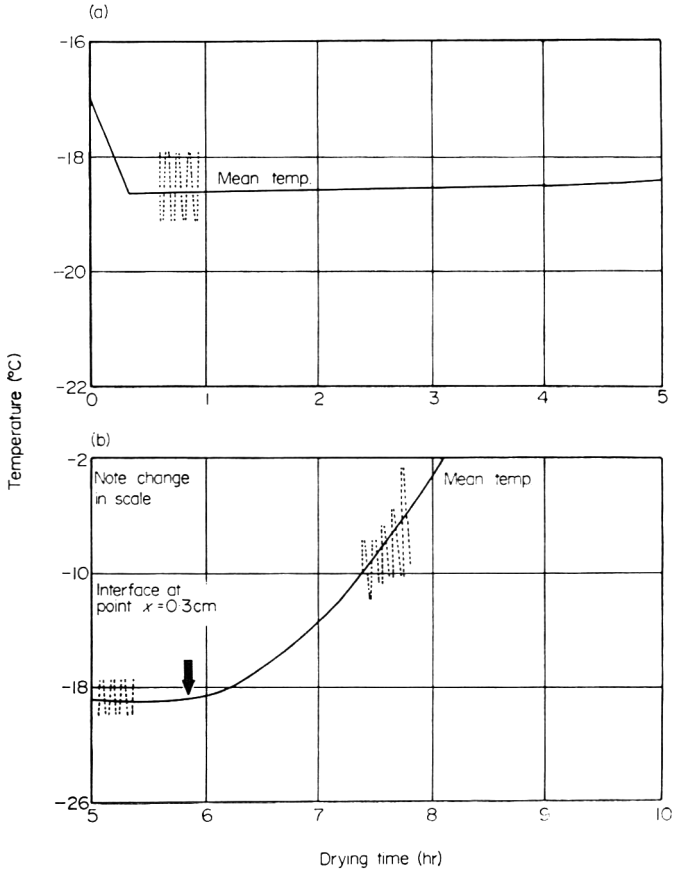

FIG. 8. Variation of temperature in product during complete freeze drying run. Cycles are not to scale.

as the recycled gas, he calculated that an upper cycle limit of 100 Torr would produce an effective gain in the thermal conductivity of only 15%, while, with a limit of 20 Torr, a gain of only 8% would be achieved. In each case, he suggested that there would be negligible increase in the drying rate. King's results are obtained by averaging the heat input over a complete cycle, and comparing this to the heat input obtained during constant pressure freeze drying, using a mean value of the thermal conductivity. In his analysis, however, he has neglected one important factor, namely, the length of the high pressure period.

As shown above the increase in the effective thermal conductivity during cyclic-pressure freeze drying compared to constant pressure operation is significant.

The oscillating pressure also affects the heater and condenser temperatures. As the sublimation reaches a maximum, the heater temperature actually drops, while the condenser temperature rises as it receives the bulk of the water vapour (Figs 6, 7). In the theoretical analysis, mean values were used to simplify calculations.

The pressure cycling is repeated throughout the freeze-drying run, and, after an initial transient period, it is found that the temperature at any point in the frozen region oscillates about a mean value which is almost constant until the interface reaches that point. Once the interface has passed, this mean temperature begins to rise while the amplitude of the oscillations increases (Fig. 8). Finally, when drying is almost completed, the slab temperatures approach that of the heating platens.

Conclusions

An oscillating pressure during freeze-drying produces a higher effective thermal conductivity in the semi-dry product than is practicable with constant pressure operation. The oscillating pressure also disturbs the quasi-steady state which exists during constant pressure drying. The temperature of the drying material oscillates with a similar frequency to that of the pressure oscillation, but lags behind because of the heat capactities of the dry and frozen layers. It was found that these oscillations occurred about a mean temperature which gradually increased as the drying progressed. It is important in processing that the maximum temperature reached during such oscillations be below the incipient melting point of the material being dried.

Notation

C, h	Constants, Torr.
P	Pressure, Torr.
P_{HI}	High pressure limit, Torr.
t_{HI}	Time for which high pressure is maintained, sec.
t_{LOW}	Time during which pumping occurs, sec.
t_{CYC}	Total cycle time $= t_{HI} + t_{LOW}$, sec.

λ Thermal conductivity, Kcal/m°Ch.
λ_g Gas phase thermal conductivity, Kcal/m°Ch.
λ_{go} Thermal conductivity of air at atmospheric pressure, Kcal/m°Ch.
λ_{HI} Thermal conductivity of vapour mixture in freeze-drying chamber at high pressure portion of cycle, Kcal/m°Ch.
λ_s Solid phase thermal conductivity, Kcal/m°Ch.
$\bar{\lambda}$ Mean thermal conductivity, Kcal/m°Ch.

Acknowledgment

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Retention of aroma components in extractive drying of aqueous carbohydrate solutions

P. J. A. M. KERKHOF AND H. A. C. THIJSSEN

Summary

A new drying technique, extractive drying, has been studied experimentally. The retention of the components methanol, *n*-propanol and *n*-pentanol in extractive drying of a model liquid food was investigated. The model liquid food consisted of an aqueous solution of mixtures of dextrin and malto-dextrin, to which the alcohols were added in low concentrations. As extraction liquid polyethylene glycol (PEG) 400 was used. The solution to be dried was dispersed in the PEG in the form of droplets.

High losses of the alcohols were found when the feed droplets were distorted upon contact with the extraction liquid. This distortion does not occur when the viscosity of the feed solution is higher than approximately 150 cP. For such feed solutions, containing 40% water and on dry basis 25% dextrin and 75% malto-dextrin, very high aroma retentions were found. The effects observed agree with the selective diffusion theory.

Introduction

The most common processes for the selective dehydration of aroma-containing liquid foods including coffee and tea extracts and fruit juices are spray-drying and freeze drying. Rulkens & Thijssen (1972a,b) showed that under optimum process conditions, high retentions of volatile compounds in dry product can be obtained with both these techniques. Owing to the elevated temperatures used in spray drying thermal degradation of heat-sensitive products may occur. Thermal deterioration can fully be avoided in freeze-drying, which thus results in better product quality. However, the cost of freeze drying is much higher than the cost of spray drying. Optimum conditions for high aroma retention in spray drying include a high dissolved solids content of the feed liquid. To satisfy this requirement preconcentration of the juices or extracts is often necessary.

Preconcentration may be done by evaporation with aroma recovery, by freeze concentration or by reverse osmosis. Evaporation may cause thermal damage to the

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food liquid, while the costs of freeze concentration and reverse osmosis are relatively high; moreover the maximum attainable dissolved solids concentration by the latter concentration techniques may not be sufficient to ensure high volatile retention in spray drying.

At present considerable interest is taken in foods to the dry product of which natural or synthetic aroma components are added. The objective of such a process can be either to give a pleasant smell to the dry product or to provide for a full flavour pattern of the product upon rehydration. In case of the former, adsorption of aroma components to the surface of the dry product gives instant aroma availability (Le Maguer, 1972), whereas in the latter case the aromas should not instantly be released from the dry product. Therefore the flavour components should be entrapped in a water soluble solid, which is inert to the flavour itself and of course fit for human consumption.

Recently micro-encapsulation processes, which generally consist of the incorporation of an active material in an inert solid medium, have attracted much interest and found very widespread application, as was shown by Balassa & Brody (1968), Balassa & Weiss (1967) and Nack (1970). An extensive review of the application of micro-encapsulation processes in the food industry was given by Balassa (1971).

Although most of the applications concern the encapsulation of discrete particles or droplets in protecting capsules, an interesting aspect of the micro-encapsulation technique is the encapsulation of volatile aroma components from aqueous solutions, which contain the volatiles and the capsule-forming solid in the homogeneously dissolved state. This aspect was patented among other things by the Balchem Corp. (1971).

Extractive drying

According to the Balchem patent aqueous solutions of dextrin with 1% added pineapple aroma were dried by dispersing them in polyethylene glycol with a molecular weight ranging from 106 to 2000. The flavour retention in the dried product was reported to be good. By analogy with spray drying and freeze drying this technique in which water is removed by means of contact with a water-absorbing second phase, will be called extractive drying.

A quantitative study has been made of the retention of aroma compounds in extractive drying of model food solutions with PEG 400 as extraction liquid. The model solutions consisted of aqueous solutions of dextrin, maltodextrin or mixtures of these carbohydrates and methanol, *n*-propanol and *n*-pentanol as model aroma compounds. These aroma compounds were in all but one experiment added in low concentrations.

Aroma retention

From experimental work reported on spray-drying (Rulkens & Thijssen, 1972a), slab-drying (Menting, 1969; Menting, Hoogstad & Thijssen, 1970a; Chandrasekaran & King, 1972a; Rulkens, 1973), freeze-drying (Rulkens & Thijssen, 1972b; Flink &

Karel, 1972) and drying of single droplets (Menting, 1969) it has become clear that under proper conditions it is possible to retain aroma components in a drying aqueous carbohydrate solution, despite the high relative volatilities of the aroma compounds with respect to the volatility of water. The cause of this is the transport mechanism of water and aroma components in the drying liquid.

As stated by Thijssen (1965) the transport of water and of aroma towards the evaporating surface is controlled by molecular diffusion. The diffusion coefficients of both water and aroma components are, for these types of solutions, dependent on water concentration (Fish, 1958; Thijssen & Rulkens, 1968; Menting, 1969; Menting *et al.*, 1970b; Chandrasekaran & King, 1972b). With decreasing water concentration both diffusion coefficients decrease; the aroma diffusivity, however, decreases faster than the water diffusivity. When water is removed at the phase boundary of the drying liquid foods, the interfacial water concentration in the drying specimen will decrease with time. If that water concentration has been reduced to a low enough level, say below 20 wt%, the diffusion of aroma components is so much slower than that of water, that the surface becomes virtually impermeable to aroma components. This theory is known as the 'selective diffusion' theory.

From the above-mentioned experimental studies and from theoretical work it has been shown that the selective diffusion concept gives a good description of the water and aroma loss from drying liquid foods for air drying and vacuum drying of slabs and droplets. For reviews of the theoretical aspects of the drying of liquid foods the reader is referred to the literature (Chandrasekaran & King, 1972a; Kerkhof, Rulkens & van der Lijn, 1972; Kerkhof & Schoeber, 1973). Theoretical considerations of extractive drying, which are strongly analogous to the description of the drying of spray drying or slab drying, will be presented elsewhere (Kerkhof, 1974).

For slab drying and spray drying both from experimental and theoretical results follows that aroma retention increases with an increasing rate of external water removal, with increasing initial dissolved solids concentration, and with increasing molecular weight of the aroma component.

In extractive drying high aroma retentions can be expected when the following conditions are satisfied.

(1) The selective diffusion behaviour found in air drying and freeze drying should be retained. This means that no appreciable amount of extraction liquid should penetrate into the drying system.

(2) The droplets should remain rigid from the beginning of their formation, so no extra loss can occur by distortion of the droplet or internal circulation. (This latter phenomenon increases the transport rate of aroma components and prevents a rapid reduction of the surface water concentration.)

(3) The water withdrawal from the droplet should be very fast with respect to the internal water transport velocity. (This means that the extraction liquid should

be hygroscopic and that the continuous phase mass transfer coefficient should be high.)

Investigations some years ago in our laboratory (van Oorsouw, 1965) into the first condition showed that the permeability of low molecular organic solvents such as iso-propanol, *n*-propanol, ethanol and ethylacetate through membranes of cellophane or dextrin was, upon direct contact with these solutes, of the same order of magnitude as the permeability of water in these membranes. This means that low molecular solvents permeate into the drying food liquid quite easily and that no selective diffusion of water occurs at the interface. On the basis of the following experimental results it will be shown that PEG 400 does not penetrate into the drying system to any significant extent and that the selective diffusion concept also holds for this drying technique.

Experimental

A schematic diagram of the experimental set-up is given in Fig. 1. All extractive drying experiments were carried out batchwise in a 1 l tank, equipped with four baffles and an eight-blade turbine impeller. The maximum impeller speed was 1500 rev/min. The feed solution was fed to the tank from a 100 ml perspex container, to which air pressure could

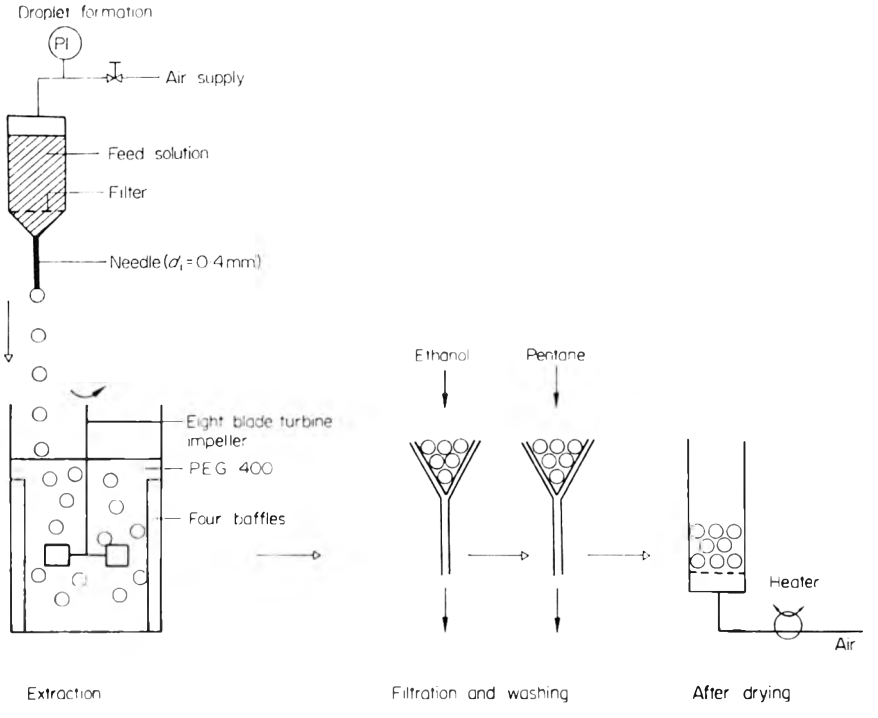

Fig. 1. Diagram of the experimental set-up for extractive drying process.

be applied. A 0·4 mm needle was used as disperser, from the end of which droplets were formed. Droplet size ranged from 0·2 to 2 mm, depending on feed viscosity.

The feed liquid consisted of an aqueous solution of a mixture of dextrin and malto-dextrin. Methanol, *n*-propanol and *n*-pentanol were added in concentrations of 0·3 wt% on dissolved solids base, except for one experiment. Water-free PEG 400 was used as extraction liquid. In each experiment about 55 ml of feed solution was used, and 1 l of PEG. Extraction took place at room temperature.

After extraction for 30 min the product was filtered off and quickly washed first with dry ethanol to remove adhering PEG and subsequently with pentane to remove the ethanol. Then the product was placed in a small bed drier and dried with dry air for several hours at a temperature of about 30°C.

Aroma retention was measured by gas chromatographic determination of the amounts of aroma present in the feed liquid and in the product.

The influence of the following conditions was investigated.

Dissolved solids composition: 0, 25, 50, 75 and 100 wt% malto-dextrin on solute basis at a total dissolved solids concentration in feed solution 50 wt%.

Dissolved solids concentration: 50 and 60 wt% total dissolved solids in feed, solute composition 75% malto-dextrin.

Stirrer speed: 150–1500 rev/min.

Aroma concentration: in one experiment 15 wt% total aroma was added to a 60 wt% dissolved solids feed with a solute composition of 75% malto-dextrin and 25% dextrin.

More detailed information is given by van Delft (1972).

Results and discussion

Aroma retention

Dissolved solids composition. In Fig. 2 the effect of the dissolved solids composition on aroma retention is shown at a total solute concentration of the feed of 50 wt%. With this solute concentration the alcohol retention attains a maximum at a solute composition of 75% malto-dextrin and 25% dextrin. The retentions found with 100% malto-dextrin are very low with respect to the optimum solute composition. Visual observation learned that at 50% solute concentration the droplets more or less retained their spherical shape upon entering the extraction liquid at up to 50 wt% malto-dextrin on dry basis, whereas at higher ratios of malto-dextrin to dextrin in the feed, deformation of the droplets occurred, which effect grew worse with increasing malto-dextrin content, resulting in full distortion to threads at 100% malto-dextrin. This is accompanied by a sharp decrease in the viscosity of the feed liquid at malto-dextrin fractions of more than 75%.

The deformation of the droplets upon contact with the PEG can be explained by the low interfacial tension between the two phases. For the system dextrin–PEG–water,

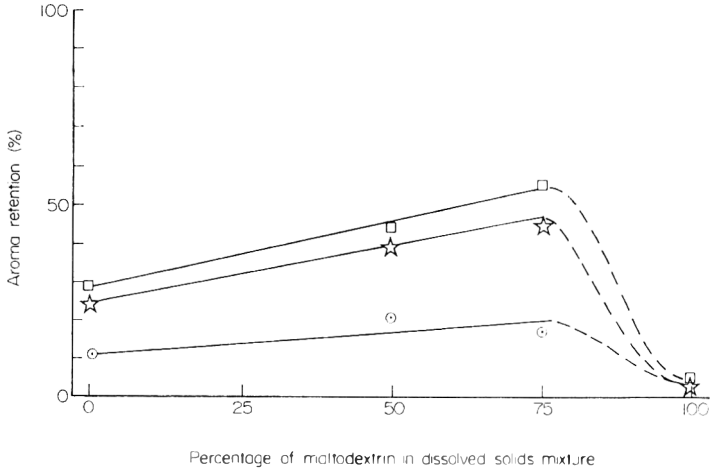

FIG. 2. Experimental values of retention of model aroma components as a function of composition of dissolved solids, for 50 wt% total dissolved solids. □, *n*-pentanol; ☆, *n*-propanol; ⊙, methanol.

Ryden & Albertson (1971) reported values of the interfacial concentration smaller than 0·1 dyne/cm. Thus a droplet can only remain spherical if its viscosity is high compared with the viscosity of the surrounding continuous phase.

The partial substitution of dextrin by malto-dextrin has a positive effect on aroma retention, provided the viscosity remains high enough. Apparently the selectivity of the surface of the drying droplet is improved by the addition of malto-dextrin. Probably the combination of the lower molecular carbohydrates with the high molecular dextrin results in a less open structure at the same water concentration, thus reducing the mobility of the aroma molecules.

The effect of the molecular weight of the homologous series of alcohols can also be read from Fig. 2. Higher molecular weight gives better retention as has also been reported in the literature for spray-drying and freeze drying (Rulkens & Thijssen, 1972a,b).

Stirrer speed. From the above it can be concluded that a fair part of the aroma loss is due to droplet deformation (and distortion). At a constant malto-dextrin fraction of 75 wt% on dissolved solids basis the effect of stirrer speed on aroma retention was investigated for total solute concentrations of 50 and 60 wt% of the feed (see Fig. 3). For the 50 wt% solution there was a definite drop in aroma retention with higher stirrer speed, whereas at 60 wt% no influence was found. Apparently the droplets formed with the 60% solution already have such a high viscosity, that the shear forces exerted on the droplet are not strong enough to induce deformation.

Dissolved solids concentration. The effect of the total solute concentration of the feed on aroma retention can also be derived from Fig. 3. At 60 wt % the retention of *n*-propanol

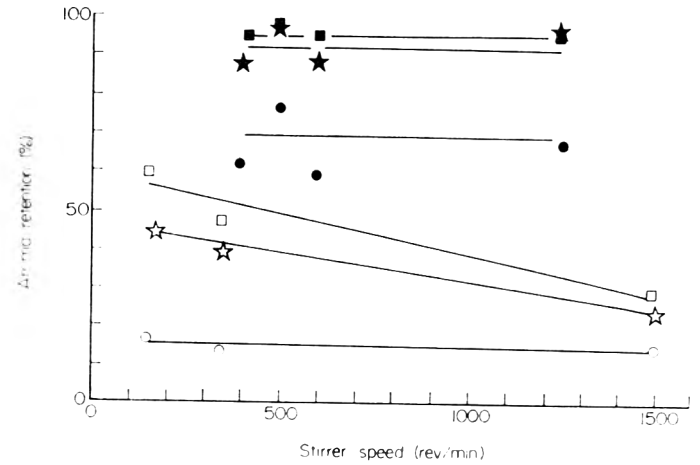

FIG. 3. Experimental values of retention of model aroma components as function of stirrer speed. Total initial dissolved solids content: ■, ★, ●, 60 wt%; ⊡, ✡, ⊙, 50 wt%. □, ■, *n*-pentanol; ✡, ★, *n*-propanol; ○, ●, methanol.

and *n*-pentanol was 90 and 95% respectively, while the retention of methanol was about 70%. These values are much higher than those found for 50 wt% dissolved solids. The retention is not only higher because of less deformation of the droplets, but also because of the shorter time needed to attain selective permeability at the surface.

Aroma concentration. To a 60 wt% dissolved solids solution with a 3 : 1 ratio of maltodextrin and dextrin in the solids an amount of 5 wt% on total dry basis of each aroma component was added. The retentions were found:

methanol 31% (0·016 g/g on dry basis);
n-propanol 59% (0·030 g/g on dry basis);
n-pentanol 55% (0·028 g/g on dry basis).

The addition of the alcohols to the mixture reduced the dissolved solids content to only 55%, which caused a drop in viscosity, thus allowing a somewhat stronger deformation. Moreover, it is not likely that at such high alcohol concentrations the selectivity of the 'dry skin' is as high as at low concentrations.

An interesting aspect of this experiment is the absolute amount of aroma retained in the product, which equalled a total aroma content of 7·4 wt% on dry basis.

Extraction rate

Extraction of water was found to be nearly complete after a few minutes in the case of thread formation. If the droplets remained spherical, the water concentration after extraction for 30 min was still about 15 wt%, and after-drying was necessary.

General conclusions

Experiments have shown that very high aroma retentions can be obtained in extractive drying with PEG 400. Moreover, it is possible to retain a large amount of aromas on an absolute basis. The primary condition for high retention is a sufficiently high viscosity of the feed to prevent distortion of the droplets. To improve aroma retention, the solute composition must be such that the solution exhibits a high selectivity at the drying interface with the PEG phase.

The influence of initial dissolved solids concentration and of aroma molecular weight on aroma retention agree with experimentally found results for spray drying (Rulkens & Thijssen, 1972a) and slab drying (Rulkens, 1973) and with the selective diffusion theory.

As the process is simple and is carried out at room temperature and as high aroma retention is obtained, extractive drying is thought to be a very promising aroma encapsulation technique for the food industry. Further study is however necessary, regarding extraction times, product-extractant ratio and the suitability of the process for other liquid foods, as these factors determine the economics and practicability of the process.

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Production of an enzymic hydrolysate of casein on a kilogram scale

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Summary

The scaling-up of a method for the production of an enzymic hydrolysate of casein is described; minor modifications of the laboratory procedure were required. The spray-dried product is relatively free of the bitterness often associated with proteins subjected to enzymic treatment. The bacteriological load of the raw materials, the intermediate stages, and the final product was monitored.

The production of hydrolysed casein in quantity is providing an opportunity to investigate the dietary potential of pre-digested protein for patients suffering from digestive disorders as a result of pancreatic malfunction.

Introduction

The need for pre-digested protein in the treatment of certain digestive disorders, and the advantages of enzymic hydrolysis, have been discussed in a previous paper (Clegg & McMillan, 1974). The formation of bitter-tasting peptides after enzymic treatment of a variety of proteins is a characteristic phenomenon (Petrischek, Lynen & Belitz, 1972). However, Clegg & McMillan (1974) described a laboratory method for the hydrolysis of casein in which the bitter peptide is degraded by utilizing the spectrum of proteases present in pig kidney for a second stage of enzymic treatment. This method has now been modified for the production of kilogram quantities of the relatively non-bitter hydrolysed powder for use in medical trials.

Experimental

In the laboratory, wet casein curd precipitated from 6 l of liquid skim milk was washed and suspended in aqueous solution and followed by hydrolysis with papain and pig kidney tissue (Clegg & McMillan, 1974). After boiling and centrifuging to inactivate the enzymes and remove insoluble materials, respectively, the supernatants from two

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batches were combined and concentrated in a falling film evaporator before spray-drying. In the course of four trials, the pre-concentration was to 13–15% solids and a range of inlet and outlet temperatures for the spray-drier was investigated. Working with such small samples in pilot scale processing equipment inevitably resulted in low yields, and the moisture content of the powder ranged from 7·0 to 9·8%.

Modification of the procedure was required when the volume of liquid skim milk for casein precipitation in a cheese-vat was increased to 50 gal. The casein curd was transferred to a thermostatically-controlled Silkeborg fermentation tank (with a capacity of 150 gal and an intermittent stirring mechanism) and suspended in 50 gal of water. The temperature control was set at 40°C for the period of enzymic hydrolysis. A replacement for toluene (which is subsequently removed as an azeotropic mixture during boiling in the laboratory) as the preservative during the enzymic treatment had to be found as the use of larger amounts in pilot plant production could present an explosive hazard. Chloroform, with its lower boiling point, was substituted at a level of 0·5% (v/v) and could not be detected in the final powder because of evaporation during the concentration process. After enzymic hydrolysis of the casein curd, the boiling and centrifuging stages as used in the laboratory were replaced by passing the

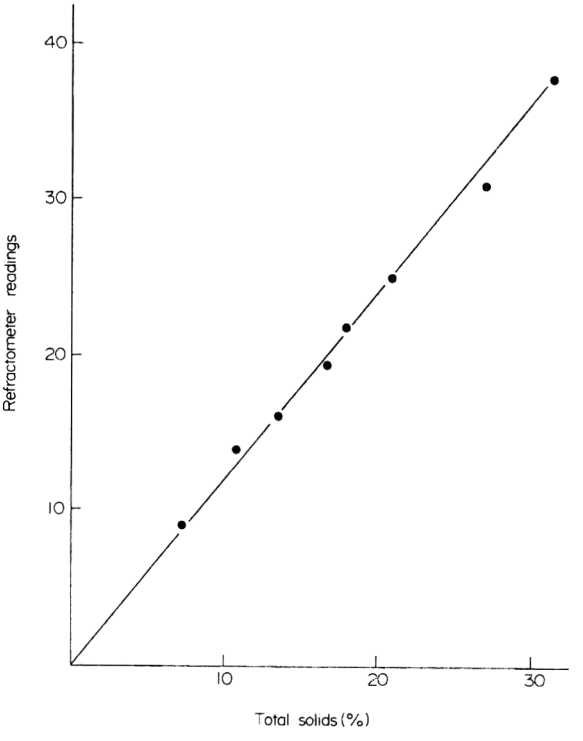
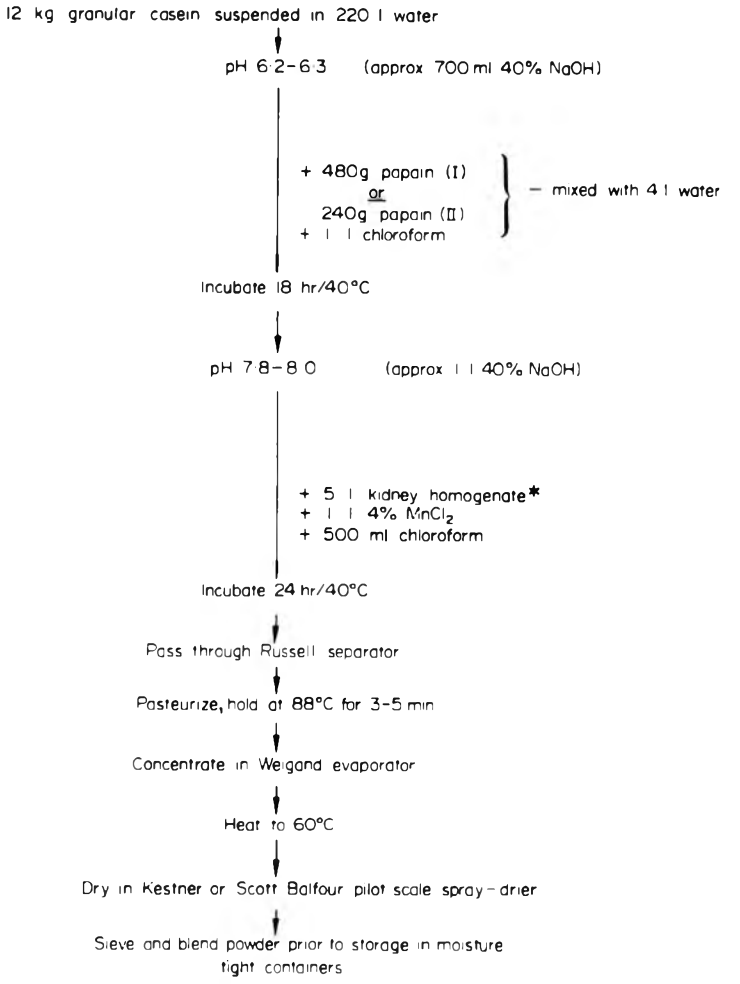


FIG. 1. The total solids content and refractometer readings of the liquid enzymic hydrolysate of casein during concentration in a falling film evaporator.

hydrolysate through a Russell separator to remove insoluble material. The product was then pasteurized at 83–88°C for 3–5 min, in an APV plate heat exchanger with holding tube, to terminate enzyme activity and reduce the microbial load.

Concentration of the warm hydrolysate was effected by a Weigand pilot plant single effect falling film evaporator with recirculation and an evaporative capacity of 60 gal of water an hour. The establishment of a linear correlation between refractometer readings and the chemically determined solids content (Fig. 1) provided a useful



* 1 l homogenate of cortical tissue and water ; thirty pig kidneys yield approximately 5 l homogenate.

FIG. 2. Flow diagram for the pilot plant production of enzymically hydrolysed casein.

method for following concentration in the evaporator from which samples were withdrawn periodically. A basic problem associated with concentration and spray-drying was observed: there was a tendency for the hydrolysate to have a high viscosity at a relatively low solids content which restricted concentration to 20–23% dry weight for efficient handling in the evaporator. The concentrated hydrolysate was held in a water-jacketed vessel at 60°C before entering the spray-drier, also to reduce the problem of viscosity.

The pale cream-coloured spray-dried products obtained after enzymic hydrolysis of wet casein precipitated from liquid skim milk, granular acid casein, and sodium caseinate powder were compared. The extent of enzymic hydrolysis was lowest with sodium caseinate. The powder from liquid milk had marginally the most acceptable taste of the three products but in the interest of shortening the process time, commercial granular casein offered the greatest scope for further development.

Using two pilot plant spray-driers concurrently, 12 kg granular casein was enzymically hydrolysed, concentrated and dried within 60 hr, as shown in Fig. 2. The gas-heated Scott-Balfour and the electrically operated Kestner proved satisfactory for spray-drying the hydrolysate concentrate, with an inlet temperature of 200–225°C and an outlet temperature of 90–100°C and a feed rate of approximately 3 l/hr per drier.

Results and discussion

The analytical and microbiological data from five batches of enzymically hydrolysed granular casein prepared on separate occasions are summarized in Table 1. The majority of these hydrolysates contained 46–50% free amino acids, as measured by the dialysable nitrogen content (Clegg & McMillan, 1974). For Batch C, the scale of operation was doubled to start with 24 kg casein using the same fermentation tank; the free amino acid content was reduced to 35%. Further investigations would be needed to establish whether this was a chance occurrence or if the ratio of volume to the surface area exposed to the atmosphere is critical for optimal enzymic activity; laboratory trials indicate that the latter is not the case.

Approximately 75% of the original 12 kg casein sample was recovered as the hydrolysed powder. Except for the small amount of solid material removed by the Russell separator, the loss was a constant factor due to retention in the processing equipment and the yield would be proportionally increased with a larger scale of production.

At the end of each stage of the process, the microbial content was analysed quantitatively and qualitatively. The chloroform preservative kept the growth of bacteria satisfactorily in check while the hydrolysate was in the fermentation tank, and the heat processes prior to spray-drying resulted in a noticeable reduction in the number of non-thermophilic organisms. The exception was with Batch B which was held in the concentrated liquid state in cold storage for 2 days before drying; this concentrate, therefore, twice passed through favourable temperature conditions for the growth of

TABLE 1. Chemical and bacteriological data for enzymic digests of granular casein

	A	B	C	D	E
Chemical					
Moisture (%)	—	4·2	3·5	2·8	3·7
Nitrogen (%)	12·2	12·0	11·6	12·6	11·9
Dialysable nitrogen* (%)	46·8	47·7	35·6	45·9	51·9
Bacteriological (count/g)					
Total viable count at 30°C ('000)	3·6	50·3	1·6	0·1	0·4
Proteolytic organisms					
(i) 37°C × 48 hr ('00)	26·4	95·0	2·7	0·4	0·5
(ii) 22°C × 72 hr ('00)	17·7	128·0	9·8	0·7	0·9
Surviving 80°/15 min					
(i) Aerobic ('00)	22·4	66·0	11·0	0·1	0·1
(ii) Anaerobic gas-producers	18	50	5	5	7

* An approximate measure of the free amino acid content (Clegg & McMillan, 1974).

surviving organisms in the cooling down and warming up stages which were incurred. Batch B was considered to be unsafe bacteriologically for oral administration and was discarded but the trial had provided additional evidence for avoiding delay between the concentration and spray-drying operations. Also, there was a possibility that contamination of the Kestner from a previous drying of a fish product accounted for the significant number of anaerobic micro-organisms in Batch B, thus confirming the need for scrupulous hygienic practice in the maintenance of equipment.

Batches A, B and C were prepared with the same commercial papain (I) whilst an alternative, bacteriologically cleaner, commercial papain (II)—a drop in total count from several thousand to < 10/g—was used in the production of Batches D and E. In addition, the second papain was more active and laboratory tests showed that the amount required could be halved.

The bacteriological count of the final spray-dried powders was determined for a range of micro-organisms. *Staphylococci* in 0·1 g, sulphite reducing anaerobes in 0·5 g, coliform in 1·0 g, and *salmonella* in 20·0 g were absent; approximately two *enterococci* in 0·1 g powder were found for all batches and this organism will require monitoring in future production. The West German regulations (Bundesgesetzblatt, 1963) lay down the following standards for dried infant foods:

(i) maximum 50,000 organisms in 1·0 g;
(ii) coliforms not detectable in 0·01 g;
(iii) maximum 150 aerobic sporeformers or other proteolytic bacteria (caseolytic) in 0·1 g;
(iv) anaerobic sporeformers must not be detectable.

When the above results and those given in Table 1 are compared with the German regulations, the batches of casein hydrolysate conform except for the presence of a very small number of anaerobic gas-producers, and this contamination has been reduced since the introduction of bacteriologically cleaner papain.

Samples of spray-dried hydrolysate were filled into polythene (250 gauge) pouches and glass jars which were packed under atmospheric conditions or flushed with nitrogen before heat seaming or screwing on the caps. The containers were stored at various temperatures within the range $-8°C$ to $+40°C$ for 3 months and the results showed that the moisture content did not change significantly and the bacteriological count of several classes of organism diminished considerably in all cases; a continued reduction in count after a further 5 months storage under these various conditions was observed. It is concluded that the spray-dried powder is a very stable product provided it is packed under moisture-tight conditions.

Two small rat feeding trials were carried out to confirm that the original nutritional quality of the casein had not been impaired by the enzymic hydrolysis and subsequent processing. Recently weaned rats were fed *ad libitum* a nutritionally complete synthetic diet in which the protein was supplied by the hydrolysate powder for the test groups and by whole casein (10%) for the control groups; both diets had the same nitrogen content. Food intake and weight gains were recorded and the results are summarized in Table 2. The rats on the hydrolysate diet showed an increased weight gain and Protein Efficiency Ratio compared with controls supplied with casein, with no symptoms of toxicity. The indication for better utilization of the pre-digested product compared with the intact equivalent protein for healthy rats could have various explanations: the small percentage of soluble kidney tissue in the hydrolysate could have a nutritional supplementary effect improving the amino acid pattern of the final product; or, the content of small peptides in the hydrolysate could be of special significance. The importance of peptides in protein digestion and absorption from the gut is a current topic of interest and research (Silk *et al.*, 1973).

TABLE 2. Weight gain and Protein Efficiency Ratio (PER) for rats fed diets containing 10% protein supplied by whole casein and enzymically hydrolysed casein

		Duration (days)	No. of rats	Av. wt gain (g/first 21 days)	PER*
I	Hydrolysate	21	9	97·7	3·04
	Casein	21	10	91·1	2·57
II	Hydrolysate	28	6	106·8	2·73
	Casein	28	6	91·7	2·48

* PER = wt gain (g)/protein eaten (g) during the trial period, i.e. 21 days for I and 28 days for II.

Medical investigations now in progress will establish whether this type of pre-digested protein offers advantages to patients with impaired digestion. If the outcome should prove successful, the present undertaking will have shown how the skills of food technology, nutrition, bacteriology and medicine can be fused to design a food product for a specific need.

Acknowledgment

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Water vapour adsorption on whole corn flour, degermed corn flour, and germ flour

MAHESH KUMAR

Summary

Adsorption isotherms for whole corn, degermed corn, and germ flours were obtained at 78°F and 122°F and at relative humidities in the range of 11–97%. Various empirical equations for the isotherms were tried and the parameters were calculated for the equations. Brunauer-Emmett-Teller (BET) surface areas of the various materials were calculated to describe the sorption phenomenon. The BET surface areas based on water sorption were higher for degermed corn flour than for germ flour. The free energy changes for whole corn, degermed corn, and germ flours were determined and represented by empirical equations.

Introduction

In the drying and storage of solids, it is important to distinguish between hygroscopic and non-hygroscopic materials. If a hygroscopic material is maintained in contact with air at constant temperature and relative humidity until equilibrium is reached, the material will attain a definite moisture content which is known as the equilibrium moisture content.

A graphic representation of the relationship between the equilibrium moisture content of a material and its relative humidity is termed the equilibrium moisture curve or the isotherm. The mathematical description of the curve is known as an isotherm equation. The shape of the isotherm reveals the manner in which the water is bound. The force of the binding of the water to the surface is indicated by the shape of the curve.

The equilibrium moisture content of a material is important to food engineers because of its relationship to storage, handling, and drying. If a dehydrated food is dried to moisture content less than it normally possesses when in equilibrium with its environment, it will return to its equilibrium value on storage or handling, unless special precautions are taken. The equilibrium moisture content is especially important in drying because it represents the limiting value for given relative humidity and temperature conditions of the drying system. In the existing theory of drying, equilibrium moisture content is an integral part of the overall drying problem.

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In this study several equilibrium moisture curve (isotherm) equations which relate the equilibrium moisture content to drying parameters are adapted to data on various corn flours.

The adsorption process may be classified as physical (Van der Waals) or chemical depending on the nature of the forces involved. Many investigators agree that adsorption of water vapour by cereals and their products is entirely of the Van der Waals type (Chung & Pfost, 1967). Intermolecular forces between molecules of water vapour and the surface of the adsorbent (polar sites of adsorbent) are increased by the physical adsorption. Physically adsorbed layers particularly those which are mainly one molecular diameter in thickness behave in many respects like a liquid.

In any solid, the atoms of molecules are held together by different forces; at the surface there are unbalanced forces of attraction normal to the surface plane; and the balance of these forces is partially restored by the adsorption of water vapour molecules. Adsorption of water vapour on a solid is a spontaneous process and decreases the free energy of the system, which is a measure of work done by the system.

Review of literature

The phenomenon of the surface adsorption of gases is explained at length by Brunauer (1945). Brunauer, Emmett & Teller (1938) found adsorption takes place in multiple layers. Ngoddy (1970) developed a general theory of sorption phenomena in biological materials. Brunauer *et al.* (1938), Henderson (1952), Day & Nelson (1965), Strohman & Yoerger (1960), Chen (1969) and Chen & Clayton (1970) developed new equilibrium moisture content equations. Some of more recent research in equilibrium moisture contents and adsorption and desorption isotherms for corn kernel and its components was reported by Thompson & Shedd (1954), Hubbard, Earle & Senti (1957), Hall & Rodriguez-Arias (1958), and Shelef & Mohsenin (1966). Kunze & Hall (1965, 1967) studied the adsorption characteristics of brown rice.

Becker & Sallans (1956), Becker (1960), Bushuk & Hlynka (1960), Young & Nelson (1956), Day & Nelson (1965) have reported sorption phenomenon for wheat. Bushuk & Winkler (1957) developed equilibrium moisture curves for wheat flour and its constituents. Winkler & Geddes (1931) and Schrenk, Andrews & King (1949) have reported the heat of hydration of wheat flours and its constituents. However, there are no data available for isotherms on whole corn, degermed corn, and germ flours.

Materials and methods

The corn used in this work was a midwest, open variety supplied by the Robinson Hybrid Company, Delaware, Ohio. The pedigree of the variety was (WF9MST × H71 × 0·1 43RF XB 37 RF). The germ was separated from whole corn by hand dissecting it

after soaking overnight. The samples of whole corn, degermed corn, and germ were ground to a less than 60-mesh. Samples were dried in air oven for 72 hr at 75°C.

Samples of whole corn, degermed corn, and germ flours were conditioned for moisture with a series of relative humidities from 11 to 97%. The desired relative humidities were maintained in desiccators by means of saturated salt solutions methods given by Wexler & Hasegawa (1954), Carr & Harris (1914) and Wink & Sears (1950). The samples attained the equilibrium moisture content in 2–3 weeks, depending on the humidity shift. Mould growth was prevented in the high humidity desiccators by placing an open dish of small quantity of toluene in it. The moisture content was determined by an air oven method (103°C for 72 hr), given by researchers at USDA (1959). All moisture percentages in this work are expressed on a dry weight basis.

Adsorption isotherms

There are several empirical equations available in the literature. Among them is Henderson's two-parameter equation (1952) which has been widely used. Day & Nelson (1965) expressed the constants in Henderson's equation as power functions of temperature to modify the temperature expression in the original equation, which was based on Gibbs thermodynamic adsorption equation.

Henderson's two-parameter equation can be written as:

$$1-h=\exp\,(-B1(m)^{B2}) \tag{1}$$

$$B1=B1'T \tag{2}$$

where $B1$ and $B2$ = constants for a material,
h = relative humidity, decimal,
m = equilibrium moisture content, decimal,
T = absolute temperature,
$B1'$ = temperature coefficient for a material.

Pitchler (1970) found that equation (1) subjected to equation (2) did not permit the mathematical prediction of the shift due to temperature for wheat.

A modified form of Henderson's equation was obtained by rotating constants $B1$ and $B2$ of equation (1) to empirical power functions of temperature as:

$$B1=a_1T^{b1} \tag{3}$$

$$B2=a_2T^{b2} \tag{4}$$

where a_1 and a_2 are temperature coefficients and b_1 and b_2 are temperature exponents for the material.

The equation (1) shows that moisture adsorption becomes zero as relative humidity becomes zero. The moisture content increases without bound as relative humidity approaches 100%, which does not appear to be in agreement with the physical pheno-

menon. Equation (1) gives a single sigmoid shape of curve provided the value of $B2$ is not one. For most food products, it is expected that $B2$ will not be one because the sigmoid shape has been observed.

In this study, equation (1) was modified from two-parameter to three-parameter equation by replacing the constant (one) with a parameter $B3$. The resulting equation

$$h = B3 - \exp\,(-B1(m)^{B2}) \tag{5}$$

will be used to fit the data in this study.

Chen (1969) developed a three-parameter equation which gives an implicit relationship between equilibrium moisture content and drying parameters. The equation derived was based upon the Gibbs–Duhem equation. This three-parameter equation shows an improved agreement over other isotherm equations for a wide range of relative humidities when adapted to published data.

Chen's three-parameter equation may be written as:

$$h = \exp\,(B3 - B1\,\exp\,(-B2(m))) \tag{6}$$

where $B1$, $B2$ and $B3$ are constants for a material at a particular temperature.

Equation (6) provides a single sigmoid shape curve which has no inherent limitation at higher relative humidities. It appears that equation (6) predicts a shape that is in agreement with the observed phenomenon.

Equation (6) can be written as:

$$h = A\,\exp\,(-B1\,\exp\,(-B2(m))) \tag{7}$$

where A is another constant for a material.

In applying the analysis to a number of materials Chen & Clayton (1970) found that the values of A are very close to one. Therefore, it is assumed that equation (7) can be written as

$$h = \exp\,(-B1\,\exp\,(-B2(m))). \tag{8}$$

This equation should have similar behaviour as that of equation (6) except where $A = 1$. It should be noted that the values of $B1$ and $B2$ in equation (6) are not necessarily the same as those of equation (8). Chen & Clayton (1970) found that values of both $B1$ and $B2$ are power functions of temperature.

$$B1 = g_1 T^{k1} \tag{9}$$

$$B2 = g_2 T^{k2} \tag{10}$$

where g_1 and g_2 are temperature coefficients and k_1 and k_2 are temperature exponents for a material.

In order to find the quality of fit for these equations as applied to corn flour data, it is necessary to have quantitative information. Therefore, 'root mean square error', RMS,

was calculated which can be given

$$\text{RMS} = \left[\sum_{i=1}^{n} (h_i - E_i)^2 / n \right]^{1/2} \tag{11}$$

where,

i = index number,
n = number of data,
E = predicted relative humidity,
h = observed relative humidity.

Equation (11) shows that the smaller the values of RMS the better it fits the data.

The other researchers calculated the isotherm equation by using two data points for the two-parameter equation and three data points for the three-parameter equation. Following this method, the resultant equation may not be true for other data points. For this reason, a computer program, an algorithm for least squares estimation of nonlinear parameters, given by Marquardt (1963) was modified to determine the constants $B1$, $B2$ and $B3$ in the isotherm equation after considering every datum point (Kumar, 1972).

Calculation of BET surface area

BET theory of adsorption leads to the equation

$$v_m = v(p_0 - p)(p_0 - p + cp)/cpp_0 \tag{12}$$

where,

v_m = volume of vapour required to form a monomolecular layer
v = volume of water vapour adsorbed at pressure p,
p = pressure of vapour,
c = constant.

If the above equation is used in the form of moisture content.

$$M_m = m(p_0 - p)(p_0 - p + cp)/cpp_0 \tag{13}$$

where,

M_m = moisture content at monolayer capacity,
m = moisture content in grams.

M_m can be evaluated by plotting the graph on normal paper between $(p/p_0)/m(1-(p/p_0))$ and p/p_0. The slope of the line and intercept of the line will be $(c-1)/M_m c$ and $1/cM_m$, respectively. From the slope and intercept M_m can be calculated.

The BET surface areas of adsorbents were found by the equation for a monomolecular layer given below.

$$S = NM_m\sigma/M \tag{14}$$

where,

S = BET surface area of the adsorbent,

N = Avagadro number,
σ = cross-sectional area of a single molecule, measured in the plane of the above surface,
M = molecular weight of water.

The value of σ was determined by the methods, given by Levingston (1949). The σ was found to be 10·5 $A^{\circ 2}$ and 10·6 $A^{\circ 2}$ at 78°F and 122°F, respectively. Then the BET surface area was calculated from equation (14).

Free energy change

The free energy change in adsorption/desorption phenomena is the energy required to transfer molecules from the vapour state to the solid surface or from solid surface to vapour state. This energy can be considered as a measure of work done by the system to accomplish the adsorption desorption process. The free energy change of sorption can be expressed by

$$\Delta F = R_g T \ln(p/p_0) \tag{15}$$

where ΔF is the free energy change of sorption, R_g is the universal gas constant, T is the absolute temperature, p is the partial vapour pressure of adsorbate, and p_0 is the saturation vapour pressure at T.

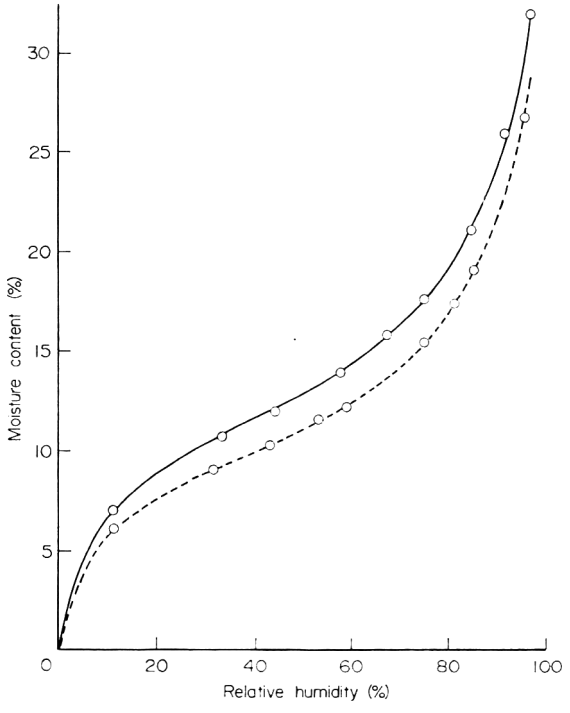

FIG. 1. Adsorption isotherm for whole corn flour at (——) 78°F and (– – –) 122°F.

Results and discussions

Adsorption isotherms

Adsorption isotherms for whole corn, degermed corn and germ flours, obtained at 78°F and 122°F, are illustrated in Figs 1–3. The force of bonding of the water to the surface is indicated by the shape of the isotherm. Water is held on polar sites of relatively high energies up to the first inflection point on the isotherm. This is the 'monomolecular region'. The region beyond the first inflection point shows the multilayer region. Within this region, the effect of surface geometry and hydrogen bonding to nonpolar sites is also operating. Above the second inflection point the water approaches the condition of a condensed phase. It is relatively free and it can be taken to reflect solution and surface capillary effects. The relationship between equilibrium moisture content and relative humidity for the above materials was found to be sigmoidal.

It was noted that the whole corn and degermed corn flours adsorbed more water than the germ flour at lower relative humidities. At the higher relative humidities, the germ flour adsorbed much more water than the whole corn and degermed corn flours. A similar type of observation was made by Shelef & Mohsenin (1966) in the case of whole corn kernel, endosperm and germ. It might be due to the fact that endosperm (starch) has more active polar sites. It was further noted that flours adsorbed more water than the corn constituents as a whole.

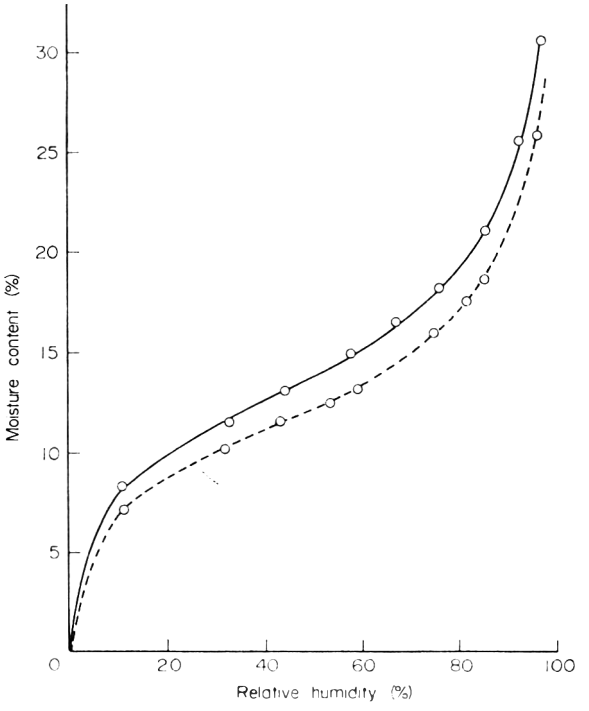

FIG. 2. Adsorption isotherm for degermed corn flour at (——) 78°F and (– – –) 122°F.

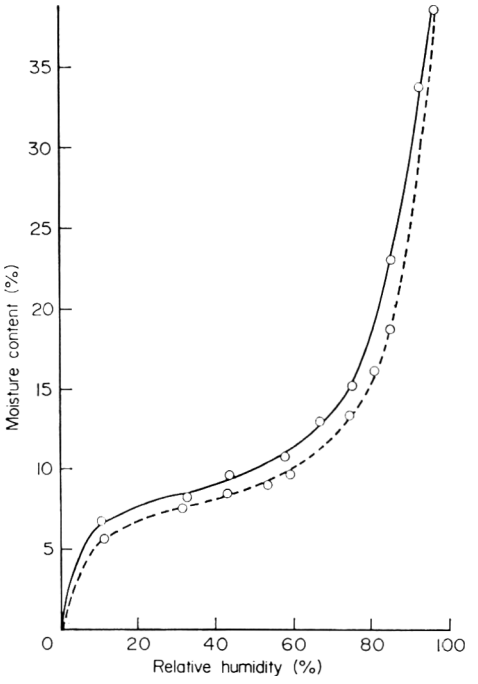

FIG. 3. Adsorption isotherm for corn germ flour at (——) 78°F and (– – –) 122°F.

Equations 1, 5, 6 and 8, each with two or three parameters, were well adapted to the data on various corn flours. The values of these constants (parameters) $B1$, $B2$ and $B3$ for various equations are given in Tables 1 and 2 at 78°F and 122°F, respectively. After considering the RMS values it was found that three-parameter equation (6) has a better fit than two-parameter equations. The equations, given by Chen (1969) and Chen & Clayton (1970) have an improved fit over Henderson's equation. It was concluded that equation (6) gave the best results. The equation was best fitted in the case of whole corn and degermed corn flours. But in the case of term flour there was a lack of agreement between the observed and calculated points. It might be concluded that oil-free materials agreed best with the predicted equations. The mathematical expressions of adsorption isotherms (equilibrium moisture curves) for whole corn, degermed corn, and germ flours permitted the analytical determination of equilibrium moisture content for various relative humidities.

BET surface areas

Monomolecular adsorption was observed up to 35% relative humidity, and BET surface areas were calculated from the isotherm below that value for whole corn, degermed corn, and germ flours were calculated from equation (14). Surface areas and

TABLE 1. Values of parameters of equations (1, 5, 6 and 8) for various corn flours at 78°F

Material (flour)	Equation	$B1$	$B2$	$B3$	RMS	Max. h_i-E_i	Min. h_i-E_i
Whole corn	1	62·97	2·22	—	0·0264	0·0526	0·0029
Degermed corn	1	118·07	2·62	—	0·0254	0·0484	0·0025
Germ	1	89·12	2·18	—	0·0574	0·1101	0·0011
Whole corn	5	81·80	2·26	0·948	0·0142	0·0236	0·0013
Degermed corn	5	152·41	2·67	0·948	0·0111	0·0223	0·0001
Germ	5	204·72	2·40	0·902	0·0419	0·0656	0·0109
Whole corn	6	8·07	19·34	−0·0252	0·0105	0·0191	0·0018
Degermed corn	6	11·93	20·62	−0·0165	0·0060	0·0111	0·0006
Germ	6	12·50	29·15	−0·0923	0·0329	0·0572	0·0055
Whole corn	8	7·31	18·18	—	0·0136	0·0241	0·0021
Degermed corn	8	11·01	19·82	—	0·0082	0·0194	0·0019
Germ	8	7·56	22·43	—	0·0449	0·1108	0·0019

TABLE 2. Values of parameters of equations (1, 5, 6 and 8) for various corn flours at 122°F

Material (flour)	Equation	$B1$	$B2$	$B3$	RMS	Max. h_i-E_i	Min. h_i-E_i
Whole corn	1	68·50	2·12	—	0·0224	0·0315	0·0030
Degermed corn	1	166·63	2·63	—	0·0241	0·0353	0·0055
Germ	1	63·96	1·93	—	0·0617	0·1172	0·0078
Whole corn	5	83·07	2·13	0·952	0·0141	0·0252	0·0005
Degermed corn	5	205·04	2·64	0·946	0·0133	0·0236	0·0030
Germ	5	326·43	2·42	0·874	0·0414	0·0842	0·0045
Whole corn	6	7·17	21·19	−0·0252	0·0106	0·0199	0·0009
Degermed corn	6	12·13	23·52	−0·0174	0·0090	0·0158	0·0002
Germ	6	14·42	35·38	−0·1153	0·0344	0·0669	0·0072
Whole corn	8	6·54	19·89	—	0·0128	0·0213	0·0009
Degermed corn	8	11·20	22·63	—	0·0099	0·0157	0·0031
Germ	8	6·33	23·41	—	0·0535	0·0823	0·0092

the constants from the BET equation are presented in Table 3. Degermed corn flour had the highest surface area, with values decreasing with increasing temperature.

The calculated BET surface areas may be affected by the following limitations when water vapour is used as an adsorbent.

(1) Water vapour may react with the materials under study.
(2) Water does not form a uniform layer when it is adsorbed by the material.

TABLE 3. BET surface areas and constants for BET isotherm

Material (flour)	Temp. (°F)	Constant c	Constant M_m (g/g)	BET surface area (m^2)
Whole corn	78	68·86	0·0726	255·5
	122	25·55	0·0696	245·3
Degermed corn	78	73·94	0·0795	279·7
	122	29·44	0·0754	267·0
Germ	78	39·11	0·0672	236·5
	122	23·06	0·0637	225·6

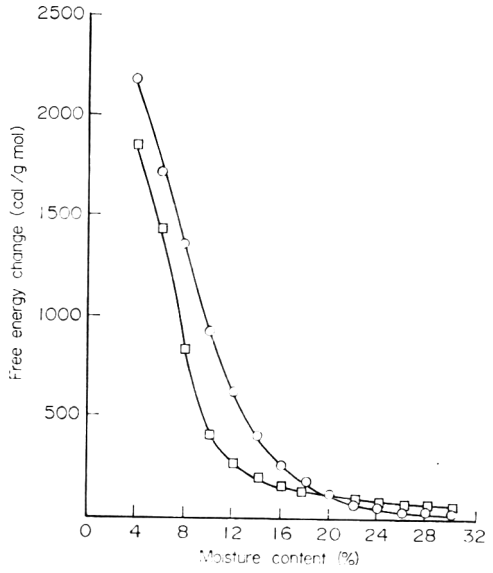

FIG. 4. Free energy change of (○) degermed corn flour and (□) germ flour at 78°F.

Free energy change

Free energy changes at 78°F for degermed corn and germ flours are illustrated in Fig. 4. Similar plots were obtained at 122°F. The free energy change of adsorption for whole corn, degermed corn, and germ flours decreased continually with increasing moisture content and the curve was hyperbolic in form. At 78°F, for each moisture content, the free energy change for degermed corn flour was higher than for germ flour up to 20% and *vice versa* for higher moisture contents.

The results could be expressed by

$$-\Delta F = A_1 \exp(-A_2 m) \tag{16}$$

where A_1 and A_2 are constants, which depended on temperature and the type of

TABLE 4. Constants A_1 and A_2 for Equation (16) of free energy change

Material (flour)	A_1		A_2	
	78°F	122°F	78°F	122°F
Whole corn	4075	4338	0·170	0·197
Degermed corn	4348	4567	0·160	0·180
Germ	4618	4982	0·214	0·246

adsorbent and m is moisture content. A_1 and A_2 were evaluated by nonlinear least squares and the results tabulated in Table 4.

Therefore, the value of the free energy change could be predicted by equation (16) using constants from Table 4.

Conclusions

(1) Adsorption isotherms (equilibrium moisture curves) were obtained for whole corn, degermed corn, and germ flours. Chen's three-parameter equation described best the relationship between equilibrium moisture content and relative humidity. The mathematical expression of isotherms of corn flours permitted the analytical determination of the equilibrium moisture content for various relative humidities.

(2) Degermed corn flour has a higher surface area than whole corn and germ flours. BET surface areas for corn flours decreased with an increase in temperature.

(3) The free energy changes are represented by a mathematical expression which will allow their calculation for corn flours at various equilibrium moisture contents at a particular temperature.

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The incidence of *Clostridium botulinum* in Danish trout farms

I. Distribution in fish and their environment

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Summary

Examination of four Danish trout farms demonstrated the presence of *Clostridium botulinum* in trout, the wet-fish feed, farm waters, pond muds and invertebrates and in the soil of the area surrounding the farm. The incidence in 530 trout examined varied from 5–100% in winter to 85–100% in late summer. *Clostridium botulinum* type E was the predominant type found.

Introduction

The contamination of live fish by the food poisoning organism, *Clostridium botulinum*, has been known for many years and has been the subject of much research. Its incidence in fish and incrimination in food poisoning vary considerably in different parts of the world and have been the subject of reviews by Dolman (1957) and Riemann (1973).

In Scandinavia the incidence of *Clostridium botulinum*, particularly type E, is high. The organism has been found in soil, dust in the streets, freshwater and marine fish and in bottom deposits of the coastal waters of the Skagerrak, the Kattegat, the Sound and the Baltic (Pedersen, 1955; Skulberg, 1961; Johannsen, 1963; Cann *et al.*, 1965; Zaleski, Fik & Daczkowska, 1973).

A recent case of fatal human botulism in Germany (Bach *et al.*, 1971) caused by smoked trout has drawn attention to the botulinogenic potential of farm raised trout. Examination of trout products on sale in Britain has demonstrated the presence of *Clostridium botulinum* in Danish, Japanese and British raised fish in that order of frequency (Jarvis & Corbett, 1972; Torry Research Station, unpublished results).

Fish farming has been practised in Denmark for centuries and, in recent years, has reached an annual production of about 12,000 tons. Most of this is exported as frozen products along with smaller quantities of iced and live fish.

Danish trout farms consist of earth ponds fed by the natural flow of rivers, streams and, in some cases, underground springs. The trout are normally fed on minced marine trash fish, which may be fortified by the addition of vitamin supplements.

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The present study has been undertaken to ascertain the incidence and distribution of *Clostridium botulinum* in Danish trout farms and to determine the ecological factors governing the presence of the organism in farm trout.

Materials and methods

Four trout farms were chosen for examination, each varying from the others in certain aspects. Farm A is a typical farm in South Jutland using surface river water and feeding minced wet fish to the trout. The farm is situated near the outlet of the river into the sea. Farm B is similar but located in North Jutland at the head of a river. Farm C is a small and very special farm in that it uses dry commercially produced pelletized feed and the water supply is from a spring originating only a few metres from the farm. Farm D is a normal trout farm in Central Jutland but was singularly different in that it experienced an outbreak of botulism in the trout stock, and samples were collected only 7 days later. Pond bottoms in farm A and D consist of earth, while in farm B and C they are mainly sand and limestone.

Whenever practical a sample size of thirty-five was chosen because the standard error of the estimated percentage is no greater than 5%; so with 95% confidence the true incidence lies within ± 10 of the observed percentage. Trout were individually taken from the ponds, stunned and placed in plastic bags of polyamide (Nylon 11, 0·04 mm) so that cross-contamination would be avoided. On return to the laboratory the fish were vacuum-packaged and incubated for 6 days at 30°C. After incubation the fish were usually almost totally liquefied. However, the contents of each bag were transferred to sterile plastic containers and any remaining fish muscle homogenized.

One part of homogenate was diluted with four parts of sterile gelatine phosphate buffer II (Nordic Committee on Food Analysis, 1971), mechanically shaken for 1 hr at 0°C, and refrigerated prior to analysis.

Pond muds were collected by means of a long-handled metal scoop and sampled in 5 g amounts. Cores of material from pond bottoms were collected from drained ponds by pressing sterile cylinders of plastic into the bottom and carefully rotating during removal. By this means the cores of material remained within the cylinders. After extrusion the cores were sampled at varying depths.

Invertebrates were collected, washed free from adherent mud by repeated gentle agitation in deionized water, and placed in sterile jars. Farm waters were examined by either the suspension overnight of sewer swabs in the water or by membrane filtration of 10 l of water followed by maceration of the filters in 100 ml of gelatine phosphate buffer and a count of *Clostridium botulinum* made using a modification of the standard Most Probable Number technique as described in the Bacteriological Examination of Water Supplies (Ministry of Health, 1956). All samples other than fish were cultured in ten times their volume of freshly made Reinforced Clostridial Medium (Oxoid) and incubated for 6 days at 30°C.

The extracted fish homogenates and broth cultures were centrifuged at 15,000 rev/min for 30 min and assayed for toxin by the procedure described by Cann *et al.* (1965).

Results

The results of the survey of four trout farms are shown in Tables 1 and 2. *Clostridium botulinum* was found to a greater or lesser degree in all materials tested with type E predominating. Other types of *Clostridium botulinum* were found on only four occasions. Once a mixture of types A and E was found and three times type B alone was found.

The incidence in trout varied from 5 to 100% during the colder months of Winter

TABLE 1. Incidence of *Clostridium botulinum* in fish from four different trout farms

	Date	No. of samples	Percentage* positive	Standard error	Date	No. of samples	Percentage* positive	Standard error
Farm A	14/12	210	60	3·4	31/5	60	100	—
Farm B	12/2	40	35	7·5	20/8	60	96	2·5
Farm C	12/2	80	5	2·4	20/8	20	85	8·0
Farm D	2/4	60	100	—	19/7	20	100	—

* All positive samples were type E with the exception of two samples in farm A; one sample containing type AE and the other B.

TABLE 2. Incidence of *Cl. botulinum* type E in the environment of four different trout farms

	Pond bottom material				Pond invertebrates	Surface water				
						Inlet			Outlet	
	Date of sampling	No. of samples	Percentage positive	Standard error	No. of positive/ No. of samples	MPN* 101	SS	Ponds SS	MPN 101	SS
Farm A	14/12	100	38	4·9	11/11	nil	0/6	6/6	nil	6/6
	26/9	25	74	8·8						
Farm B	12/2	40	0	—		NT	NT	NT	NT	NT
	20/8	10	0	—	0/4					
Farm C	12/2	80	7	2·9		NT	NT	NT	NT	NT
	20/8	35	0	—	0/1					
Farm D	2/4	40	80	6·3	5/5	2·3	NT	NT	8·5	NT

* MPN = Most probable number.
SS = Sewer swabs.
NT = Not tested.

and early Spring, but by the end of the Summer there was a uniformly high incidence—85–100%—in all four farms.

The findings with samples taken from the bottom of the ponds did not always parallel those in trout. Although *Clostridium botulinum* was found in three farms, it was not found in farm B, even when its presence in trout was as high as 96%. It appears that in farm B and C, where pond bottoms mainly consist of sand and limestone, the conditions are not favourable for the establishment of permanent contamination with *Clostridium botulinum.*

The three types of invertebrates tested—snails (*Limnea pereger*), Lumbriculus and small nematode worms from farm A and D—all carried *Clostridium botulinum* and the organism was also readily detected in pond waters of farm A, and in countable numbers in the inlet (2·3/10 l) and outlet waters (8·5/10 l) of farm D.

Table 3 shows the distribution of *Clostridium botulinum* in the pond and ground soils of farm A and of nearby agricultural land and virgin woodland. *Clostridium botulinum* type E is always present with the highest incidence in the mud of the trout ponds.

TABLE 3. Comparison of incidence of *Cl. botulinum* in pond muds and soil during September, 1973

Sample	No. of samples	Percentage positive	Standard error	Types
Fish pond muds	25	74	8·8	E
Fish farm soils	20	55	11·1	E
Agricultural farm soils	10	30	14·5	2 E, 1 B
Virgin woodland soils	10	50	15·8	4 E, 1 B

Examination of cores of material taken from one pond in farm A showed that *Clostridium botulinum* type E could be found to a depth of 14 cm.

Detailed examination of trout taken from farm D yielded Most Probable Number counts of 0·34, 0·5, 1·5, 1·9, 2·3 and 5·3/g of *Clostridium botulinum* type E in whole fish and the organism was demonstrated in the gills of two and in all the intestinal tracts of four aseptically dissected fish. The organism was not found in the liver, spleen, kidney, head, tail or in the remaining cleaned carcasses.

Discussion

From the data presented it is clear that all Danish trout farms can be expected to be contaminated with *Clostridium botulinum* type E to some extent, dependent on their situation along the rivers, the presence of the organism in the soil, the type of feed used and the time of the year. The contributary factors are shown in the postulated cycle presented in Fig. 1, and from time to time one source or another may be responsible for the high incidence in the fish.

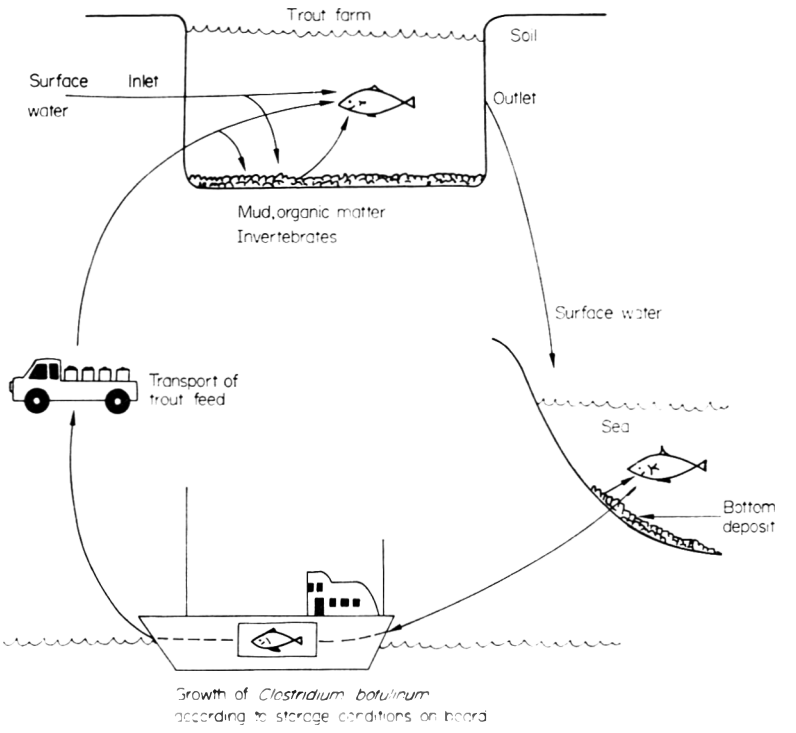

FIG. 1. Probable sources of contamination of a trout farm by *Clostridium botulinum*.

The minced marine trash fish used as feed, presents, in our opinion, the most significant source of contamination. Depending on where trash fish is caught (Pedersen, 1955; Johannsen, 1963; Cann *et al.*, 1965) and how it is handled on board the trout feed may contain large numbers of *Clostridium botulinum*. Until recently, no or insufficient chilling has been applied to this type of catch. The outbreak of botulism, which will be fully reported on elsewhere, in the stock of trout in farm D is classical proof of this source of contamination. Samples of remnants of feed were shown to contain not only the organism but toxin at a level of 50 Mouse Lethal Doses per gram. Detectable toxin was also found in the stomachs of dying and dead fish.

Also surface water has been shown to present an obvious source of contamination. The presence of *Clostridium botulinum* in the pond and outlet water of farm A and in countable numbers in both inlet and outlet water of farm D indicates a heavy contamination of the water passing through the farms.

Whatever the initial source of contamination of a given farm the specialized conditions existing are ideal for establishment and growth of the organism in the fish ponds. Commercial profitability requires a high population density in fish ponds and concentrated feeding to establish a good growth rate. Excess feed and fish faeces lying in

the bottom of ponds provide ample nutrients for growth of *Clostridium botulinum*. Its presence in mud to a depth of 14 cm and in invertebrates suggests a continual propagation of the organism regardless of routine cleansing of fish ponds by drainage and removal of all loose mud and other material. Growth of *Clostridium botulinum* is possible whenever conditions of temperature and anaerobiosis are suitable. Such a time has been during the long dry and warm summer which Denmark experienced in 1973 with resultant high water temperatures and water supplies so low that recirculation became necessary.

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The incidence of *Clostridium botulinum* in Danish trout farms

II. Measures to reduce contamination of the fish

H. H. HUSS, ANETTE PEDERSEN AND D. C. CANN*

Summary

A number of experiments have been carried out to develop methods for the reduction of the contamination of farmed trout by the food poisoning organism *Clostridium botulinum*.

Starvation studies of fish gave variable results. When carried out under hygienic conditions in artificial containers and a clean water supply the contamination was reduced. Gutting of fish, either by hand or machine, always reduced the contamination to a low level.

Quick liming effectively reduced the contamination of pond bottoms and may be useful as a control measure against *Clostridium botulinum*.

Introduction

From the data presented in Part I of this paper (Huss, Pedersen & Cann, 1974) it is clear that the incidence of *Clostridium botulinum* type E in certain Danish trout is high. Wet fish feed, surface waters and soil were all found to be possible sources of contamination. It was also shown, in agreement with other workers (Johannsen, 1963; Craig, Hayes & Pilcher, 1968; Bott *et al.*, 1966; Bach *et al.*, 1971), that the contamination is closely related to the intestinal tract and to a lesser extent the gills. In the trout ponds the organism can become established in mud and bottom living invertebrates.

Although such a high contamination of fish with a lethal food poisoning organism is highly unsatisfactory, it is nevertheless true that Danish trout products are remarkably free from incrimination in food poisoning outbreaks of this type. The only recorded case was in 1963 (V. Møller, Danish Government Serum Institute, Personal Communication). The bulk of the industry's production is sold as the frozen and gutted product and is cooked before eating. It should also be remembered in the case of raw trout that the demonstrations of toxicity reported here were made after the fish had been incubated far beyond the stage at which they could ever be expected to be viewed as a foodstuff for human beings.

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Nevertheless, products such as hot smoked and 'gravad' trout, a Danish delicacy comprising sugaring, salting and spicing of the fish, are potentially dangerous, as spoilage is delayed and the ultimate safeguard of destruction of toxin by cooking is absent.

It is therefore necessary to take measures for the elimination or reduction of the level of contamination in the fish, preferably before dispatch from individual farms. The German workers (Wenzel, Bach & Müller-Prasuhn, 1971) recommended starvation for 5 days before processing as a means of reducing the contamination of live fish. In the present study this recommendation has been evaluated and developed along with further measures to reduce the incidence of *Clostridium botulinum* in the farms and in the final processed products.

Materials and methods

Starvation of fish

A number of experiments were set up to study the effect of starvation of trout on the incidence of *Clostridium botulinum* in fish. The fish were held for 3, 6–7 and 14 days in various types of container and differing supplies of water as shown in Table 1. Initially the studies were made indoors in small containers using filtered hatchery water and, at the processing plant and the Copenhagen laboratory, using mains water. Later studies were made outdoors in the natural ponds of farm A.

Treatment of ponds

An empty pond in farm A, known to be contaminated with *Clostridium botulinum*, was used to assess the effect of two treatments of the pond bottoms. The pond had been empty for 1 month but was not completely dry.

(i) *Heat treatment.* Direct heat from a horticultural flame thrower was applied for 2 min to an area of a pond bottom. Twenty surface samples from the pond bottom were collected before and after heat treatment.

(ii) *Lime treatment.* The bottom was treated with 1·6 kg quick lime (CaO) per m^2 and the lime raked into the surface layers of pond bottom. Bottom material was collected before, 18 hr and 7 days after the lime treatment. Before culture the samples were neutralized to pH 7·0 with sterile normal hydrochloric acid.

Effect of processing

Fish were processed in two ways. In the first, gutting and cleaning were carried out on the farm using fish direct from the ponds without any period of starvation. One lot of hand-processed fish was taken to a commercial smoking plant and after brining for 90 min in 21% brine given the normal hot smoke treatment (3 hr in the kiln with the temperature raised to 80°C for the last half hour).

In the second, fish were commercially processed involving mechanical removal of all

TABLE 1. Measures to reduce the contamination of live fish by *Cl. botulinum*

Holding conditions for fish	Water supply	Days of starvation	No. of samples	Percentage positive	Standard error
In cage suspended in stainless steel tank	Mains water	0	40	100	—
		3	40	83	5·9
		6	40	68	7·4
In cage suspended in concrete tank	Mains water recirculated	0	40	100	—
		3	40	100	—
		6	40	100	—
In plastic basin	Direct from source, filtered	0	50	64	6·8
		7	50	26	6·2
		14	47	30	6·7
In cage suspended in concrete basin	Direct from river, filtered	0	50	64	6·8
		7	58	21	5·3
		14	60	20	5·2
In earth pond	Direct from river	0	60	100	—
		7	60	53	6·4
In cage suspended in earth pond	Direct from river	0	60	100	—
		7	60	25	5·6
In cage suspended in earth pond	Slow-running recirculated farm water	0	40	100	—
		3	40	100	—
In cage suspended in earth pond	Slow-running recirculated farm water	0	40	68	7·4
		3	36	86	5·8
		6	36	83	6·3
		14	40	100	—
In cage suspended in earth pond. Fed laxative (10% $MgSO_4$) before starvation	Direct from river	0	40	100	—
		3	40	100	—
		7	20	100	—
In cage suspended in earth pond	Direct from river	0	40	100	—
		6	40	100	—

internal organs, including the gills and kidney blood (trout gutting machine from BAADER) with subsequent cleaning in a large tumbler washer which has a continuous throughput of water.

In all cases fish and samples of pond bottom material were collected and tested for the presence of *Clostridium botulinum* as described in Part I of this paper (Huss *et al.*, 1974).

Results

Measures to reduce the contamination of live fish

The effect on the incidence of *Clostridium botulinum* in trout starved under different

holding conditions is shown in Table 1. The results varied considerably. Using artificial containers and a fast, clean, water supply there was a reduction in the contamination of the fish. However, when studies were carried out under natural conditions in earth ponds and in two cases using recirculated water this result could not be readily achieved. Indeed, in four trials out of six the contamination of the fish remained at a high level or even increased with the time of holding in the ponds. Neither starvation beyond 7 days nor the use of a laxative had any beneficial effect.

Measures to reduce the contamination of the environment

(*i*) *Direct heat.* Direct application of heat to an area of a pond bottom raised the temperature to 70°C at a depth of 1 cm.

Examination of the area before and after treatment showed no reduction in the incidence of *Clostridium botulinum*. Of twenty samples tested in each case, 70% were positive before and 75% 1 hr after treatment.

(*ii*) *Quick liming.* One day after liming of the bottom of the pond there was no reduction in the incidence of *Clostridium botulinum*. 80% of twenty samples tested were positive, compared with 72% of forty samples positive before treatment. However, after 7 days the organism could not be detected in twenty-two samples that were tested. The pH of the mud before and after treatment was 6·8 and > 12, respectively.

Reduction of the contamination by processing

Removal of gills and guts whether by hand or by machine was invariably followed by a substantial reduction of the contamination of the fish by *Clostridium botulinum* (Table 2). Even in batches of heavily contaminated fish *Clostridium botulinum* is only found in a few percent of the fish after processing. In the small number of fish examined there was no further significant reduction demonstrated as a result of smoke curing. Dipping the gutted and washed fish in chlorine solutions increased the number of positives found.

Discussion

Measures to reduce the contamination of farmed trout by *Clostridium botulinum*, although very necessary, should be practicable and economically acceptable to the industry.

Ideally the sources of contamination should be removed. However, the commercial viability of the trout farm industry in Denmark is based on simple conditions of rearing fish together with a cheap supply of food in the form of marine trash fish. Any changes, for instance from wet feed to manufactured pellets, would increase production costs considerably.

As long as wet marine trash fish is used, contamination by *Clostridium botulinum* cannot be completely eliminated, but gross effects can be avoided by keeping the feed chilled at all stages from catch to use on the farms.

TABLE 2. The reduction of *Clostridium botulinum* contamination of trout by processing

Type of processing	No. of samples	Percentage positive	Standard error
Controls, untreated	40	68	7·4
Hand-gutted and cleaned on farm	40	5	3·4
Hand-gutted, beheaded and cleaned on farm	40	5	3·4
Hand-gutted, cleaned on farm and commercially smoked*	20	5	4·9
Controls, untreated, 7 days starved	58	21	5·3
Commercially machine-gutted and cleaned	60	0	—
Commercially machine-gutted and cleaned by hand	60	3	2·2
Controls, untreated, 6 days starved	36	83	6·3
Commercially machine-gutted and cleaned	40	2	2·2
Controls, untreated, 6 days starved	40	100	—
Commercially machine-gutted and cleaned	60	20	5·2
Commercially machine-gutted and cleaned. Samples taken at random during normal production	113	12	3·1
Controls, untreated, 3 days starved	40	100	—
Commercially machine-gutted and cleaned	40	10	4·7
Commercially machine-gutted and cleaned. Dipped in water containing 50 ppm chlorine	40	25	6·8
Commercially machine-gutted and cleaned. Dipped in water containing 200 ppm chlorine	40	33	7·4

* 2·40% NaCl in water phase.

Elimination of *Clostridium botulinum* from the environment of a trout farm also presents a very difficult problem. As a normal routine the ponds are periodically emptied and all loose matter is removed. Once or twice a year the pond bottoms are treated with approximately 0·33 kg slaked lime ($Ca(OH)_2$) per m^2. Our experimental work showed that quick lime (CaO) used in an amount of 1·6 kg m^2 and left in contact with the surface layers for 1 week was followed by a marked reduction in the number of organisms present. It is possible then that more frequent treatments would prevent any build-up in the number of *Clostridium botulinum* present in the pond bottoms and pond invertebrates.

The possibility of the use of horticultural flame throwers has been mooted by biologists for the combined control of weed and parasite spores in pond muds. Evaluation of this treatment showed that the heating effect is minimal and unlikely to be of value.

The relationship of *Clostridium botulinum* when present in fish to the intestinal tract has been further evaluated. Under experimental conditions it has been found that in some cases fish fed an estimated 1000 type E spores cleared themselves of *Clostridium botulinum* spores in 2 days and by the sixth day almost all the fish were free of the

organism (Sugiyama, Bott & Foster, 1970). The same pattern occurred when spores were inoculated on the surface of the fish. Similar results are reported in experimental feeding of trout with *Salmonella typhimurium* (Hagen, 1966).

The knowledge that the gastro-intestinal tract of fish is probably the largest reservoir of contamination led the German workers (Wenzel *et al.*, 1971), to recommend the 'self cleaning' of fish by starvation for 5 days before sale.

This practice has been in use in Denmark for a number of years but for another reason. Trout are normally dispatched live from the farm to the processing factories so that their quality is assured and processing can be achieved before the onset of rigor mortis makes machine gutting difficult. As trout do not travel well when recently fed, the fish are usually starved for a period of time before dispatch. This period varies from a minimum of 2 days during summer to 1–2 weeks during the colder winter months. Any extension of this starvation period would keep ponds out of normal use and cause unnecessary loss of weight of the starving fish.

Examination of the data presented shows that starvation can reduce contamination of fish by *Clostridium botulinum* but appears to be very dependent on the type of container and water supply used during the self cleansing process. In the open farm it is of limited value, especially when there is heavy contamination of the environment as shown in late summer. Prolonged starvation is of no value and it is further debatable if holding fish for more than 3 days is justified as by this time most of the contents of the gastro-intestinal tract have been excreted. In addition, recontamination may occur as the hungrier fish get the more likely they are to ingest snails and any shape and size of suspended particulate matter. Cannibalism has also been noted.

Gutting of the fish always resulted in a marked reduction in the contamination of the fish. Thus the gut and its contents must be regarded as a most important reservoir of *Clostridium botulinum*. This reservoir should preferably be removed immediately after killing, and utmost care must be taken to avoid cross-contamination during the gutting operation. Application of preservation methods such as marinating or smoke curing to ungutted fish would seriously increase the risk of botulinal toxin being formed.

Commercial smoke curing of gutted fish as currently practised does not eliminate the organism. Indeed, it has been demonstrated by other workers (Pace *et al.*, 1967) that the percentages of fish carrying type E spores can increase during the stages of the smoke curing process. From a public health point of view any smoked trout should be assumed to carry *Clostridium botulinum* and the prevention of botulism from smoked fish depends on proper refrigeration until consumption. Best assurance would be obtained if this product was sold only in the frozen state as recommended by Kautter (1964).

Chlorine has been used in the food industry as a sanitizing agent for many years and it has been reported (Ito *et al.*, 1967) that exposure of type A, B and E spores to 4·5 ppm of free chlorine in a phosphate buffer, pH 6·5, caused a 99·99% reduction in 4–6 min for type E and in 3–8 min for type A and B. It is rather surprising therefore that our use of chlorine dips led to an increase in the percentage of fish found to be carrying

Clostridium botulinum type E. This phenomenon needs further examination but it may be due to interference with the balance of the normal microbial flora existing on the fish with the creation of better growth conditions for the spores of *Clostridium botulinum.*

Conclusion and recommendations

Elimination of *Clostridium botulinum* from trout raised under current farming practices is probably impossible. The contamination can, however, be reduced to a low level by a combined effort at all stages of production and processing. New regulations requiring chilling of trout feed are already in force in Denmark and are expected to prevent any gross contamination of the farms. The use of lime, which is a common practice for the destruction of fish parasites, appears to have distinct possibilities as regards the prophylactic treatment of fish ponds against contamination with *Clostridium botulinum.* The amounts of lime used and the frequency of treatment require further study. A terminal period of starvation of trout in specially constructed delivery ponds is a valuable measure to reduce the contamination of the fish. Much importance must be attached to the hygienic condition of the delivery pond, and the use of recirculated farm water should not be permitted. Amendments of the present Danish regulations to cover these points are in preparation. It is finally recommended that the total evisceration of the fish under hygienic conditions be carried out immediately after killing and prior to sale.

Acknowledgments

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Effect of electrolytic tinplate and pack variables on the shelf life of canned pure citrus fruit juices

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Summary

Corrosion performance in citrus fruit juices of five different batches of electrolytic tinplates having values of special properties test outside of type K tinplate specifications was studied. The effect of A.T.C. values was followed in order to find out the precise limits for an adequate shelf life in citrus fruit juice packs. The effect of different levels of nitrate and sulphur dioxide in relating with A.T.C. values was investigated. It was shown that (1) certain A.T.C. values are necessary in order to obtain a good shelf life in pure juices and those values are different for orange and grapefruit juices; (2) while the A.T.C. value can affect the detinning rate in pure citrus juices, the addition of nitrate and sulphur dioxide at concentrations used, overrides this effect.

Introduction

There have been several reports during recent years concerning research on corrosion aspects of tinplate cans containing citrus fruit juices. Many factors may influence this process; the quality of the citrus fruit juice product, its processing as well as the quality of the tinplate itself.

Much of the published literature is concerned with corrosion as influenced by quality of the product (Strodtz & Henry, 1954; Farrow, Charbonneau & Lao, 1969; Farrow, Lao & Kim, 1970; Farrow *et al.*, 1971; Horio, Iwamoto & Oda, 1964/65, 1966; Clough, Shostrom & Clark, 1924, 1930; Morris & Bryan, 1931; Hartwell, 1951; Hirst & Adam, 1945) or its processing (Bakal & Manheim, 1966).

The tinplate most used in the last years for canning citrus fruit juices, is electrolytic tinplate. This tinplate has a thin alloy layer and research (Kamm & Willey, 1961a,b; Kamm *et al.*, 1961; Carter & Butler, 1961; Gabe & Mort, 1965; Landau & Manheim, 1970; Endle, 1972) has shown that variability in corrosion resistance of the electrolytic tinplate is related to the continuity of this layer. Simple electrochemical laboratory tests were developed to assess the continuity character of the alloy layer (the alloy tin

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couple–A.T.C.-test) as well as other properties of the tin coating or of the steel base, which may affect the performance of electrolytic tinplate: the tin crystal size (McKirahan, Connell & Hotchner, 1959), the iron solution–ISV-test (Willey, 1956) and the pickle lag–PLV-test (Willey, 1956). By selecting tinplate which reaches certain values in all these tests, improved corrosion performance can be obtained. American Can Company selected a tinplate meeting specific values for all four tests and designated it type K tinplate. This type K tinplate is most used in canning citrus fruit juices. Most of the published results relate special property values to shelf-life terminated by formation of hydrogen swells and information about relation of the values to rules of dissolution of tin is sparse.

While it is known that all the factors—tinplate, product and processing—may affect shelf life of canned citrus products, the manner in which they act, particularly with respect to each other is as yet unknown. The object of this paper is to report on results of corrosion performance of standard electrolytic tinplate with special properties as tested by previous methods—outside of type K tinplate specifications—in citrus fruit juices. The ultimate aim is to increase shelf-life, whether this is terminated, by excessive dissolution of tin or by formation of hydrogen swells.

These preliminary trials are part of a continuing study being made by the Israel Can Company. The variables studied here were different A.T.C. values in order to find out which are the limits that affect the corrosion rate in canned orange and grapefruit juices. Experiments have been done too, in order to find out the extent to which A.T.C. value is affected by the presence of depolarizers nitrates and sulphur dioxide.

Materials and methods

Five different batches of electrolytic tinplates were used for these trials. These covered normal production electrolytic tinplate at three different A.T.C. values. In addition, some experimental tinplate using a novel pre-pickling procedure (Beard, Gabe & Warner, 1971) plus control material for this experimental tinplate were tried.*

Table 1 summarizes the properties of these tinplate materials in terms of tin coating weights (free and alloyed), A.T.C. and other special property values. The A.T.C. values are means of ten results. Measurements were made on tinplate as produced and on the interior of unfilled cans. The other special property values refer to the unformed tinplate only.

A2 size cans were made from those tinplate types and were packed by the Israel Can Company with orange and grapefruit juices. Two levels of nitrate—30 and 60 ppm NO_3—and two levels of sulphur dioxide (added as sodium bisulphite)—10 and 20 ppm SO_2 (actual levels 5–10 ppm and 10–20 ppm respectively)—were studied.

* None of the tinplate types tested were within the type K tinplate specifications.

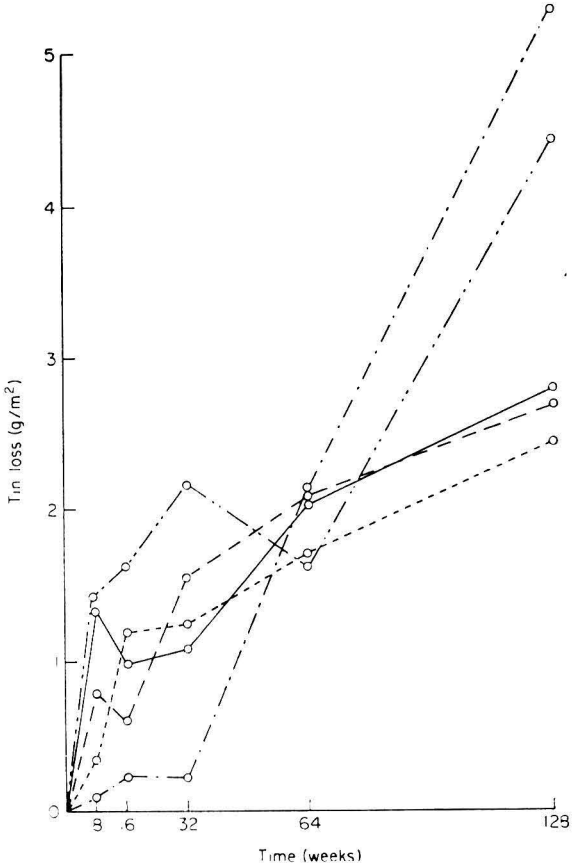

FIG. 1. Tin loss (g/m²) of five different electrolytic tinplate in A2 cans containing orange juice, as a function of storage time at 25°C. Tinplate: ——— type 1; - - - - type 2; —·—·— type 3; — — — type 4; —··—··— type 5.

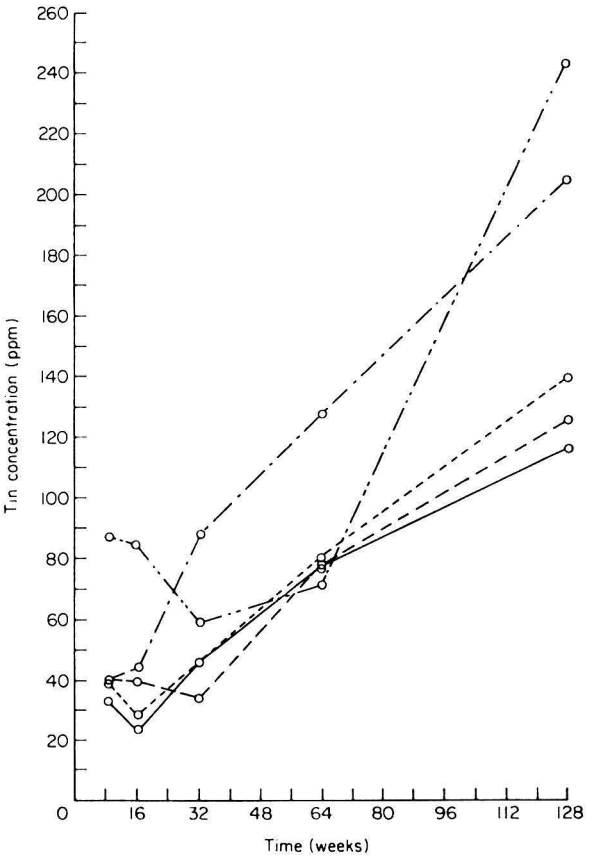

FIG. 2 Dissolved tin (ppm) in orange juice packed in A2 cans of five different electrolytic tinplate, as a function of storage time at 25°C. Tinplate: ——— type 1; - - - - type 2; —·—·— type 3; — — — type 4; —··—··— type 5.

TABLE 1. Tinplate specification values

Type no.	Electrolytic tinplate	Coating weight g/m²		Special property values			
		Free tin*	Alloy*	Alloy tin couple A.T.C. (μmA/cm²)	Iron soln value I.S.V. (μg Fe^{2+} /50ml)	Tin crystal size T.C.S.†	Pickle lag value P.L.V. (sec)
1	Standard production	10·36	1·04	0·13	34	8	4
2	Standard production	10·12	0·77	0·26	31	8	133
3	Standard production	10·06	0·69	0·37	41	10	36
4	Experimental	7·94	1·22	0·13	12	8	6
5	Control	8·06	1·04	0·22	26	9	6

* One side only.

† Tin crystal size values, ASTM standard scale.

Can ends on all experiments were made from one batch of plain hot dipped tinplate. Filled cans were stored at a controlled temperature of 25°C.

At suitable intervals samples of each variable were taken from storage and cans content analysed for dissolved tin.

The internal appearance of the cans was noted and measurements of the residual tin coating weight remaining on the inside of the cans were made using the coulometric method.

At each storage period one can per variable was analysed by duplicate analysis.

A.T.C. measurements were made on the inside of the cans after 128 weeks storage.

Results

Tinplate quality and detinning in pure citrus fruit juice

Results on corrosion performance of five tinplate quality cans packed with orange juice and grapefruit juice are summarized in Figs 1 and 2 and Figs 3 and 4 respectively.

They are expressed as tin loss (g/m²) of the can internal surface (Figs 1 and 3) or dissolved tin (ppm) in the fruit juice (Figs 2 and 4) as a function of storage time at 25°C.

For orange juice, the tin losses after 64 weeks were similar for all five tinplate quality experimented, except for tinplate type 3; this was confirmed by dissolved tin analysis. After this period, cans of tinplate type 1, 2 and 4 were essentially similar but better than types 3 and 5. These results fit in reasonably well with the initial A.T.C. values, except that cans of tinplate type 5, which behaved rather less well than those of tinplate type 2, despite possessing similar special property values. Cans of tinplate type 5 should, in fact, have behaved significantly better than cans of tinplate type 3, if performance depended

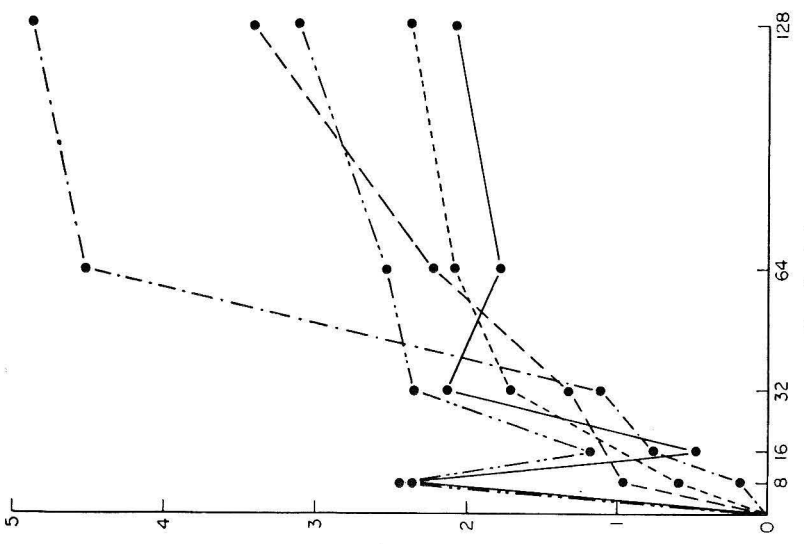

FIG. 3. Tin loss (g/m²) of five different electrolytic tinplate in A2 cans containing grapefruit juice, as a function of storage time at 25°C. Tinplate: ——— type 1; - - - - type 2; —·—·— type 3; — — — type 4; —··—··— type 5.

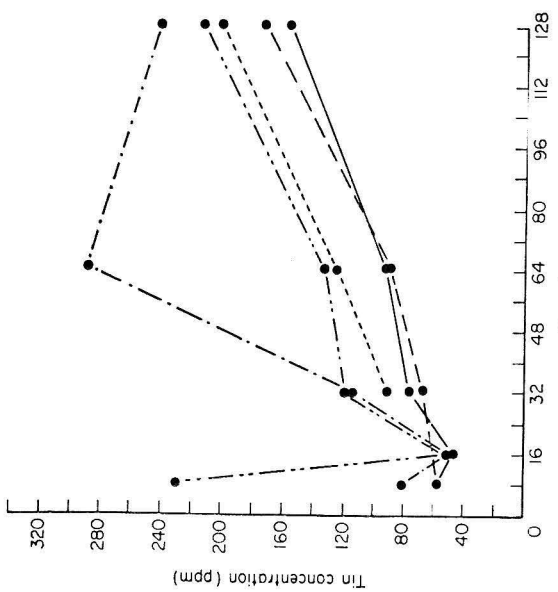

FIG. 4. Dissolved tin (ppm) in grapefruit juice packed in A2 cans of five different electrolytic tinplate, as a function of storage time at 25°C. Tinplate: ——— type 1; - - - - type 2; —·—·— type 3; — — — type 4; —··—··— type 5.

on the A.T.C. values. Cans of tinplate type 5 did in fact exhibit hydrogen swells after 110 weeks storage. The results for cans of tinplate type 5 may be spurious and were not confirmed by cans of the same tinplate type quality packed with grapefruit juice.

Dissolved tin values after 2 years (104 weeks) were all well below 250 ppm for these orange juice packs.

For grapefruit juice, the results again showed a reasonable relationship between A.T.C. values and tin dissolution or coating weight loss. The dissolution values were generally higher than in orange juice, but after 2 years storage only the worst system, tinplates type 3, had reached the 250 ppm level.

Tin coating weight loss results showed a less clear relationship. Whilst the tinplate with the lowest (type 1) and highest (type 3) A.T.C. values gave the least and greatest detinning rates respectively, the results for the intermediate A.T.C. levels do not present a clear picture.

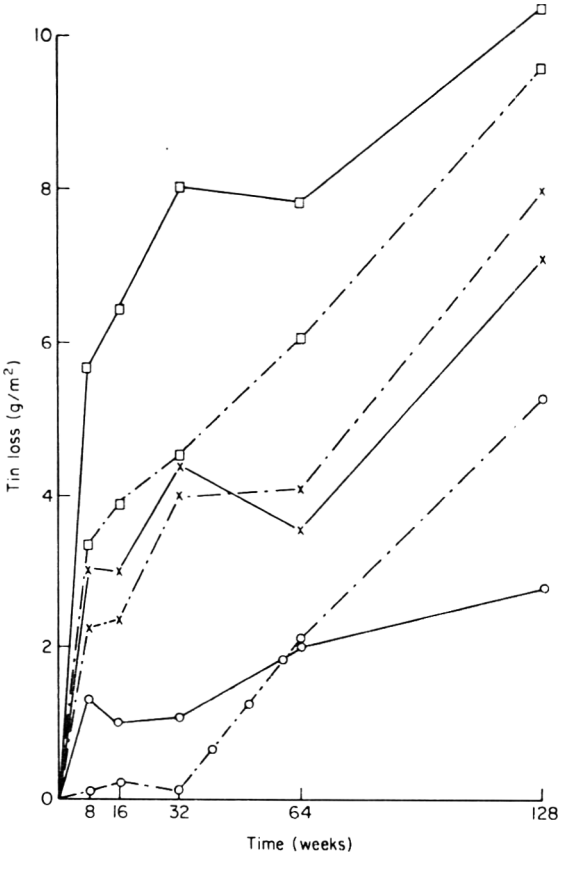

FIG. 5. Effect of nitrate concentration on the tin loss (g/m²) of two different electrolytic tinplate in A2 cans containing orange juice, as a function of storage time at 25°C. Tinplate: ——— type 1; —·—·— type 3. NO_3 concentrations: ○, 0 ppm; ×, 30 ppm; □, 60 ppm.

TABLE 2. A.T.C. values on interior of cans packed with pure citrus fruit juices after 128 weeks storage

Type no. of tinplate	After 128 weeks A.T.C. (μmA/cm^2)	Initial average value A.T.C. (μmA/cm^2)
1	0·10	0·13
2	0·21	0·26
3	0·33	0·37
4	0·09	0·13
5	0·25	0·22

Cans of tinplate type 3, packed with grapefruit juice, exhibited hydrogen swells after 64 weeks indicating a shelf-life of about 1 year.

Effect of nitrate on detinning

Data on the detinning rate of two tinplate types (type 1 and type 3) quality cans packed with orange juice containing 0, 30 and 60 ppm nitrate levels are given in Figs 5 and 6. They are expressed as tin loss (g/m^2) of the can internal surface (Fig. 5) or dissolved tin (ppm) in the orange juice (Fig. 6) as a function of storage time at 25°C.

The data in Figs 5 and 6 showed very clearly that the addition of 30 ppm nitrate

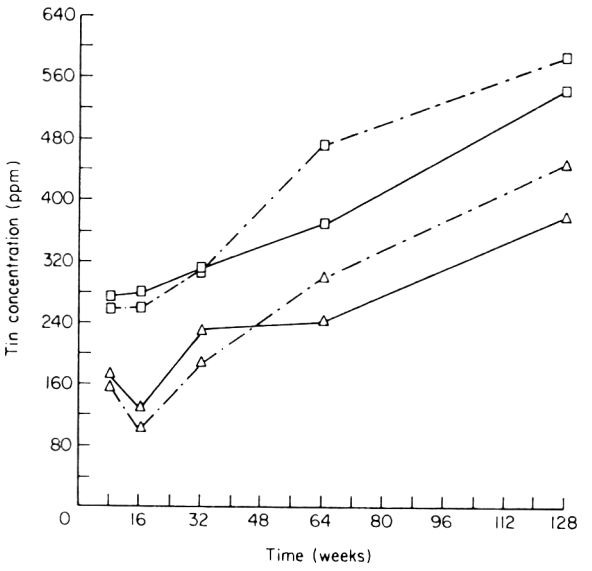

FIG. 6. Effect of nitrate concentration on tin dissolution (ppm) in A2 cans of two different electrolytic tinplate containing orange juice, as a function of storage time at 25°C. Tinplate: ——— type 1; —·—·— type 3. NO_3 concentrations: △, 30 ppm; □, 60 ppm.

increases the detinning rate very markedly and is apparently immediately effective. With additions of 60 ppm, dissolved tin levels were greater than 250 ppm after only 8 weeks storage.

Effect of sulphur dioxide on detinning

Figures 7 and 8 show the effect of SO_2 at two concentration levels (5–10 ppm and 0 ppm) on tin loss (g/m^2) and tin dissolution (ppm) in cans of tinplate type 1 packed with citrus juices as a function of storage time at 25°C.

It can be seen from those corrosion curves in Figs 7 and 8 as well as results from Table 3, that small concentrations of sulphur dioxide have marked effect on corrosions: 5–10 ppm of SO_2 was sufficient to cause hydrogen swells after only 21 weeks of storage. The effect seemed most severe with grapefruit juice packs.

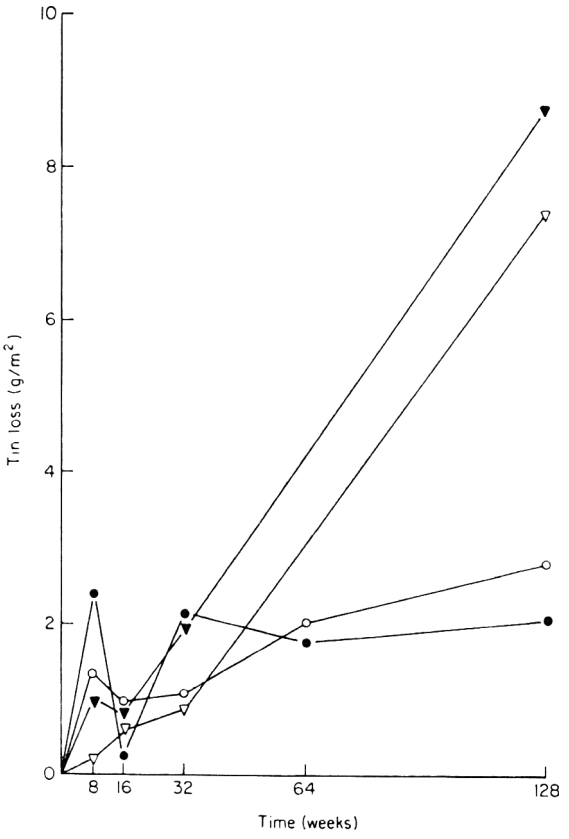

FIG. 7. Effect of sulphur dioxide concentration on the tin loss (g/m^2) of electrolytic tinplate type 1 A2 cans, containing citrus juices, as a function of storage time at 25°C. Sulphur dioxide concentrations: ○, ●, 0 ppm; ▽, ▼, 10 ppm. Orange juice, ○, ▽; grapefruit juice: ●, ▼.

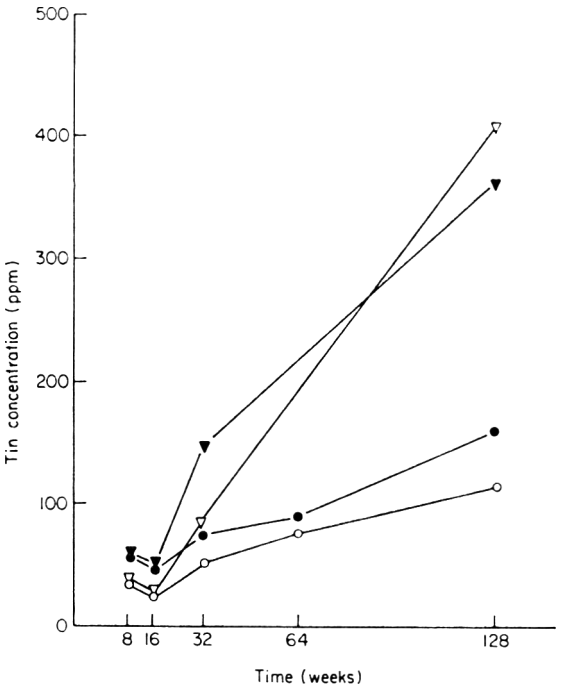

FIG. 8. Effect of sulphur dioxide concentration on tin dissolution (ppm) in A2 cans of electrolytic tinplate type 1, containing citrus juice, as a function of storage time at 25°C. Sulphur dioxide concentrations: ○, ●, 0 ppm; ▽, ▼, 10 ppm. Orange juice ○, ▽; grapefruit juice ●, ▼.

TABLE 3. Time to first observed can failure* for cans with added SO_2 levels

Type no. of tinplate	Citrus juice	SO_2 level (ppm)	Storage period (weeks)
1	Grapefruit	10	16
1	Grapefruit	20	21
3	Grapefruit	10	21
3	Grapefruit	20	21
1	Orange	10	40
1	Orange	20	40
3	Orange	10	21
3	Orange	20	40

* Failure as hydrogen swell.

Early failure due to hydrogen swells is not reflected by corrosion of the tin coating which only shows a sharp increase in detinning after some 32 weeks storage.

Can internal appearance and hydrogen swells

Throughout the shelf-life period and at each opening, examinations were made of the internal appearance of the cans. Those cans containing pure orange and grapefruit juice generally showed a gradual increase in tin etch throughout the whole storage period. The major exceptions were cans of tinplate type 3 packed with grapefruit juice which at 64 weeks were severely etched, in some areas completely detinned and exhibited hydrogen swelling, as well as cans of tinplate type 5 packed with oranges juice which exhibited a hydrogen swell after 110 weeks storage.

The cans with added nitrates showed severe etching after only 8 weeks storage with the alloy layer exposed by 32 weeks. Cans with 60 ppm added nitrate were hydrogen swells after 110 weeks storage and cans with 30 ppm added nitrate had virtually completely detinned between 64 and 128 weeks storage.

Cans containing added sulphur dioxide exhibited a rather different picture. After only 8 weeks, all cans showed some tin sulphide staining and by 16 weeks severe local tin etching. Peculiarly, the cans with the highest SO_2 contents were least detinned. By 32 weeks all of the grapefruit juice cans and orange juice cans exhibited hydrogen swells with severe detinning and staining. After 64 weeks storage all cans with added SO_2 exhibited hydrogen swells except cans of tinplate type 1 packed with orange and grapefruit juice containing 10 ppm SO_2 which were retained in storage. All cans with hydrogen swells showed severe patchy detinning and this pattern was still present in these cans after 128 weeks storage.

Discussion

The limited number of cans used in this preliminary exercise would normally allow only general comments to be made and conclusions drawn. However, the effects observed in some instances were so marked, and trends so clear that more definite comment is in fact possible.

The results provide strong evidence that the Alloy Tin Couple value of tinplate plays an important part in determining the rate of dissolution of tin by orange and grapefruit packs. It is clear that for packs of pure citrus juices cans of tinplate type 1 (A.T.C. value 0·13 μmA/cm^2) perform much better than cans of tinplate type 3 (A.T.C. value 0·37 μmA/cm^2), while the performance of tinplate with intermediate A.T.C. values is rather less clear.

Generally, it is observed that for both orange and grapefruit packs the A.T.C. value only becomes influential after 32 weeks storage. The indications are that tinplate with A.T.C. values of 0·26 μmA/cm^2 will give satisfactory shelf-life, i.e. 2 years storage with under 250 ppm dissolved tin for both citrus juices, while tinplate with A.T.C. values

below 0·20 μmA/cm^2 will give satisfactory behaviour for periods exceeding 2 years. Tinplate with A.T.C. values of 0·37 μmA/cm^2 gives a shelf-life of only about 1 year for grapefruit juice, but up to about 2 years for orange juice.

The effect of the addition of nitrates and sulphur dioxide tc the citrus fruit juices resulted in the well known accelerated corrosion of the containers. The nitrate caused an even detinning of the surface while the sulphur dioxide caused the characteristic patchy detinning and staining. Detinning was more severe in packs containing nitrate while early hydrogen swells appeared as more characteristic to the SO_2 corrosion.

The effect of A.T.C. value in the presence of these depolarizers, was marginal; i.e. improvement of tinplate qualities will not prevent corrosion in high concentrations of these depolarizers as studied in these trials. It is obviously possible that at lower levels (say 1–10 ppm nitrate and 1–5 ppm sulphur dioxide) the effect of A.T.C. would be more marked. Experiments are in hand covering these aspects.

The conclusion must also take into account the possible influence of the other 'special property' values of the tinplate. Because of variations in these properties, it is difficult to interpret the results in terms of overall tinplate quality.

However, it is probably fair to deduce, from the results obtained, that a satisfactory shelf-life may be expected using tinplate with A.T.C. values outside the type K specifications as laid down by the American Can Company. Further experiments with tinplate at various A.T.C. values and with other special property values held constant will help towards defining more exact requirements for satisfactory shelf-life for orange and grapefruit packs.

The differences between cans of tinplate type 4 and 5, the special treatment and control tinplate, for both orange and grapefruit juices were not always clearly related to the A.T.C. values. With both fruit juices, cans of tinplate type 4 behaved generally very similarly to cans of tinplate type 1 as might be expected from the A.T.C. values. However, cans of tinplate type 5 produced somewhat variable results, albeit generally poorer than cans of tinplate type 4. It is possible to say from this trial that the nitric acid pickling treatment is effective in producing tinplate which gives a satisfactory shelf-life for both orange and grapefruit juices.

The results in Table 2 are also worthy of comment in that they tie in very closely to the initial average values, thus indicating the possibility of obtaining meaningful measurements after some 2 years storage. A.T.C. values obtained on cans with added NO_3 or SO_2 are not quoted as these were extremely high indicating the abnormal effect of these additives.

Conclusions

The indications are that certain A.T.C. values are necessary in order to give adequate shelf-life for citrus juices, and those values are different for orange and grapefruit juices. Tinplate with values of A.T.C. outside the type K specification will give a satisfactory performance.

Nitrates and sulphur dioxide, in the concentrations considered, generally override the effect of A.T.C. value and reduce shelf-life considerably. The presence of nitrate results in severe even detinning while sulphur dioxide produced tin sulphide staining, patchy detinning and early failure due to hydrogen swells.

Further work is necessary in order (a) to establish more precise limits for A.T.C. value for various packs and (b) determine the influence in relation to A.T.C. value of nitrate and sulphur dioxide at lower concentrations. Future work should also involve tinplate with special property values other than A.T.C. value held constant.

Acknowledgments

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Pectinesterase activity in the component parts of different Israeli citrus fruit varieties*

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Summary

Pectinesterase activity in whole fruits and single fruit components was established for grapefruit and orange (shamouti and valencia) varieties. It was found to range, in terms of pectinesterase units per gram of test material, from PE.u. $\times 10^4 = 5{\cdot}1$ for hand-reamed or 11·2 for commercially extracted juice to PE.u. $\times 10^4 = 821$ for pulp and rag. Enzyme activity in each of the different components was highest in valencia. The comparative distribution of component activity was similar for all three fruit varieties. When test materials of low pH were allowed to stand even for short periods prior to examination, partial inactivation occurred, whereas at pH 3·5 and above, pectinesterase activity remained stable for 8 hr or more. Therefore, in order to obtain reproducible results, stabilization of the more acid samples by raising the pH level to at least 3·5 was necessary.

Introduction

Distribution of pectinesterase activity in the different fruit components was established by MacDonnell, Jansen & Lineweaver (1945) for navel and valencia oranges and by Rouse (1953) for different Florida orange varieties, i.e. Hamlin, pineapple, temple, and valencia. Seasonal changes in enzyme activity in the component parts were shown by Rouse, Atkins & Moore for the valencia (1962) and pineapple (1964), orange varieties, and for silver cluster grapefruit (1965), and by Rouse & Knorr for Florida lemons (1969). Till now no data have been presented for Israeli citrus varieties.

We have studied pectinesterase activity in some local citrus fruits and its distribution in the single fruit components, in order to obtain data that may contribute toward establishing optimal pasteurization conditions for different types and varieties of citrus products having varying juice, peel or pulp contents.

* This work was partly supported by a grant from the Israel Ministry of Commerce and Industry.
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Materials and methods

Test materials

Test materials consisted of commercially or laboratory extracted juices as well as laboratory prepared whole fruits, peels, and pulps from the valencia, shamouti and grapefruit varieties. The factory-line juices were extracted by FMC juice extractors and strained through a 24 mesh Sveco vibrator screen 48 in. in diameter. Laboratory samples were prepared as follows. About 2 kg of fruit were cut in halves and divided into two portions, each containing the same amount of stem and stylar ends. One portion was used as raw material for the 'whole fruit' sample. From the second, after removing the peel (flavedo plus albedo) juice was extracted by a hand reamer from the edible portion and strained through a 24 mesh sieve. The remainder, comprising cells, membranes and some residual juice, was termed pulp and rag. All raw materials were macerated in an electrical blender at high speed for 3 min, after addition of pasteurized juice of the respective variety, to four times the volume in the case of peels and two-fold in the case of whole fruits and pulp and rag.

Analytical procedure

Pectinesterase activity was determined by the method described by Rouse & Atkins (1955). Test materials having higher activity than unpasteurized juice were diluted prior to analysis with pasteurized juice to bring them to within the same activity range; thus all analyses were conducted with equal amounts of the test sample and within the same activity range. The blank values for pasteurized juices were deducted from the titration values obtained.

Results

Pectinesterase activities of commercially extracted juices, whole fruits, and single fruit components are summarized for the grapefruit, shamouti and valencia varieties in Table 1. From the minimum, maximum and average values of pectinesterase activities presented in the table, it may be seen that, whereas the activity of any particular component differed considerably among the different varieties, the comparative activities of the various components followed a similar pattern for all varieties. Thus the average activity of factory-line juices ranged from PE.u. $\times 10^4 = 16{\cdot}4$ to $28{\cdot}5$, of whole fruits from 166 to 259, of peels from 181 to 248, and of pulp and rag from 388 to 677. However, considering factory-line juice as the standard base, the average activities of whole fruit was in the range nine to ten fold, of peels eight to eleven fold, and of pulp and rag seventeen to twenty-four fold their respective standard base value for the particular fruit variety.

Analyses of orange (shamouti and valencia) samples were easily reproducible, but for the more acid fruit varieties (grapefruit and lemon), a reduction in initial enzyme activity occurred during the interval between replicate determinations. The degree of inactivation increased with decreasing pH and increasing length of exposure (Fig. 1).

TABLE 1. Distribution of pectinesterase activity in different components of grapefruit and shamouti and valencia orange varieties

	Pectinesterase activity (PE.u. × 10^4/g)		Activity ratio average (factory line juice taken as standard base)
	Range	Average	
Grapefruit			
Factory line juice	11·2–23·0	16·4	1
Whole fruit	149–195	166	10
Peel	162–215	181	11
Laboratory prepared juice	6·1–10·7	8·5	0·5
Pulp and rag	336–509	400	24
Shamouti			
Factory line juice	11·6–28·4	22·7	1
Whole fruit	188–308	225	10
Peel	152–231	183	8
Laboratory prepared juice	5·1–12·0	9·1	0·4
Pulp and rag	266–502	388	17
Valencia			
Factory line juice	21·2–35·8	28·5	1
Whole fruit	234–287	259	9
Peel	214–279	248	9
Laboratory prepared juice	12·3–18·6	15·7	0·6
Pulp and rag	566–821	677	24

Pectinesterase activity is expressed as pectinesterase units (PE.u), denoting the mEq of ester hydrolysed from a pectin substrate per min per g of test material.

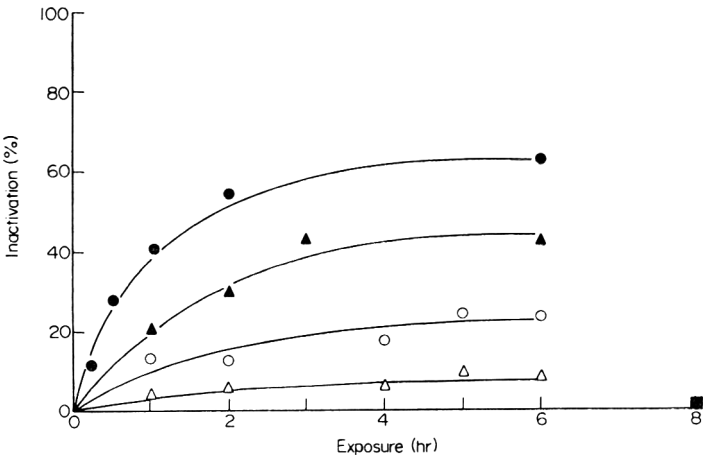

FIG. 1. Influence of pH on inactivation of pectinesterase. ●, pH 2·4–2·5; ▲, pH 2·7–2·8; ○, pH 3·0; △, pH 3·25; ■, pH 3·5. ●, ▲, lemon; ○, grapefruit; △, ■, orange.

However, by adjusting the pH to about 3·5–3·6, i.e. the level of orange juice, with dilute sodium hydroxide, enzyme activity could be stabilized for many hours (Table 2). Results for grapefruit in Table 1 refer to pH adjusted test materials; for the lemon variety, no data were recorded.

TABLE 2. Stabilization of pectinesterase activity by raising pH

Standing time at room temp. (hr)	Remaining activity (%)	Remaining activity (%)
	Grapefruit juice	
	Original pH 2·95–3·0	Adjusted pH 3·5–3·6
0	100	100
1	88	—
2	87	97
3	81	—
4	77	100
6	77	—
24*	—	99
48*	75	98
	Lemon juice	
	Original pH 2·4–2·5	Adjusted pH 3·5–3·6
0	100	100
0·25	88	—
0·5	69	102
1	59	100
2	48	100
24*	54	106

Average values from three to five experiments.
* After 8 hr kept at 4–6°.

Discussion

The highest activities in all varieties examined was found in pulp and rag, and the lowest in juices. Factory-line juices possessed generally about twice the activity of laboratory-prepared juices which may be explained by the different pressures applied in extraction. The influence of type of juice extractor employed has been investigated by Rouse & Atkins (1935). These authors obtained juice from the same lot of oranges using four different juice extractors, and found respective pulp contents of 8, 10, 11 and 12% with corresponding activities of 17·6, 22·5, 32·4 and 34·5 PE.u. $\times 10^4$. Rouse & Knorr (1969) reported that a high hydrogen ion concentration usually inhibits pectinesterase activity; from our results, it appears that the initial pectinesterase activity, e.g. of lemon juice at pH 2·4–2·8 decreased after standing for 1 hr by 20–40%, explain-

ing lack of reproducibility of data for this material. On the other hand, it was observed that pectinesterase activity of orange juice at pH 3·5 remained stable for 8 hr or more. The decrease of activity in highly acidic test materials could be avoided or stopped by raising the pH level to at least 3·5. Since no reactivation occurred within several hours after stabilization was carried out when some decrease of activity had already taken place, partial destruction of the pectinesterase enzyme seems indicated rather than inhibition of activity.

The method employed for estimation of pectinesterase activity involves a reaction time of 30 min so that there is an interval of probably 1 hr between consecutive analyses. Since in test materials of low pH, a decrease in initial activity occurred during standing, raising the pH levels to 3·5–3·6 with dilute alkali (a method applied successfully, e.g. for grapefruit constituents) is considered essential in order to obtain reproducible results.

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Sampling of potato tubers – a method based on a mathematical interpretation of gross potato tuber anatomy

A. R. QUARMBY

Summary

Evidence is presented to support the observation that within potato tubers, the mean solids concentration gradient in the perimedullary zone within the xylem 'ring' is linearly related to its mean spatial distance from the xylem 'ring'. Within any tuber the point most remote from the xylem 'ring', irrespective of tuber shape, is its geometric centre. If, for any tuber, this point is taken as the zero mean radius, then data obtained by a concentric peeling technique (*vide* Appendix I) may be used to construct a mathematical model of the mean solids concentration from the geometric centre to the periderm. A sampling technique is proposed whereby the mathematical parameters describing the tuber may be used to transform quantitative data from discrete tissue samples to values representative of whole tubers.

Introduction

Much of the experimental work concerned with the assessment of potato tuber quality relies upon tests carried out upon discrete tissue samples taken from individual tubers viz. slices, cubes etc. However, because of the heterogeneity of potato tubers, discrete samples can be generally considered non-representative of the tubers from which they are drawn. It would be expected that the degree of correlation between objective measurements made on tissue samples and subjective quality assessments made on whole tubers would be influenced therefore by the sampling technique employed, when taking samples for objective testing.

Alternate methods of sampling potato tubers were discussed by Glynne & Jackson (1919) who also reviewed sampling methods employed by investigators as long ago as 1892. Several recently published papers on aspects of potato texture and physiology appear ineffectual because of the sampling techniques employed and the authors have demonstrated their lack of knowledge of gross tuber anatomy which has been described thoroughly by Reeve, Hautala & Weaver (1969a, b, 1970); Reeve, Weaver & Timm (1971).

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The data presented in this paper are the partial result of concentric peeling experiments (Quarmby & Ratkowsky, 1972) carried out during the development of a sampling technique whereby tests, especially specific gravity (SG) determinations, made on small tissue samples, could be transformed to values representative of the tuber from which they were taken.

The examination of data obtained by concentric peeling many tubers from a number of different cultivars and oven drying the peel samples, showed that for all tubers the relationship between dry matter (DM) concentration and mean radius through the perimedullary zone was linear, or near-linear (two examples are given in Fig. 1).

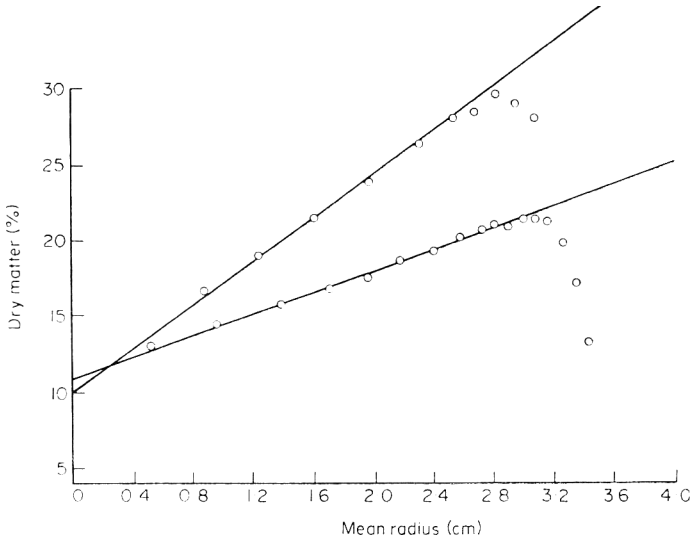

FIG. 1. Two examples of the relationship between mean radius and dry matter.

Although deviations from this pattern occurred with some tubers, especially in respect to data points influenced by pith tissue, no consistent non-linear pattern could be recognized. It also appeared that if the solids gradient from the geometric centre of any tuber to its xylem 'ring' was considered linear, then statistically significant differences existed between the characteristics of solids gradients, when groups of tubers from different cultivars were compared. It was considered furthermore that if an empirical mathematical expression for the mean solids gradient in tubers could be made comprehensive and include both perimedullary and cortical tissue, then such equations could be used to calculate intratuber solids distributions etc.

It then followed, that useful comparisons between individual tubers could be made by making successive SG determinations only, and the burden of oven drying concentric peel samples omitted. This could be done, provided a mathematical relationship between the original experimental data, viz. successive values of weight in air (W_a)

and weight in water (W_w), and the intratuber SG gradient or instantaneous SG (SG_I) was known.

By adopting Archimedes principle, specific gravity determinations on whole tubers and tissue samples may be carried out readily, and the dimensionless quantity thus obtained may be used to assess the solids content of the sample under examination.

It is generally assumed that solids content, and/or starch content and SG are related linearly, and many derived equations have been published, the most well known of these being that of von Scheele, Svensson & Rasmusson (1936) (*vide* Burton, 1966). It has been shown, however, by Wilson & Lindsay (1969), and discussed by Quarmby & Ratkowsky (1972), that the relationship between solids* or dry matter* and SG in vegetative tissue, is only near-linear and the true linear relationship lies between DM and the reciprocal of SG or specific volume (SV).

For the purpose of calculating some gross anatomical features of elongated tubers, Reeve *et al.* (1971) considered tubers as a simple geometric shape consisting of a cylinder having hemispherical ends. In this paper, however, all tubers will be considered as spheres as a compromise between elongated varieties and those whose mean lateral diameter is sometimes greater than their longitudinal axis. Previously Quarmby & Ratkowsky (1972) have considered tubers as ideal ellipsoids of equivalent volume and their mean radii as the geometric mean of their three major radii. It can be shown, however, that the mean radii values obtained using this procedure, are the same as the mean radii values (R) obtained when tubers are considered as spheres of equivalent volume.

The equations given in this paper have been derived using the principle that for any potato tuber, the mathematical relationship between the mean DM concentration gradient and the mean distance between the tuber's geometric centre and its periderm should be expressed as simply as possible. As will be shown, this results in the mathematical expressions for the experimental data from concentric peeling procedures (progressive or cumulative weights in air and water) being comparatively complex, and therefore the model parameters can only be derived from the experimental data indirectly.

To demonstrate the shape of the curves (profiles) when the various dependent variables to be considered are plotted against mean radii, Fig. 2 has been constructed from data pertaining to a hypothetical tuber having an exaggerated mean DM gradient from centre to periderm.

It may be seen that the equation for each profile can be considered as having two parts, the first embracing R values from zero to a point in the perimedullary tissue approaching the mean R value for the xylem 'ring'. The second part of the equation, which has a negative sign, can be considered as the difference between the first part and the profile of the xylem 'ring' and cortex. Throughout this paper this two-part form will be retained.

* In this paper these two terms will be synonymous.

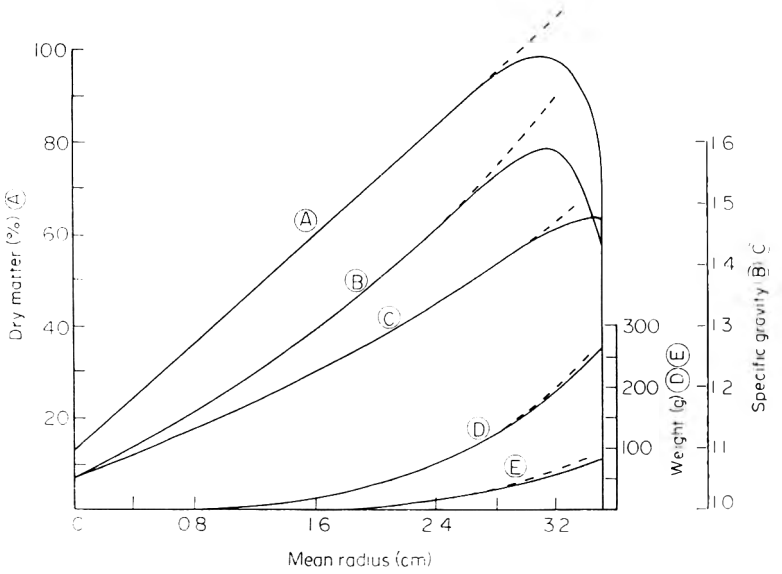

FIG. 2. Changes of various dependent variables with mean radius.

Experimental

Two well-shaped medium sized tubers from each of the three varieties Sebago, Brownell, 9–1 (unnamed) and two each from two different lots (O & N) of Kennebec (a total of ten tubers), were selected from the current season's crop and concentrically peeled according to Quarmby & Ratkowsky (1972). The major features of the ten experimental tubers are given in Table 1. The average solids content of 9–1 tubers is high

TABLE 1

Tuber		Weight (g)	Volume (cc)	SG	Major axes (cm)			Shape factor	Description
					a	*b*	*c*	$a(bc)^{-1/2}$	
Sebago	1*	198·05	180·49	1·0973	8·7	7·0	5·4	1·42	White skinned
	2	212·00	191·85	1·1050	8·8	6·9	5·5	1·43	elongated
Brownell	1	159·40	142·66	1·1173	7·1	7·0	5·6	1·13	Red skinned
	2	142·52	128·52	1·1090	6·7	6·8	5·0	1·15	globular
9–1	1	224·14	200·17	1·1197	7·6	8·2	5·7	1·11	Redskinned
	2	152·56	136·22	1·1199	7·1	7·0	4·9	1·21	globular
Kennebec (0)	1	162·15	146·38	1·1077	7·9	6·6	5·0	1·38	White skinned
	2	191·86	174·71	1·0981	7·9	7·0	5·6	1·26	elongated
Kennebec (N)	1*	228·76	208·65	1·0964	9·8	6·7	5·5	1·61	White skinned
	2	200·20	180·87	1·1069	8·7	6·9	5·5	1·41	elongated

* Tubers used in Table 3 and Figs 3 and 4.

and frequently the ratio of their stem end to apical end length (a) divided by the geometric mean of their other two axes ($a/(bc)^{-1/2}$) is $< 1{\cdot}0$.

All experimental work was carried out at constant temperature (20°C), and weighings in air and water were made on a top pan balance reading to 10 mg. Before use, all weight data were corrected for air buoyancy.

Development of mathematical model

If

$$\mathrm{DM} = \mathrm{A} - \frac{\mathrm{B}}{\mathrm{SG}} \tag{1}$$

(Wilson & Lindsay, 1969; Quarmby & Ratkowsky, 1972)

then

$$\mathrm{SG} = \frac{\mathrm{B}}{(\mathrm{A} - \mathrm{DM})}. \tag{2}$$

And so if the first part of the intratuber DM concentration gradient is considered linear (profile A, Fig. 2), then the first part of the $\mathrm{SG_I}$ gradient must be non-linear (profile B, Fig. 2), and of the form $1/(a - bR)$. The second part of the $\mathrm{SG_I}$ profile, i.e. $e^{(dR-c)}$, is a purely empirical term found most suitable to fit the data.

$$\mathrm{SG_I} = \frac{1}{(a - bR)} - e^{(dR-c)} \tag{3}$$

(Profile B, Fig. 2)

If volume

$$V = \frac{4\pi R^3}{3} \tag{4}$$

$$\mathrm{d}V = 4\pi R^2 \mathrm{d}R \tag{5}$$

then weight

$$W_a = \int_0^R \mathrm{SG_I}\,\mathrm{d}V \tag{6}$$

substituting (3) and (5)

$$Wa = 4\pi \int_0^R \left[\frac{R^2}{(a - bR)}\right]\mathrm{d}R - 4\pi \int_0^R \left[R^2 e^{(dR-c)}\right]\mathrm{d}R \tag{7}$$

$$= 4\pi\left[\frac{a^2}{b^3} \ln \frac{a}{(a - bR)} - \frac{R}{b^2} - \frac{R^2}{2b}\right] - 4\pi e^{(dR-c)}\left[\frac{R^2}{d} - \frac{2R}{d^2} + \frac{2}{d^3} - \frac{2}{d^3 e^{dR}}\right] \tag{8}$$

(Profile D, Fig. 2)

The second part of equation (8) has a negligibly small value when $R=0$.

The equation for calculating SG cumulative (SG_c) values which are the determinations made during the concentric peeling technique may be obtained by dividing weight from equation (8) by the volume from equation (4).

$$SG_c = \frac{W_a}{V} \qquad (9)$$

(Profile C, Fig. 2)

Curve fitting procedure

To obtain initial estimates of the four parameters a, b, c and d by standard curve fitting techniques (Johnson, 1952), it was first necessary to divide each set of data from the ten individual tubers (*c.* 20 points each) into two sets, viz. the first embracing the perimedullary tissue and the second the vascular region and cortex. This division was made at the point where all plots of W_a on R (profile D, Fig. 2) visually changed slope.

Specific gravity of successive peelings (SG_I) were calculated from the difference between successive weighings in air (W_a) and water (W_w), and mean R values from the volume of the remainder of the tuber, plus an addition equal to half of the last peel removed. Using the first set of data (SG_I') from each tuber, weighted regressions of the reciprocal of SG_I' on R were made to obtain initial estimates of the parameters a and b thus if

$$\frac{1}{SG_I'} = (a - bR) \qquad (10)$$

$$SG_I' = \frac{1}{(a - bR)}. \qquad (11)$$

Using the R values from the second set of data from each tuber, equation (11) was used to extrapolate the SG_I' curve (dashed lines Fig. 2), and the natural logarithm of differences between these calculated data (SG_I') and the experimental data i.e. ΔSG_I, regressed on the appropriate R values to obtain the parameters c and d thus:

$$(SG_I' - SG_I) = \Delta SG_I \qquad (12)$$

if

$$\ln \Delta SG_I = (dR - c) \qquad (13)$$

$$\Delta SG_I = e^{(dR-c)} \qquad (14)$$

and thus from (12)

$$SG_I = SG_I' - \Delta SG_I$$

and substituting (11) and (14)

$$SG_I = \frac{1}{(a - bR)} - e^{(dR-c)}. \qquad (3)$$

Weighting factor

The main objective of the curve-fitting procedure was to develop an equation which would relate peeled tuber weight and mean radius. The parameters a, b, c and d were estimated however, from data corresponding to the slope of the weight on mean radius curve i.e. SG_I on R, it was therefore considered appropriate when estimating the parameters, to weight the SG_I data in proportion to the weight of the interval which each SG_I data point represented.

The weighting factors used were thus $4\pi R^2 SG_I$ i.e. dW_a from equation (6), the element of weight at each R value used. The validity of this weighting factor increases as the thickness of peel removed decreases, and although used in this experiment a more appropriate factor could be the actual weight of the tissue from which each data point was obtained. In this experiment the result of weighting the regressions was to emphasize data points from near the outside of a tuber, compared with data points from near the pith by a factor of *c.* 10.

Non-linear least squares fit of data

As the final stage in the development of a mathematical interpretation of gross tuber anatomy the estimates of the four parameters obtained by linear methods *sup.*, were used as initial values for a computerized, weighted, non-linear, least squares fit of each set of SG_I data (Draper & Smith, 1966). The final values of the parameters and their standard errors are given in Table 2.

Comparison of calculated experimental data

From the ten experimental tubers, two were selected for further consideration on the basis of their difference in SG_I profiles on one hand, but similarity in morphology and

TABLE 2

Tuber		Maximum radius	Parameter *a*		Parameter *b*		Parameter *c*		Parameter *d*	
			Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
Sebago	1*	3·5058	0·9772	0·0019	0·027751	0·000750	40·90	1·56	11·08	0·45
	2	3·5779	0·9611	0·0034	0·024332	0·001292	43·50	2·44	11·67	0·69
Brownell	1	3·2414	0·9227	0·0038	0·014336	0·001581	44·63	3·61	13·16	1·13
	2	3·1306	0·9355	0·0039	0·017611	0·001678	46·13	3·77	14·13	1·22
9–1	1	3·6289	0·9623	0·0030	0·028814	0·001141	37·41	1·88	9·79	0·52
	2	3·1919	0·9385	0·0051	0·021627	0·002166	47·96	5·53	14·39	1·74
Kennebec (O)	1	3·2694	0·9544	0·0034	0·024365	0·001440	36·39	2·35	10·50	0·72
	2	3·4680	0·9673	0·0028	0·025239	0·001172	30·92	1·66	8·30	0·48
Kennebec (N)	1*	3·6794	0·9544	0·0024	0·017679	0·000917	38·28	2·18	9·79	0·60
	2	3·5083	0·9517	0·0021	0·021215	0·000856	30·68	1·45	8·08	0·42

* Tubers used in Table 3 and Figs 3 and 4.

specific gravities (see Table 1), on the other. Figure 3 shows the calculated SG_I profiles for these two tubers compared with the data from which their parameters were estimated.

The differences between experimental weights, and weights calculated using equation (8), are too small to demonstrate graphically, and so these data for the two tubers are compared in Table 3.

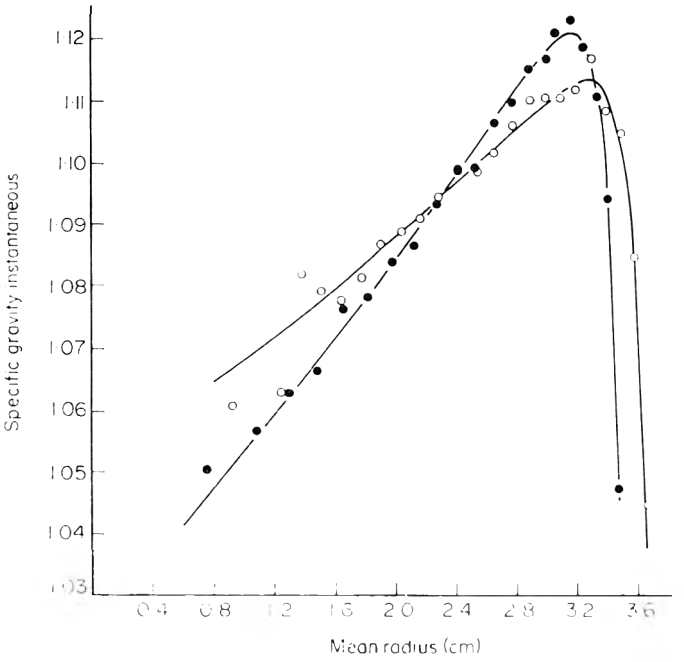

FIG. 3. ●, Sebago 1; ○, Kennebec (N) 1.

Dry matter distribution curves

It is not intended to give practical applications of the tuber model in this paper, however, to illustrate the implication of the differences in the value of parameter b for the two tubers now being considered, distribution curves of intratuber tissue DM content, were constructed as follows.

For a given value of SG_I it is not practicable to solve equation (3) for the variable R, therefore, for constant intervals of SG_I (0·00125), values of R were determined graphically (from Fig. 3). Equation (8) was then used to calculate the weight of tissue between successive values of R corresponding to the regular SG_I intervals. The distribution curves in Fig. 4 show each of these weight increments plotted as a percentage of the total fresh weight of the tuber. Although the distribution curves were initially constructed from SG_I classes, this scale was converted to percentage DM using the von Scheele equation (*vide* Burton, 1966) so that the curves could be considered in terms of the distribution of solids within the tubers.

TABLE 3

Mean radius (cm)	Sebago 1 Cumulative weight (g)			Mean radius (cm)	Kennebec (N) 1 Cumulative weight (g)		
	Calculated	Experimental	Difference		Calculated	Experimental	Difference
3·51	197·80	198·05*	−0·25	3·68	229·44	228·76*	+0·68
3·44	187·42	187·20	+0·22	3·62	217·55	218·49	−0·94
3·37	175·67	175·71	−0·04	3·53	203·00	203·52	−0·52
3·29	163·63	163·37	+0·26	3·44	187·65	188·03	−0·38
3·19	148·86	149·71	−0·85	3·34	171·75	172·20	−0·45
3·10	136·85	137·22	−0·37	3·25	156·93	157·18	−0·25
3·01	125·61	125·47	+0·14	3·15	142·99	142·98	+0·01
2·93	114·98	114·68	+0·30	3·04	129·34	129·31	+0·03
2·82	102·91	102·62	+0·29	2·94	117·38	115·97	+1·41
2·70	89·53	89·99	−0·46	2·83	102·42	103·08	−0·66
2·58	77·72	77·85	−0·13	2·71	89·58	90·69	−1·11
2·46	67·57	67·68	−0·11	2·59	79·44	79·21	+0·23
2·33	56·86	57·12	−0·26	2·47	69·62	68·74	+0·88
2·20	47·64	47·78	−0·14	2·35	58·66	58·68	−0·02
2·03	37·54	37·73	−0·19	2·22	49·28	49·41	−0·12
1·90	30·61	30·60	+0·01	2·08	40·56	40·79	−0·23
1·72	22·35	22·48	−0·13	1·96	32·79	33·81	−1·02
1·56	17·09	16·99	+0·10	1·82	26·45	27·31	−0·86
1·38	11·62	11·52	+0·10	1·70	22·19	21·98	+0·21
1·17	7·35	7·12	+0·23	1·58	18·29	17·54	+0·75
0·95	3·58	3·75	−0·17	1·44	13·67	13·31	+0·36
0	0	0	0	1·31	9·99	9·87	+0·12
				1·16	8·11	7·00	+1·11
				0	0	0	0

Difference: Mean = −0·0659; SD = 0·2778; SE = 0·0592. Difference: Mean = −0·0367; SD = 0·6687; SE = 0·1365.

* Unpeeled weights.

The mean R value dividing the cortex and the perimedullary zones was calculated by utilizing the equation for the slope of SG_I, i.e. equation (15), and a series of R values (in increments of 0·01) covering the range where SG_I (Fig. 3) showed its maximum value.

From (3)
$$\frac{dSG_I}{dR} = \frac{b}{(a-bR)^2} de^{(dR-c)}. \qquad (15)$$

The R value which gave the minimum value for dSG_I/dR was taken as the division between the two major tissue zones. Furthermore this R value, representing the position

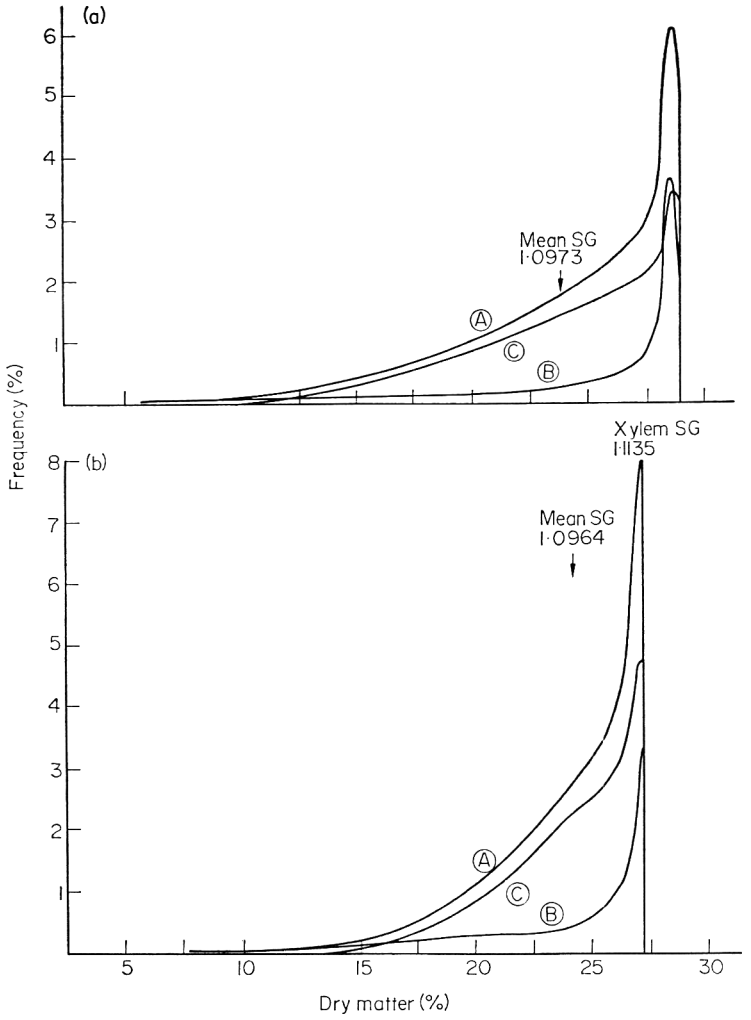

FIG.4. (a) Sebago 1. A, Total tuber 100·0% w/w. B, Cortical tissue 26·0% w/w. C, Remainder 74·0% w/w. (b) Kennebec (N) 1. A, Total tuber 100% w/w. B, Cortical tissue 28·4% w/w. C, Remainder 71·6% w/w.

of the xylem 'ring', subtracted from the *R* value corresponding to the periderm of the tuber (maximum radius, Table 1) was taken as the mean thickness of the cortex. For the one Sebago tuber and the one Kennebec tuber under discussion, these thicknesses were 0·34 and 0·39 cm respectively.

As well as the total intratuber distribution of DM, Fig. 4 shows the distribution of DM each side of the mean *R* value corresponding to the maximum concentration of solids (xylem 'ring') within each tuber.

Discussion—DM distribution curves

From the data used to construct Fig. 4 it can be readily calculated that 33% w/w of the Sebago tuber had a solids content greater than, and 6% w/w less than, the maximum and minimum levels found anywhere (excluding periderm) in the Kennebec (N) tuber.

On the proviso that the level of starch concentration within individual cells (or a dependent factor) is correlated with cell adhesion, (cf. Reeve, 1972; Hoff, 1972) it would not then have been unexpected if the response of these two tubers (of comparable SG) to cooking, differed greatly.

Unpublished experimental data to hand indicates that if mathematical models for groups of tubers of different cultivars are compared, then significant differences exist between their grouped parameter b values. These differences are in turn reflected in differences in the spread of solids distribution curves. If constructed from the combined data from a population of tubers, these curves do of course (unlike Fig. 4) develop a right hand 'tail', but remain however, negatively skewed.

Proposed sampling method for tubers

If firstly, the experimental evidence presented in this paper of a linear or near-linear solids gradient from any tuber's centre towards its xylem 'ring' is acceptable, and secondly, if in an experiment utilizing a statistically sufficient number of tubers, the required precision of fit of the models to the concentric peeling data could be somewhat less than that demonstrated, then an approach to tuber sampling, based on gross tuber anatomy can be suggested.

If each tuber was concentrically peeled through its cortical region to a point inside its xylem 'ring' (*c.* six peelings) and an additional SG determination made on an equiaxed plug (*c.* 2 g) from its centre, then the data from these operations would suffice for the estimation of the parameters a, b, c and d for the tuber concerned.

This method would have the advantage of preserving the integrity of at least 50% of the tuber's main storage tissue, which could then be used for both objective and subjective tests. Discrete tissue samples from the remains of the tuber after peeling could also be used to determine the SG differential between stem and bud ends, and so add another parameter to the mathematical model.

If tissue samples (e.g. plugs or cubes) when taken from the perimedullary zone were spatially located in respect to the centre of the tuber, then quantitative data from these samples which correlated with SG, could be transformed to values representative of the whole tuber from which they were taken.

Conclusion

The main interests of the author have been discussed in the sections dealing with DM distributions and sampling. However, the comparison of SG_I–R profiles from different tubers suggests that the changes in solids concentration, especially in the cortical regions

of tubers could be of interest to those concerned with commercial peeling losses etc., and to those concerned with the textural behaviour of tubers during canning.

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Appendix I

Concentric peeling of tubers—details of methods

After being taken from storage at 7°C tubers were washed, dried, and if necessary, sprouts removed by hand. The three major axes of each tuber were then recorded to the nearest mm by the use of an adjustable gauge.

Tubers were then weighed in air and water using a top pan balance reading to the nearest 10 mg. For weighing in water the tubers were suspended by a fine stainless steel wire provided with a short spike. The usual precautions pertaining to water temperature and density were observed.

Successive layers of peel were then removed using a domestic hand peeler, the remains of the tuber being weighed in air and water between peelings. Between all operations, which were carried out as rapidly as possible, the tuber was dried with a cloth. For some

experiments the addition to the water bath of a small quantity of dye (Erythrosine) was found to assist uniformity of peeling. It was found in practice that by using a technique of peeling each tuber from approximately the mid-point of the long axis back towards the operator the original ratio of the three axes was preserved.

When peel samples, which averaged approximately 15 g each, were required for analysis or drying they were transferred rapidly and completely to tared containers, weighed and preserved by freezing. Peeling operations on any one tuber ceased when the weight of remaining core tissue approximated 15 g. This last sample, if required for drying, was sliced thinly by hand and then treated in the same manner as the peeled samples.

The dimensions of the tuber and the successive weighing data were processed by computer to give the specific gravity, mean thickness of each layer, and mean distance from geometric centre or periderm, in addition to various other statistics.

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The vitamin C and thiamin contents of quick frozen peas

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Summary

The effect of maturity, variety, post-vining delay and various stages in commercial processing, including cleaning, blanching, freezing and end-cooking, on the vitamin C and thiamin contents of quick frozen peas has been studied. As the peas matured, the vitamin C content decreased but there was no correlation between maturity and thiamin content. The vitamin C content of six varieties was approximately constant, but one variety had an unexpectedly low thiamin content. In most cases, post-vining delay had a detrimental effect on the vitamin C content whereas immature (but not mature) peas showed an increase in thiamin content. On average, frozen peas retained 80% of the original vitamin C content and 90% of the thiamin content. Cooking caused a further loss of vitamin C so that, at the point of consumption, the frozen peas retained approximately 55% of the fresh value for this nutrient.

Introduction

The nutrients examined in this evaluation of the critical points of the freezing process under factory conditions were vitamin C and thiamin; both vitamins are water soluble and can be lost by leaching, also, enzymic or chemical oxidation during processing may cause additional losses. Peas served with a meal can contribute at least one third and one tenth of the total daily requirement for vitamin C and thiamin, respectively, so any modification which could be made to commercial procedure to reduce nutrient loss would further help to improve the nutritional value of the national diet.

The main objective has been to study the effects of variety, maturity, post-vining delay and factory processing, including washing, blanching, cooling and freezing, on the vitamin levels. Although information about nutrient changes during the preservation of green peas has been reported (Lee, 1958; Lynch, Mitchell & Casimir, 1959), blanching has been the focal point; simulated laboratory conditions have often been used to investigate the effect of blanching and other points in the processing line have

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been neglected. The present study was carried out in a commercial factory during the summer of 1972 and covered all aspects of freezing peas; the effect of cooking on the vitamin C content of fresh and frozen peas was not studied at the same factory until the summer of 1973.

Experimental and results

Analytical methods

(i) *Vitamin C content.* Duplicate 30 g samples of peas were ground with a small quantity of sand and made up to volume with 3% metaphosphoric acid–acetic acid solution. The extracts were centrifuged, in place of filtering, and assayed in duplicate by the microfluorimetric method (AOAC, 1970). The fluorimetric method determines vitamin C after a preliminary oxidation to dehydroascorbic acid and, therefore, is a measure of the total vitamin C activity in the product, i.e. ascorbic acid + dehydroascorbic acid.

(ii) *Thiamin content.* Duplicate 30 g samples of peas were assayed fluorimetrically (AOAC, 1970) but, from preliminary investigations, the initial digestion with acid was found to be unnecessary. The extracts were centrifuged to provide clear solutions for the subsequent analyses in duplicate.

(iii) *Solids content.* Approximately 500 g samples of peas were minced and 10 g sub-samples were dried at 80°C for 18–24 hr. The vitamin contents have been expressed on a dry weight basis to overcome fluctuations in the moisture content of the peas. The solids content of blanch water was determined in the same manner, taking duplicate 20 ml samples, to establish the state of cleanliness of the water.

Harvesting and processing procedure

The harvested pea plants were vined in the field and the shelled peas were transported by lorry to the factory in bulk loads of 2–3 tons. The delay time between vining and transfer to the hoppers for the first stage of the factory process varied but was seldom more than 90 min. From the hoppers, the peas travelled through cold water washers and then into a water blancher at 96–98°C for 1 min. On emerging from the blancher, the peas were cooled with water sprays for 6 sec which was followed by air cooling. Finally, the peas entered the freezing tunnel which operated at approximately −20°C.

Factors investigated

(1) Maturity. On 12 consecutive days, vines of the variety, Sparkle, were taken from the same plot of ground to eliminate agricultural variation. On arrival at the factory, the vines were podded in a miniature viner and the maturity of the peas was determined in duplicate with a tenderometer. The vitamin content was determined immediately so that the post-vining delay was zero. The results are summarized in Table 1 and show that as the peas matured the tenderometer readings (TR) rose from 77·5 to 123·5. Over

the same period, the vitamin C content decreased from 222 to 119 mg/100 g dry weight although the moisture content of the peas only decreased from 81·3 to 75·7% with increasing maturity. No correlation between the thiamin content of 1·60–1·70 mg/100 g dry weight and maturity was observed.

TABLE 1. Effect of maturity on tenderometer readings (TR) and vitamin C and thiamin contents of fresh peas (variety, Sparkle)

Day	TR value	Vitamin C (mg/100 g dry wt)	Thiamin (mg/100 g dry wt)	Total solids (%)
1	77·5	221·8	1·71	18·7
2	84·5	186·0	1·38	18·8
3	83	195·2	1·63	19·2
4	85	191·0	1·68	20·0
5	88	200·8	1·56	20·0
6	91	164·5	1·28	20·8
7	95	160·7	1·67	20·9
8	98	161·1	1·44	21·3
9	93	171·9	1·68	21·0
10	103	155·4	1·68	21·8
11	110·5	127·6	1·60	22·8
12	123·5	118·9	1·63	24·3

(2) Post-vining delay. On two occasions, samples of the variety, Sparkle, were transported to the factory on the vine. Some of the vines were handpodded and the remainder were podded mechanically in the miniature viner. Tenderometer readings of the peas were recorded immediately and the remainder were kept in the laboratory and analysed for vitamin content at intervals during a delay time of 0–120 min. Commercially, vined samples were not investigated as it was difficult, in practice, to control the delay time.

The batches of immature (TR-90) and mature (TR-110) peas, subjected to handpodding and miniature vining, showed a progressive decrease in vitamin C content with time except for the handpodded immature sample which tended to remain constant (Table 2). The thiamin content of the same samples of peas showed no conclusive pattern in relation to delay time (Table 2) except for an indication that the level could increase in some circumstances.

(3) *Various cultivars and the effect of blanching.* Six varieties (Sparkle, Swift, Swan, Scout, Dark Skinned Perfection (DSP) and Puget) were analysed after commercial vining in the field with a delay time of 70–80 min and equivalent vines were brought to the factory for miniature vining to provide reference values with a delay time of zero. Ideally, the TR values for all varieties should have been the same but, in practice, the

TABLE 2. Effect of post-vining delay on the vitamin C and thiamin contents of handpodded and miniature vined peas (variety, Sparkle)

	Delay (min)	Handpodded		Miniature vined	
		Vitamin C (mg/100 g dry wt)	Thiamin	Vitamin C (mg/100 g dry wt)	Thiamin
(a)	0	230·0	1·28	222·2	1·28
	30	214·8	1·25	202·3	1·39
	60	224·3	1·26	201·8	1·49
	90	230·1	1·36	201·7	1·51
	120	235·8	1·44	193·9	1·71
(b)	0	206·8	1·52	179·1	1·63
	30	193·0	1·56	177·5	1·94
	60	178·4	1·22	177·8	1·23
	90	177·7	1·50	174·4	1·48
	120	169·4	1·62	168·1	1·52

(a) Handpodded TR-90·5, miniature vined TR-89·5.
(b) Handpodded TR-110, miniature vined TR-100·5.

peas from the hoppers had TR values of 95–110 and the miniature vined samples recorded TR values of 90·5–96. Samples of the six varieties were also withdrawn from the air cooler after blanching for vitamin analyses.

The vitamin C content, based on the miniature vined samples with zero delay time, came within the range 154–170 mg/100 g dry weight for five varieties; the variety, Swift, contained 202 mg vitamin C/100 g dry weight. The corresponding values for the commercially vined samples were 125–160 mg vitamin C/100 g dry weight for five varieties and 181 mg vitamin C/100 g dry weight for Swift. The vitamin C content of the peas after blanching and air cooling has been expressed as a percentage of the amount present in the commercially vined peas (Table 3); retention values for four varieties came within the range 77–83%, the varieties Scout and Swan retaining 72% and 90% respectively.

Contrary to the findings for vitamin C, the thiamin content of the commercially vined samples was always slightly higher than the corresponding miniature vined peas (Table 3). Five of the varieties contained 1·51–1·71 mg thiamin/100 g dry weight prior to processing, Scout being the exception with 1·18 mg thiamin/100 g dry weight. The retention of thiamin at the air cooling stage after blanching was 91–99% for four varieties; retention values for Scout and Swift were 64% and 82%, respectively.

(4) *Other processing variables.* The intention was to study whether the state of cleanliness of the washing and blanching waters influence the level of retention of vitamin C and thiamin in peas during processing. Theoretically, four permutations are possible, viz.

TABLE 3. Comparison of the vitamin C and thiamin contents of six varieties of peas subjected to miniature vining, commercial vining and blanching. (Retention after blanching expressed as a percentage of the commercially vined values)

Variety	Sample	Content (mg/100 g dry wt)		Retention (%)	
		Vitamin C	Thiamin	Vitamin C	Thiamin
Sparkle	Miniature vined	170·4	1·59		
	Commercially vined	125·4	1·69	83·4	91·1
	Blanched	104·6	1·54		
Swift	Miniature vined	201·8	1·36		
	Commercially vined	180·8	1·57	76·7	82·2
	Blanched	138·6	1·29		
Swan	Miniature vined	168·9	1·51		
	Commercially vined	138·8	1·53	90·4	98·6
	Blanched	124·8	1·51		
Scout	Miniature vined	154·1	0·58		
	Commercially vined	137·1	1·18	72·3	63·6
	Blanched	99·1	0·75		
Puget	Miniature vined	155·4	1·49		
	Commercially vined	129·8	1·51	80·4	98·6
	Blanched	104·4	1·49		
DSP*	Miniature vined	164·4	1·63		
	Commercially vined	160·0	1·71	80·6	92·4
	Blanched	120·0	1·58		

* DSP: Dark Skinned Perfection.

clean wash/clean blanch, clean wash/dirty blanch, dirty wash/clean blanch and dirty wash/dirty blanch, but in the factory procedure for changing the processing water the situation of clean wash/dirty blanch did not arise. Samples of the variety, DSP, were withdrawn at six points on the processing line as indicated in Fig. 1 but the results in Table 4 record only four critical points. The delay time and TR values could not be kept constant for all the processing runs but in most cases were 90 min and TR-100, respectively, except that the clean wash/clean blanch combination was investigated with immature peas (TR-89·5). The samples of peas were collected from the processing line in aluminium sieves, so that excess water drained away, and were stored at 4°C in tied polythene bags. Samples taken after leaving the blancher, but before entering the air cooler, were chilled quickly by placing the bag in a mixture of solid CO_2, acetone and water. All samples were analysed on the day that they were taken and were stabilized in metaphosphoric acid within 2 hr of leaving the processing line. The 'dirtiness' of the water was assessed from the solids content, a sample of blanch water being withdrawn from the overflow pipe. Clean blanch water contained 0·2% solids

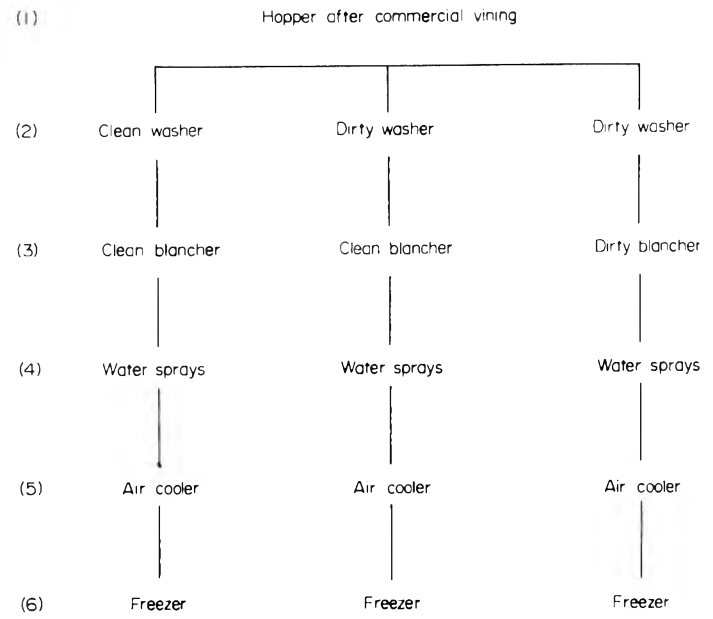

FIG. 1. Exit points of processing line from which samples of peas of variety, Dark Skinned Perfection, were withdrawn for vitamin analyses.

TABLE 4. Retention of vitamin C and thiamin after various stages of processing peas of variety, Dark Skinned Perfection

Stage in processing	Retention (%)					
	Clean wash/clean blanch		Dirty wash/clean blanch		Dirty wash/dirty blanch	
	Vitamin C	Thiamin	Vitamin C	Thiamin	Vitamin C	Thiamin
Commercial vining	100	100	100	100	100	100
Washing	97	100	95	94	94	98
Blanching	74	100	78	88	82	93
Freezing	75	91	78	92	82	90

which increased to approximately 2·0% solids when dirty and on the point of being discarded.

Individual analyses showed values ranging from 91 to 99% retention of vitamin C after the peas were washed but the average values were 94–97% retention showing that the condition of the washing water had little, or no, effect on retention; similarly, 94–100% retention of thiamin was observed at the same stage of processing, Table 4. When the peas emerged from the blancher, the vitamin C content was reduced to

average values of 74–78% retention with clean blanch water, and 82% for dirty blanch water; the subsequent stage of a 6 sec passage through water sprays, air cooling and freezing resulted in no further loss of vitamin C. The overall retention of thiamin after blanching, cooling and freezing was 90–92%. These results for the retention of vitamin C and thiamin in frozen DSP peas are in agreement with the values previously found for this variety, Table 3.

(5) *Cooling losses.* Only one trial was possible from the same batch of peas of variety, Puget, to examine the vitamin C content of fresh, frozen, fresh/cooked and frozen/cooked peas. To prepare the frozen/cooked samples, 8 oz of frozen peas were added to ½ pint of slightly salted boiling water and simmered for 3 min after returning to the boil. Fresh handpodded peas from the same field and of the same tenderometer reading were boiled under similar conditions to the same texture as the frozen cooked peas as assessed by a taste panel. In addition, further data on cooking losses only, with no allowance for the earlier reduction during commercial processing, were obtained by analysing the vitamin C content of frozen peas before and after cooking, following the same procedure described above. The overall level of vitamin C in the fresh/cooked peas and the frozen/cooked peas (Table 5) was similar despite the variation in cooking procedures, i.e.

1·1 min to boil		freezing process + 3·0 min to boil
+	=	+
6·3 min further boiling		3·0 min further boiling
(Fresh peas)		(Frozen peas)

TABLE 5. Effect of cooking on the vitamin C content of peas of variety, Puget

Sample	I* (mg vitamin C/100 g dry wt)	II†
Commercially vined	123·2	
Frozen	109·8	99·8
Frozen/cooked‡	75·7	68·1
Handpodded	139·4	
Handpodded/cooked§	76·9	

* I: duplicate analysis of one trial.
† II: duplicate analysis of three trials.
‡ 3 min to return to the boil + 3 min simmering.
§ 1·1 min to return to the boil + 6·3 min simmering.

Discussion

The reduction in the vitamin C content of peas with increasing maturity which was found in the present investigation (Table 1) is in agreement with the previous work of

Mack & Tressler (1936), Clifcorn & Heberlein (1944), Kramer *et al.* (1950) and Robertson & Sissons (1966). As peas mature, the skin/cotyledon ratio decreases but the content of vitamin C in the skin is twice that in the cotyledons which would account for the lower level of vitamin C in more mature peas (Todhunter & Sparling, 1938). No correlation between thiamin content and maturity has been found which is contrary to the reports of Clifcorn & Heberlein (1944) and Heinze, Hayden & Wade (1947) who observed that thiamin levels were affected by maturity. The percentage increase of total solids which was found with increasing maturity agrees favourably with the results of Lynch *et al.* (1959).

Two conclusions can be drawn from the study of the effect of delay on the vitamin C content of peas: ignoring differences in TR values and the method of podding, in three out of four cases the level of vitamin C in the peas showed a progressive decrease with time which is in agreement with Jenkins, Tressler & Fitzgerald (1938) who found a 14% loss after holding for 3 hr; the miniature vined peas had somewhat lower contents of vitamin C than the comparable handpodded samples (Table 2). Holdsworth (1970) has suggested that mechanical damage leads to loss of vitamin C because of increased enzymic oxidation by ascorbic acid oxidase; miniature vining is likely to inflict more damage to the peas than handpodding. Similarly, the investigations with different varieties of pea showed a large difference between the vitamin C content of miniature and corresponding commercially vined samples (Table 3) which could not be attributed entirely to the post-vining delay when analysing the latter; more severe bruising during commercial vining than with miniature vining is a probable explanation.

The effect of delay time on the thiamin content of peas was variable with increased and decreased levels being recorded, although it was the immature peas which tended to show the former characteristic (Table 2). Moyer & Tressler (1943) found no loss in thiamin during a 3 hr delay between vining and washing whereas Ingalls *et al.* (1950) observed an apparent increase which could not be attributed to changes in total solids. It is interesting to note that the thiamin content of the six varieties of peas was always higher in the commercially vined samples, with an unavoidable delay time of 70–80 min, than in the corresponding miniature vined samples with a delay time of zero (Table 3).

The microfluorimetric method of analysis, which measures the dehydroascorbic acid content in addition to the fully reduced ascorbic acid, was employed in the present investigation and may account for the range of values for the vitamin C content of six varieties of peas (Table 3) being close to the upper limit of previously reported figures (Mack & Tressler, 1936; Robertson & Sissons, 1966). However, Mapson (1961) has reported that the concentration of dehydroascorbic acid in fresh vegetable material is small. After blanching, the vitamin C retention ranged from 72–90% which indicates a possible difference in processing characteristics between varieties.

With the exception of the very low level in the variety, Scout, the thiamin contents of the other five varieties were in agreement with previously reported values (Clifcorn

& Heberlein, 1944; Ingalls *et al.*, 1950). In most cases, over 90% of the thiamin of commercially vined peas was retained after the blanching process.

Although vitamin loss during processing can arise from chemical and enzymic oxidation, leaching is likely to be the major factor. A theory has been proposed that the leaching effect will be reduced if the processing water contains a proportion of soluble solids. Allen, Barker & Mapson (1943) reported that serial scalding in the same water minimized losses during blanching whereas Guerrant *et al.* (1947) found no improvement in vitamin C retention by repeatedly passing samples through the same blanch water. Lynch *et al.* (1959) showed that it was the immature peas which lost most vitamin C during blanching. The present study has shown that the state of the washing and blanching waters had little influence on the retention of vitamin C and thiamin (Table 4); although the average retention value for vitamin C was highest after dirty wash/dirty blanch treatment, the range of values obtained from replicate analyses of the different combinations overlapped and a significant claim cannot be made. After blanching, the peas were only in contact with water for 6 sec before being air cooled and frozen, and no further loss of vitamin content was found; Hard & Ross (1956), Mapson (1956) and Joslyn (1961) recorded losses of water soluble nutrients when water cooling was employed. Mapson (1956) and Cain (1967) found no evidence that nutrients were lost during the freezing process and this observation was confirmed in the present study. The overall retention of vitamin C in frozen peas (Table 4) is in agreement with the value of 75% recommended for good commercial practice in the freezing of vegetables by the International Institute of Refrigeration (1971) when it is remembered that the current analyses include the dehydroascorbic acid content which contribute approximately 2–3% to the vitamin C activity in peas. Losses of thiamin, regardless of the condition of the processing water, were small and confirmed previous findings which have been reviewed by Lee (1958).

The retention of vitamin C after the cooking of fresh and frozen peas is of interest (Table 5). The 55% retention when fresh peas are boiled approximates to the 80% retained after the commercial freezing process followed by a 25–30% reduction during the milder cooking treatment for the frozen peas. The retention of vitamin C when boiling frozen peas appears to be a constant factor; if the frozen product has a lower vitamin C content, the level of this nutrient will be correspondingly lower after cooking under standardized conditions. The percentage retention of vitamin C after cooking fresh and frozen peas in this investigation was of a higher order than that reported by Mapson (1956).

Conclusion

The vitamin C content and thiamin content of peas through all stages from vining to the frozen product have been followed under factory conditions. The situations where loss of vitamin C is most likely to occur have been pinpointed to mechanical damage

during vining, delay in holding the shelled peas before entering the production line, blanching, and end-cooking; post-blanching cooling with water was not employed in the factory studied but could provide an additional area for loss of vitamin C. Because of the reduced time needed to cook frozen peas compared with fresh peas, the vitamin C content of both at the point of consumption was similar. The overall loss of thiamin was relatively small during the freezing of peas and, under some conditions, increases in thiamin levels were observed which must be attributed to anabolic enzymic activity as opposed to the catabolic action of ascorbic acid oxidase.

Acknowledgments

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Collagen fibre arrangement in intramuscular connective tissue. Changes associated with muscle shortening and their possible relevance to raw meat toughness measurements

R. W. D. ROWE

Summary

Collagen fibres of ovine and bovine perimysium are shown to have a crimped appearance. The size of the crimp is smaller than in the collagen fibres from tendon (e.g. perimysium 19 μm; tendon 79 μm). The collagen fibres are laid down in a well-ordered criss-cross lattice at an angle to the muscle fibre long axis. Muscle contraction changes the angles of this lattice and also changes the crimp length. These changes are related to differences between samples of meat of uniform size such as are tested for toughness. It is demonstrated that any explanation of changes in meat toughness resulting from shortening can no longer be related only to changes in 'myofibrillar toughness', but must also include extensive changes in 'connective tissue toughness'.

Introduction

Muscle can be regarded as a two-component system, i.e. the muscle fibres and the intramuscular connective tissue. The roles of these two components in contributing to the overall toughness of a piece of meat have been studied for some years. Initial work led to the conclusions that the quantity and strength of the connective tissue determined the toughness of the meat (Lehmann, 1907; Mitchell, Zimmerman & Hamilton, 1927; MacKintosh, Hall & Vail, 1936). However, following the work of Locker (1960), which showed that there was a relationship between meat toughness and the state of contraction of the muscle fibres, it became more popular to look to this component for an explanation of variability in meat toughness resulting from contraction (see Newbold & Harris, 1972, for a list of references linking toughness with muscle fibre shortening). The role of connective tissue toughness was then considered to be confined to one of providing 'background toughness' which may or may not be predominant; but which varies in relation to the quantity and age of the collagen (Goll, Hoekstra & Bray, 1964; Goll, Bray & Hoekstra, 1964; Hill, 1966; Herring, Cassens & Briskey, 1967) and not with muscle shortening. This trend towards emphasizing 'muscle fibre' or 'myofibrillar toughness' variability could be regarded as predictable when it is remembered that the

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muscle fibres are the structures responsible for actively bringing about the contraction of a muscle. More recently some authors have obtained data which they have not been able to explain by assuming only an increase in the toughness of the myofibrillar component and have indicated that muscle shortening is affecting the connective tissue component (Bouton & Harris, 1972; Pfeiffer *et al.*, 1972).

The changes in meat toughness accompanying changes in muscle length have repeatedly been linked directly with changes occurring in the myofibrils of the muscle fibres (see Newbold & Harris, 1972). Indeed it has been stated that, 'the entire relationship can be accounted for in terms of the widely differing degree of overlap of myosin and actin filaments as the muscle length changes. The observed toughening pattern is due solely to the varying extent of cross-linkage formation between these two sets of primary components, and there is no need to invoke any other constituent or change to explain the effect . . . the form of toughness which is induced by early post-mortem chilling or freezing is a function solely of actin-myosin interaction, and connective tissue does no more than change the level of the base-line toughness, on to which the contractile protein effect is superimposed' (Marsh, 1972).

All of these statements have been made and the conclusions reached as a result of testing meat toughness by subjective and/or objective methods. Ideally, any measure of toughness (strength) of a material should be considered in relation to (i) the composition (ii) the structural organization (iii) the physical state (iv) the environmental conditions at the time of measurement and (v) the effects the testing procedure has on the above four points during the course of the test. Because of the lack of data on some of these points, particularly for the connective tissue component, they cannot have been taken into full consideration.

The composition and structural organization of muscle fibres has been well documented (e.g. Cassens, 1970). This could in part account for the emphasis which in recent years has been placed on 'myofibrillar toughness'. On the other hand the composition and structure of intramuscular connective tissue specifically has received relatively little attention. The purpose of the present paper is to report on the work which is being carried out on the structural organization of collagen fibres in intramuscular connective tissue, and to point out how the findings to date must have far reaching implications on the interpretations of the factors influencing meat toughness.

Materials and methods

Bovine and ovine muscles including *semitendinosus*, *semimembranosus*, *longissimus dorsi* and deep pectoral muscles were allowed to go into rigor mortis in various states of contraction. The muscles were then examined in the fresh state and after fixation in buffered formalin (pH 6·5). Only the epimysial and perimysial intramuscular connective tissues were examined and the work reported here deals only with structural organization of the collagen fibres contained within this particular connective tissue.

Initial examination of the intramuscular connective tissue and measurement of the angle between the collagen fibres and the long axis of the muscle fibre was carried out under a dissecting microscope whilst it was still attached to the muscle fibres. The sheets of connective tissue were then isolated by micro-dissection and mounted on glass slides, in some cases following staining using Periodic Acid Foot stain (Lhotka & Myhre, 1953). The isolated connective tissue was then examined at higher magnifications on a Leitz Ortholux microscope. The collagen fibres of the connective tissue were photographed and measurements of crimp length were taken from enlarged prints.

Sarcomere lengths were measured for all samples of muscle (except cold shortened muscle, see page 7). The technique used was that of optical diffraction outlined by Sandow (1936).

Results

The collagen fibres found in the epimysium and perimysium of all samples examined had a remarkably well ordered structural organization. There is a basic pattern common to both the epimysium and the perimysium for all muscles examined, both within a species and between different species of animals. Figure 1 shows a very simplified diagram of the organization within a muscle. The surface of the muscle is covered by the epimysium whereas the perimysium is seen when the muscle is cut open. When it is at right angles to the long axis of the muscle fibres, the perimysium is seen to divide the muscle into fibre bundles. In some instances subdivision of larger bundles into successively smaller bundles can be seen to occur by finer and finer sheets of perimysium (this is not shown in Fig. 1). In Fig. 1 the sheets of connective tissue making up the epimysium and the perimysium are represented as having a criss-cross lattice of collagen fibres. In the diagram the lattice has been drawn very open, whereas *in vivo* the sheets of collagen are almost solid. It is the criss-cross pattern which is common to all the samples examined. Meat toughness assessment invariably involves sub-sampling a large muscle. This usually means that the epimysium is not included in the sample and for this reason, results will be confined to properties of the perimysium even though they apply equally well to the epimysium.

Plate 1 shows a piece of sheep *semitendinosus* perimysium which has been stained by the Periodic Acid Foot method. The criss-cross pattern can readily be seen. In addition each collagen fibre is seen to be crimped. The crimp of collagen fibres from tendon has been commented on by Rigby *et al.* (1959) and studied in some detail by Diamant *et al.* (1972). These latter workers found the crimp length to be of the order of 42 μm in tendon from young rats increasing to approximately 220 μm with increasing animal age. The crimp length in the perimysium collagen fibres examined here was found to be much shorter than that of tendon collagen from the same muscle (e.g. ovine *semimembranosus* perimysium 19 μm, tendon 79 μm). Although the material which has been stained has greater contrast (cf. Plates 1 and 2), the tissue processing which included

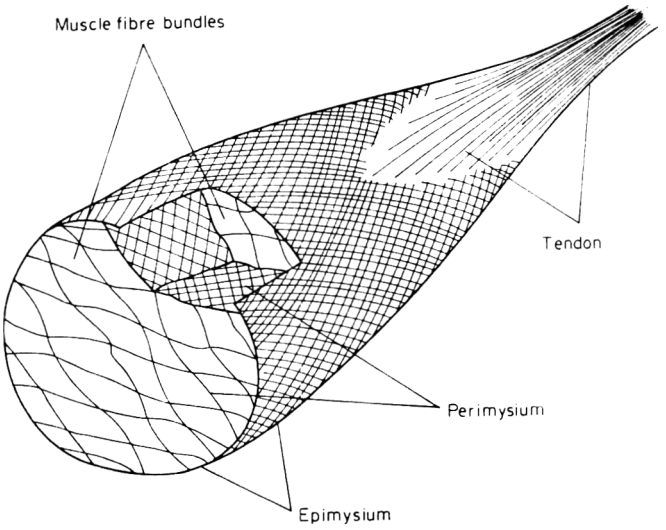

FIG. 1. Diagram of a muscle cut transversely to show the subdivision of the muscle into fibre bundles by the perimysium. The perimysium and epimysium on the surface of the muscle are made of criss-cross networks representing the collagen fibres.

dehydration was considered likely to have led to appreciable shrinkage and possible distortion of the specimen. For this reason work was concentrated mainly on hydrated, unstained tissue. Plate 2 shows unstained sheep *semimembranosus* perimysium. In unstained tissue the criss-cross pattern is more difficult to visualize and does not always show up on the micrographs, although it is still present and can be seen in the microscope by focusing at different levels through the thickness of the perimysium. Note how dense the collagen sheet is, with little space between collagen fibres.

Plate 3a shows unstained sheep *semitendinosus* perimysium in which the criss-cross pattern is visible. The axis of the muscle fibres has been marked on the photomicrograph with a single arrow. Note the angle between the collagen fibres and the muscle fibres. The muscle providing the material shown in Plate 3 was allowed to enter rigor mortis on the carcass which was hung by the Achilles tendon. The muscle from the contralateral side of the same carcass was excised pre-rigor and allowed to enter rigor mortis at 0°C. This resulted in cold shortening (Locker & Hagyard, 1963). The muscle contracted to less than 50% of its excised length. In this case sarcomere lengths could not be determined reliably by the optical diffraction method, presumably because of the great disarray of sarcomeres and variability of sarcomere lengths found in cold shortened muscle (Voyle, 1969). The perimysium from the cold shortened muscle (Plate 4a,b) is strikingly different from its control (Plate 3a,b). There has been a large change in the angle between the collagen fibres and the long axis of the muscle fibres. In addition the crimp has been totally eliminated in one direction of the criss-cross and extensively drawn out in the other direction of the criss-cross (Plate 4b).

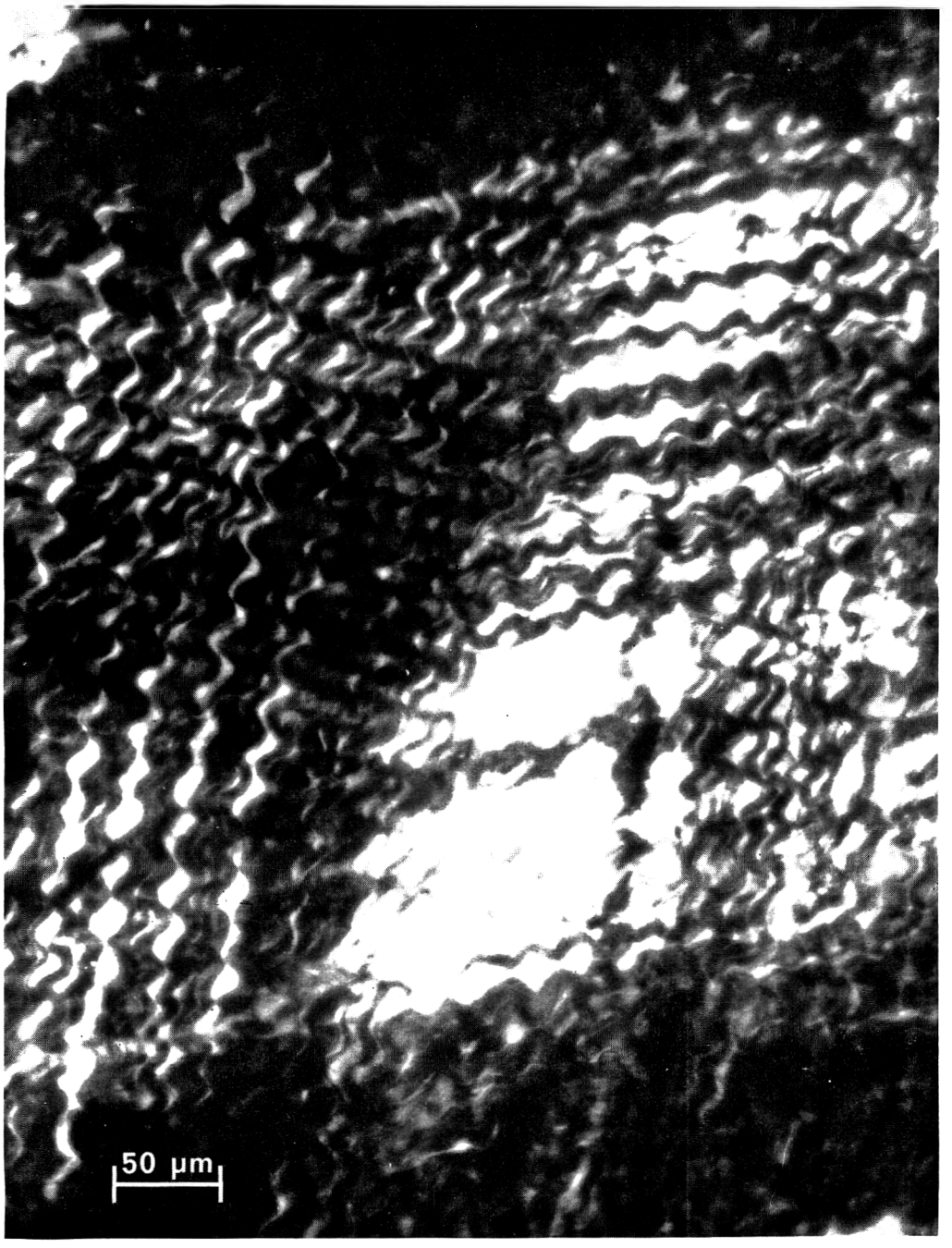

PLATE 1. Formalin fixed ovine *semitendinosus* perimysium stained by the Periodic Acid Foot method and dehydrated. Note the criss-cross lattice of crimped collagen fibres.

(*Facing p.* 504)

PLATE 2. Unstained, hydrated, formalin fixed ovine *semimembranosus* perimysium. In comparison with Plate 1 note that the sheet of collagen fibres does not have extensive spaces.

PLATE 3a

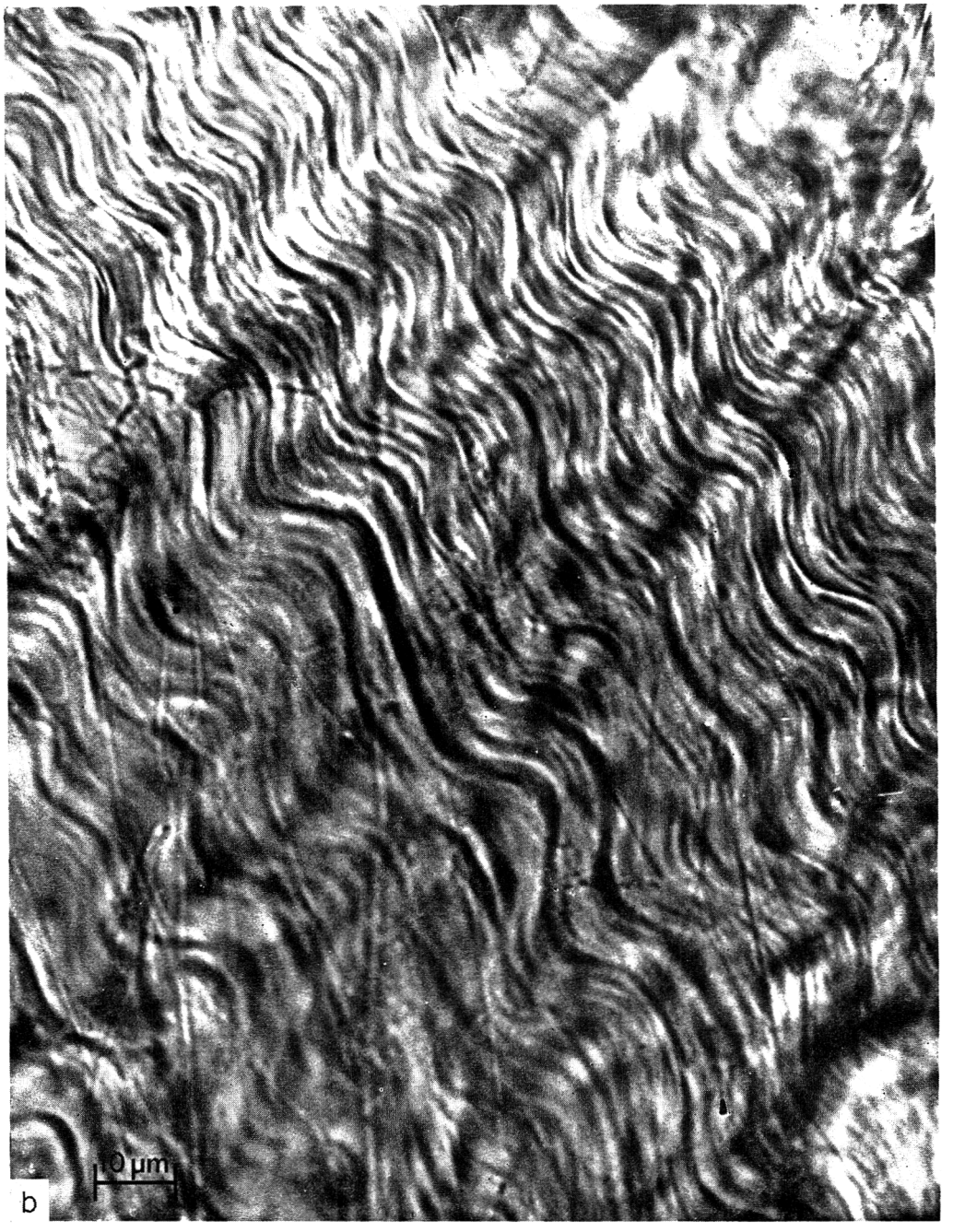

PLATE 3b

PLATE 3. Unstained, hydrated, formalin fixed ovine *semitendinosus* perimysium. (a) Low magnification. The double arrows show the criss-cross lattice of collagen fibres at an angle to the long axis of the muscle fibres (single arrow). Fat cells can be seen at both sides of this figure. They occur at the points where two sheets of perimysium meet. (b) High magnification of part of (a).

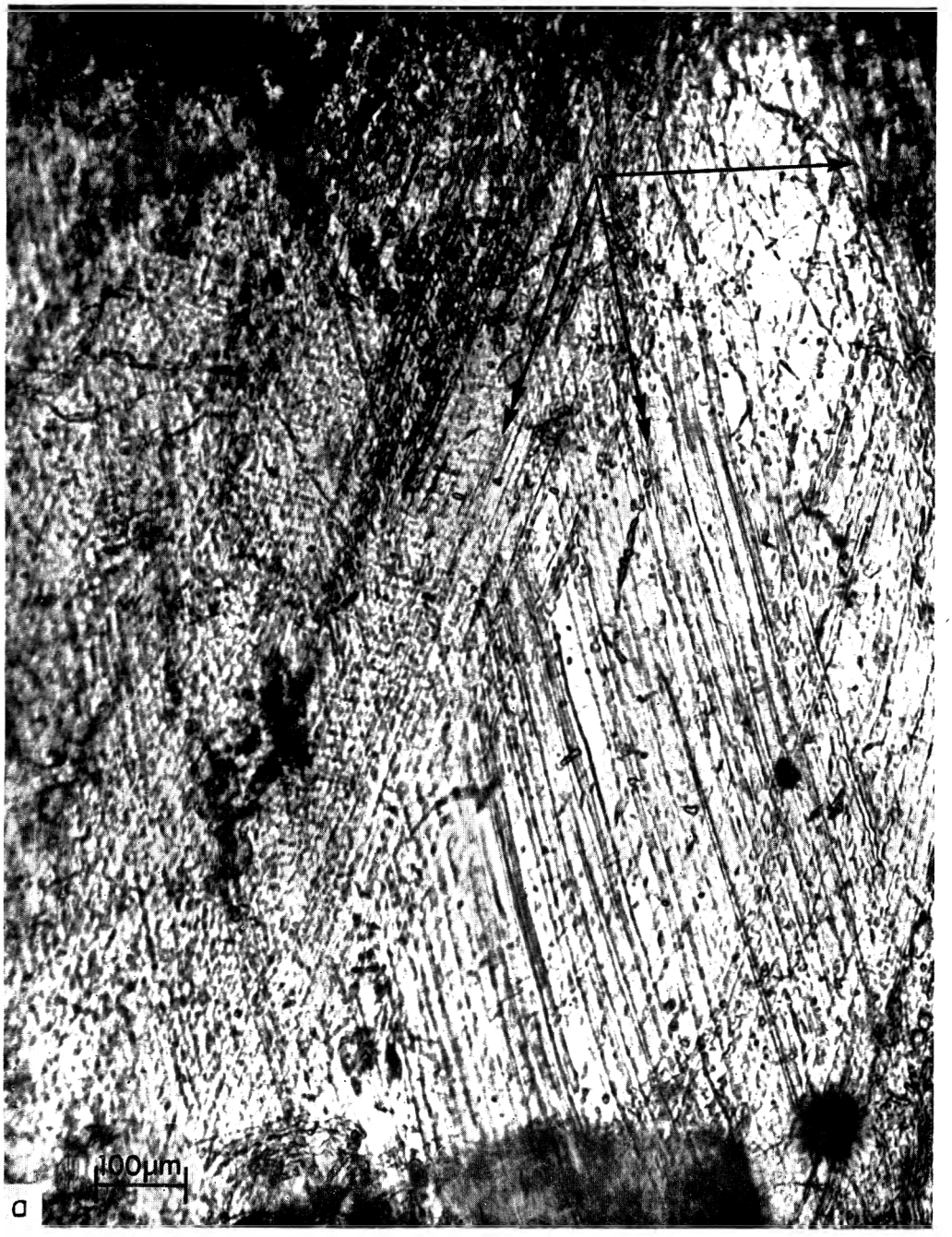

PLATE 4a

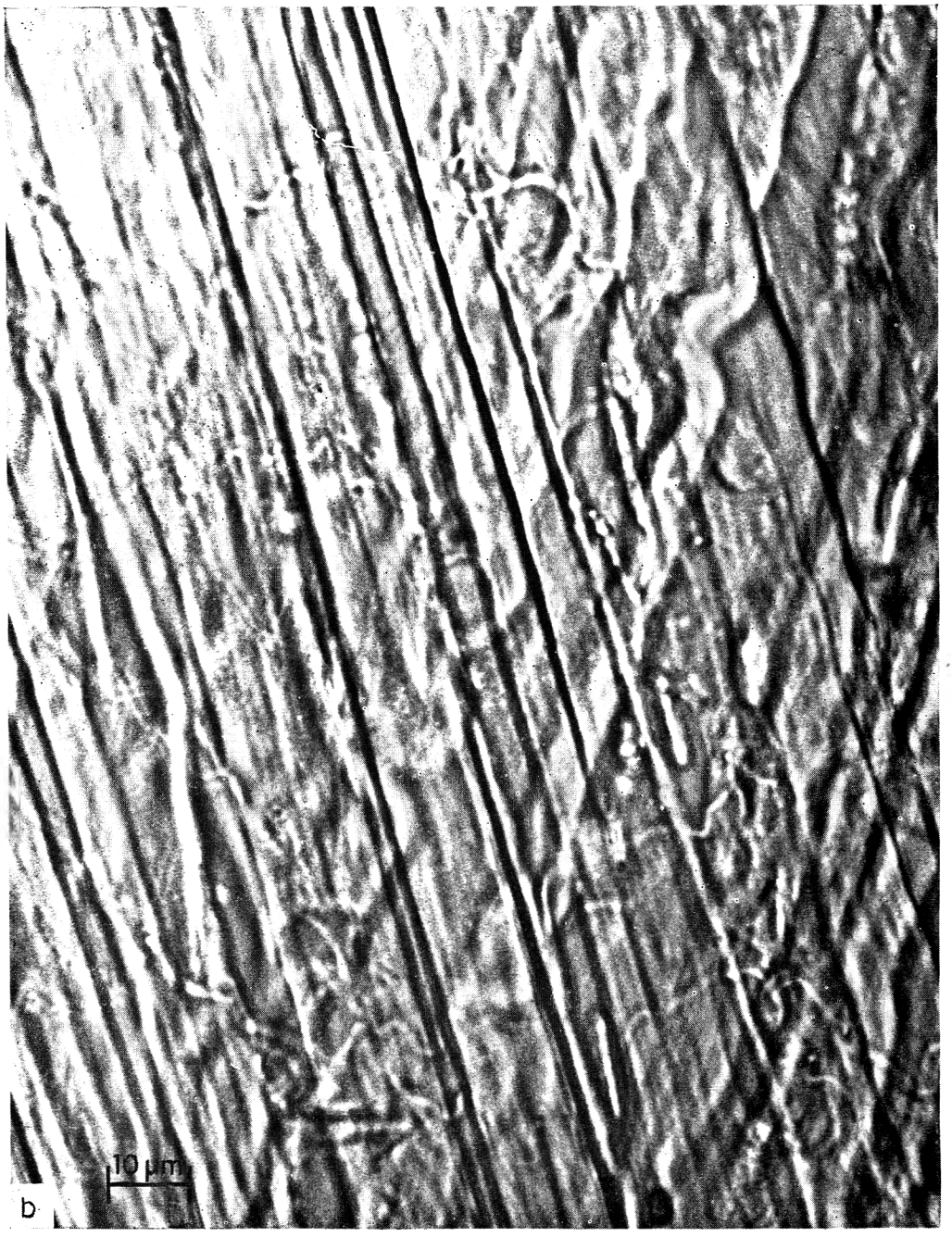

PLATE 4b

PLATE 4. Unstained, hydrated, formalin fixed ovine *semitendinosus* perimysium from the contralateral side of the carcass providing the material in Plate 3. Here the muscle was allowed to cold shorten. (a) Low magnification (arrows as for Plate 3a). Note the difference between the angles of the lattice relative to the muscle fibre long axis in comparison with those of Plate 3a. (b) High magnification of part of (a). Note that the crimp has been straightened out.

Samples of muscles have been examined in various states of contraction and changes in the crimp length and angle between collagen and muscle fibres examined (see Table 1). In order to negate any possible animal age differences, paired muscles from the same animal are compared. There are two changes taking place in the collagen networks with muscle shortening. Firstly the crimp length changes, i.e. it appears that the collagen fibres are pulled out and lengthened at the expense of the crimp. Secondly, the angles of collagen fibres relative to the long axis of the muscle fibre change with contraction.

TABLE 1. Sarcomere length, collagen lattice angle and crimp length data for bovine and ovine muscle

Muscle	Sarcomere length ($\mu m \pm$ s.e.)	Angle between muscle fibres and collagen fibres	Crimp length ($\mu m \pm$ s.e.)
Semitendinosus (O)	$3{\cdot}08 \pm 0{\cdot}01$	50°	$27{\cdot}1 \pm 1{\cdot}7$
Semitendinosus (O)	$2{\cdot}97 \pm 0{\cdot}04$	51°	$31{\cdot}9 \pm 1{\cdot}5$
Semitendinosus (O)	$2{\cdot}78 \pm 0{\cdot}03$	45°	$16{\cdot}3 \pm 0{\cdot}6$
Semitendinosus (O)	$1{\cdot}86 \pm 0{\cdot}02$	55°	$33{\cdot}4 \pm 0{\cdot}4$
Semitendinosus (O)	$2{\cdot}85 \pm 0{\cdot}01$	50°	$22{\cdot}9 \pm 0{\cdot}7$
Semitendinosus (O)	$< 1{\cdot}0$	75°	$\times\times\times$
Semimembranosus (O)	$2{\cdot}04 \pm 0{\cdot}04$	60°	$28{\cdot}7 \pm 0{\cdot}8$
Semitendinosus (B)	$2{\cdot}41 \pm 0{\cdot}04$	52°	—
Deep pectoral (B)	$2{\cdot}62 \pm 0{\cdot}04$	50°	$31{\cdot}2 \pm 0{\cdot}8$

Paired muscles are from the same animal.
$\times\times\times$ See Fig. 5 and the text for the effect of cold shortening on crimp length.
(O), Ovine.
(B), Bovine.

Discussion

To date muscle contraction has only been shown to change the structure of the muscle fibre component. The lateral expansion accompanying shortening has long been recognized as changing the fibre packing density with a concomitant change in the area of connective tissue per unit cross-sectional area; however, no change in the actual structure of the connective tissue component has been envisaged at the perimysium level. A consideration of the well ordered changes reported here which occur in the collagen network of the perimysium, in relation to meat toughness assessment makes it obvious that some of the explanations for changes in toughness put forward previously (see Introduction), need to be reconsidered.

If we consider Fig. $2A_1$ and B_1 as diagrammatically representing a muscle in two

cross-sectional area. Figure $2A_2$ and B_2 show one sheet of the perimysium of the two muscles exposed with the criss-cross pattern of the collagen fibres visible. In the contracted muscle B_2 the angle between the long axis of the muscle fibre and the collagen fibres has increased. Samples of meat tested for meat toughness are cut to a uniform different states of contraction, both have the same volume, i.e. the contracted muscle B_1 has expanded transversely and therefore the fibre bundles (top surface) are larger in

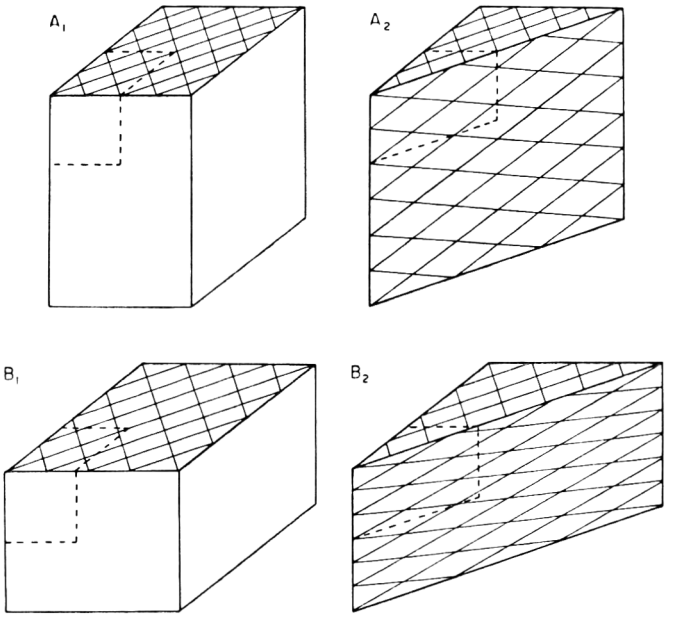

FIG. 2. Diagram of a muscle in two states of contraction. The top surface of A_1 and B_1 shows the outlines of the muscle fibre bundles i.e. perimysium. In A_2 and B_2 part of the muscle has been cut away to expose one sheet of perimysium with its criss-cross lattice of collagen fibres. The dotted lines represent uniform size of samples of the two muscles A_1 and B_1. See text for full explanation.

size regardless of the state of contraction, they have fixed areas and according to Davey & Gilbert (1969) should have fixed lengths. The broken lines in Fig $2A_1$, A_2 and B_1, B_2 represent outlines of samples cut from muscles such as A_1 and B_1. Here the samples have the same cross-sectional area and the same length.

Consider firstly the collagen components contributing to the strength of the sample in the direction parallel to the long axis of the muscle fibres. In the sample from A_1 there are the outlines of eight complete fibre bundles in transverse section (top surface), whereas there are less than eight in the sample from B_1. For example let us assume there to be three collagen fibres to each side of the square of the perimysium surrounding one fibre bundle. Therefore in the sample from A_1 there are a total of forty-eight collagen

fibres in the transverse plane of the sample, whereas in the sample from B_1 there are less (approximately thirty-four). Let each collagen fibre have the same tensile strength along its long axis. Then as the angle between the direction of applied force (in this case parallel to the long axis of the muscle fibres) and the long axis of the collagen fibres increases, the force, which has to be applied to bring the component of such a force in the direction of the collagen long axis up to the breaking strain of the collagen fibre, also becomes greater.

If we now consider the collagen components contributing to the strength in the direction at right angles to the muscle fibre long axis, again there are important changes. From Fig. 2 it can be seen that in the sample from A_1, the less contracted muscle, four sheets of perimysium insert on the side surface of the sample (i.e. four of the outlines of the perimysium on the top surface meet the side of the sample). In Fig. $2A_2$ one sheet of perimysium is shown with the collagen fibre lattice represented. From this figure it can be seen that four collagen fibres are included in the sample for one sheet of perimysium. Therefore the sample from A_1 has a total of sixteen collagen fibres inserting on the side surface. From Fig. $2B_1$ and B_2 it can be seen that there are three sheets of six collagen fibres, i.e. a total of eighteen collagen fibres in the more contracted muscle sample. In this case however, the angle between the collagen fibres and the direction of applied force decreases with contraction.

The overall change in the strength of the collagen system, either in line with the muscle fibre long axis or at right angles to this is therefore dependent on the relative change in both collagen fibre number and the collagen fibre angle. The overall collagen toughness of the sample will then be some permutation of the strength in these two directions.

Even a brief consideration of just the collagen system of the perimysium, as outlined above, makes it obvious that the role of intramuscular connective tissue as providing merely 'background toughness' which changes very little with contraction is no longer tenable. In addition, there are factors which have not been taken into account such as changes in the crimp, or other potentially important components of the connective tissue, e.g. elastin, reticulin, mucopolysaccharides etc. Marsh & Leet (1966) and Davey, Kuttel & Gilbert (1967) reported a gradual increase in meat toughness with contraction (up to 40% shortening) and in cases of extreme contraction (in excess of 40% shortening) there was a decline in toughness. In material examined here it is of interest to note that with the shortening of the muscle, the crimp of the collagen fibres was gradually pulled out and that in extremely shortened muscle samples it was completely eliminated in one direction of the criss-cross. It has been shown in the case of tendon collagen that the crimp can be straightened by stretching the collagen fibres (Rigby *et al.*, 1959). This effect is reversible so long as the stretch is not excessive. In addition Rigby (1964) showed that mechanical extension of collagen fibres decreased their thermal stability and that the greatest effect occurred after the crimp had been eliminated.

Further work is being carried out in order to follow more closely the changes occurring in the intramuscular connective tissue with muscle contraction.

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The ante-rigor excision and air cooling of beef semimembranosus muscles at temperatures between −5°C and +15°C

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Summary

Excision of the M.semimembranosus early post mortem substantially reduced the temperature gradient throughout the whole muscle during subsequent cooling. This resulted in a retardation of the biochemical changes associated with rigor mortis, with the following effects on the quality of the meat; a substantial reduction (30–90%) of the loose drip in the vacuum packed meat with cooling between +5°C and +15°C, minimal discolouration, a marginal saving in the drip loss from the retail joints, an improvement in tenderness with cooling at +5°C to +15°C, and progressively increased bacterial counts at the surface and in the loose drip as the cooling temperature was raised. Bacterial contamination of the interior of the meat was only marginally increased at the higher cooling temperatures.

Introduction

The low temperatures currently employed commercially for cooling beef sides are necessary to attain rates of cooling in the deep musculature that eliminate the once familiar bone taint in the region of the Aitch Bone (Nottingham, 1960), and equally important commercially, effects a substantial reduction in drip loss from the meat after retail prepacking (Disney, Follett & Ratcliff, 1967; Taylor & Dant, 1971). With the advent of vacuum packed deboned primal cuts of beef, the problems of drip and discolouration of the meat in the evacuated packs can all too often lead to gross economic losses as well as difficulties in handling. As much as 7% of the weight of a vacuum packed cut of beef can be present as loose drip after 7 days' chill storage.

Experiments carried out by the present authors on the excision and vacuum packing of semimembranosus muscles prior to the onset of rigor have produced encouraging results in both drip and overall meat quality, providing muscles are removed from the sides within 1 hr of death (Follett, unpublished data). In these investigations the air cooling temperature was maintained at 0 ± 1°C, in line with present commercial practice of hanging beef sides.

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The tenderness of these muscles was not appreciably different from that of muscles cooled in the intact sides for 36 hr prior to butchery and vacuum packing.

Shortening within a muscle during the initial cooling can affect the ultimate tenderness. Locker & Hagyard (1963) showed that minimum shortening for excised muscle strips occurred in the range 14°C–19°C and Marsh & Leet (1966) have subsequently demonstrated the relationship between shortening and tenderness.

Other workers (Busch, Goll & Parrish, 1967) have shown that holding excised muscles at +15·5°C for 2 days produced meat which was more tender than that from excised muscles held at 2°C for 16 days.

The present investigation was designed to study the effects of cooling early excised, vacuum packed semimembranosus muscles in air between −5°C and +15°C for the initial 12–24 hr after death, followed by storage at 0°C for a further 12 days. The advantages and disadvantages of the different cooling temperatures with respect to ultimate meat quality are discussed in the light of similar data obtained from corresponding muscles cooled and matured in the conventional way.

Materials and methods

Animals and slaughter procedures

Twenty-four North Devon steers aged 2–3 years were stunned, exsanguinated and dressed by conventional methods. Dressed carcass weights were within the range 580–610 lb.

Excision of muscles

(*i*) *Ante-rigor excised* (*AE*) *muscles.* Semimembranosus muscles were excised from twenty-four left sides at 1 hr* and placed in large plastic (nylon-polythene laminated) bags. Half of the bags were evacuated and heat-sealed whilst the remainder were left unsealed to permit removal of samples for biochemical analysis, tenderness assessment and oxygen uptake at various times throughout cooling and storage.

(*ii*) *Post-rigor excised* (*PE*) *muscles.* Semimembranosus muscles from the right sides of the same carcasses were excised at 36 hr. Half of these muscles (corresponding to the vacuum-packed AE muscles), were vacuum packed immediately after removal, and the remainder stored in unsealed bags for oxygen uptake measurement, and tenderness assessment during maturation.

Cooling procedures

The AE muscles were divided equally into four groups, each group comprising three vacuum-packed and three unsealed bagged muscles. The six muscles of any one group were cooled in air for 24 hr (with the exception of group IV muscles which were cooled at −5°C for only 12 hr) and then stored at 0 ± 1°C for up to 12 days. Air cooling

* Times recorded in this paper refer to the period from death of the animals.

temperatures used were: Group I, +15°C; Group II, +10°C; Group III, +5°C; Group IV, −5°C.

The corresponding PE muscles were cooled in the intact sides for 35 hr in a chiller room at 2–3°C and then stored alongside the AE muscles.

Sampling procedures

Samples for biochemical analyses were removed from the exposed internal surface of the three unsealed bagged AE muscles of each group, and from a corresponding (8 cm) depth in the PE muscles. Sampling times were at 1, 3, 5, 8, 12, 24 and 36 hr. These samples were immediately deep frozen in liquid nitrogen and stored below −30°C until required. Additional samples were removed from both AE and PE muscles at 36 hr, stored in vacuum-sealed plastic pouches at 0 ± 1°C and later used for biochemical analyses and determinations of oxygen uptake.

Temperature recordings

Temperatures were recorded between 1 and 36 hr using a Grant multipoint instrument. In the PE muscles, temperatures were taken 2 cm and 8 cm below the external surface, and in the AE muscles at 2 cm and 5 cm (corresponding to the centre of the muscle) below the exposed internal surface.

Objective tenderness measurement and cooking loss

Tenderness was assessed at 3, 7 and 13 days on the non-vacuum packed AE and PE muscles (three of each per group) by the following method.

A sample 3·5 × 3·5 × 7·0 cm was cut from each muscle with the fibre direction parallel to the longest side. These samples were placed in small polythene bags which were evacuated and then immersed in a bath of water at 15°C. The water temperature was raised to 80°C within 1¼ hr to bring the centre of the meat to 75°C. The cooked meat sample was then brought to room temperature and the outer surfaces trimmed to give a strip 1·0 × 2·0 × 5·0 cm. Four shear force measurements were carried out on this strip, using an Instron Universal Tester equipped with a Wolodkewitsch jaw (2 cm width), the latter being perpendicular to the fibre direction.

Results, as a mean of three samples (four readings per sample), have been expressed as a percentage increase or decrease in tenderness relative to that observed in the corresponding PE muscles.

The exudate in the polythene bag after cooking was measured volumetrically and expressed as a percentage of the original weight of meat (v/w).

Drip loss

Drip loss from the vacuum packed muscles was measured volumetrically after 7 days. Each muscle was then cut parallel to the fibre direction into three portions and these

were trussed and cut into retail joints (10–12 per muscle). The joints were weighed, wrapped in oxygen permeable film and stored at 0 ± 1°C for 48 hr. Mean drip loss was calculated from the total weight difference of all the joints before and after storage.

Results for each group of AE muscles have been expressed as a percentage increase or decrease compared with values from the corresponding PE muscles.

pH measurement

Determinations of pH were carried out on 1 g samples of frozen meat (Disney *et al.*, 1967). Sampling times were those used for biochemical analyses.

Sarcomere length determination

The sarcomere lengths in individual fibres were determined after 3 and 7 days by phase contrast and photographic techniques on uncooked samples of muscle (Herring, Cassens & Briskey, 1965).

Biochemical analyses

One gram of frozen sample was analysed for ATP*, glucose, G-6-P, lactate and NAD by enzymatic methods (Follett & Ratcliff, 1969). Results for the samples removed between 1 and 36 hr (mean of three muscles in each AE group and the mean of twelve PE muscles) are expressed as a percentage of (i) the highest observed value (lactate and NAD), (ii) the initial value recorded (ATP and glucose) or (iii) as μmol/g wet tissue (G-6-P, and the initial and maximum concentrations of all metabolites). Concentrations recorded at 3 and 7 days have been expressed as μmol/g wet tissue.

Oxygen uptake

The unfrozen, vacuum-sealed samples from each group were used to determine the oxygen uptake at 7 days (Atkinson, Follett & Ratcliff, 1969). Results, taking the mean of three AE muscles in each group, have been presented as the percentage increase in uptake compared with the mean value of the corresponding three PE muscles.

Bacteriological examination

All vacuum-packed muscles were examined at the surface, within the deep musculature, and in the loose drip at 7 days. Bacterial counts have been expressed as the $\log_{10}$ colony count per g or ml, after incubation at 22°C or 37°C for 48 hr.

Results

Temperature recordings

Temperatures in the AE and PE muscles are shown in Fig. 1a–c. The initial temperature in the deep musculature of the PE muscles was 41·5°C, and 8°C lower at 2 cm

* The following abbreviations have been used throughout: ATP = adenosine triphosphate; G-6-P = glucose 6 phosphate; NAD = nicotinamide adenine dinucleotide (oxidized form).

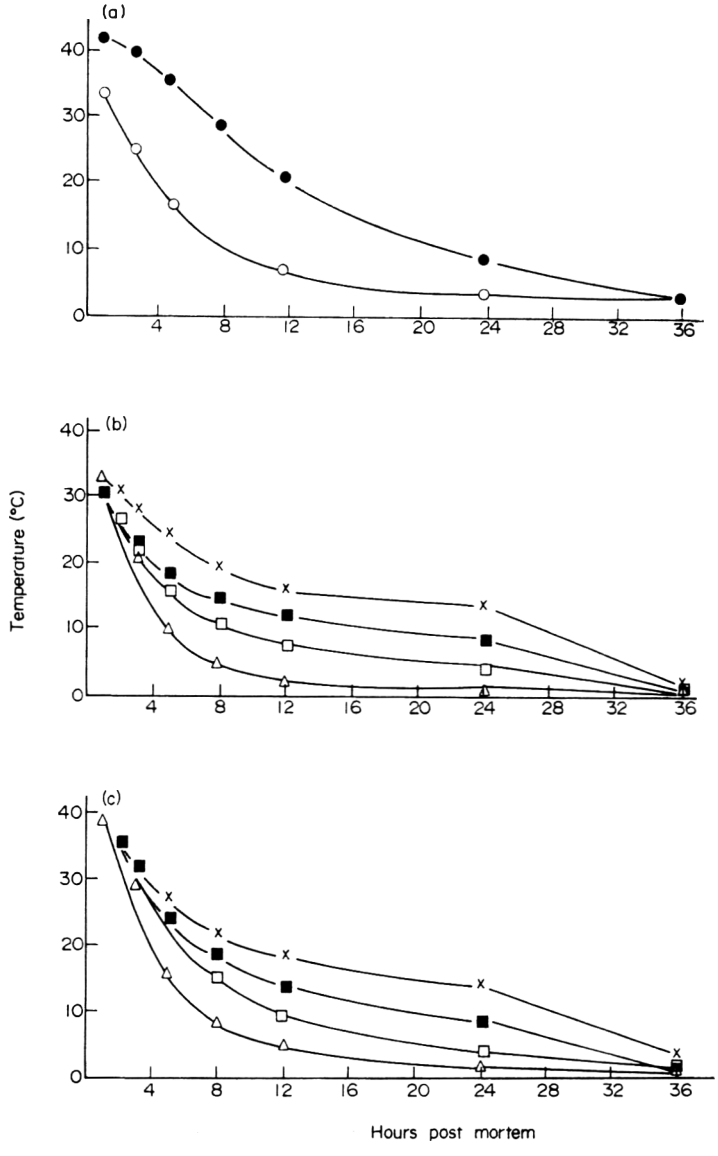

FIG. 1. Temperatures recorded in semimembranosus muscle of beef (a) in the PE muscles at 8 cm (●) and 2 cm (○), (b) and (c) in the AE muscles at 2 cm and 5 cm depths respectively. Group I (×); Group II (■); Group III (□); Group IV (△).

depth. Times required for equilibration to 2–3°C in the deep and shallow muscles were 35 and 23 hr respectively. The effects of the excision procedure rapidly became apparent; the cooling rate in the anatomically deep tissue (read at 2 cm depth) now equalled that observed in the anatomically superficial tissue (also read at 2 cm depth).

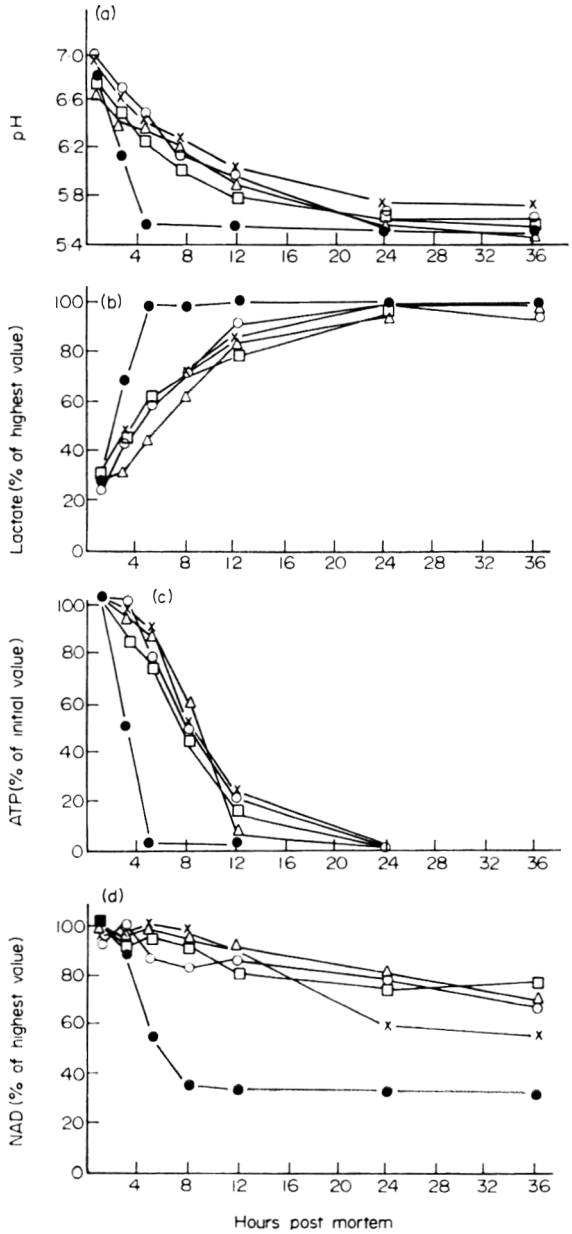

FIG. 2. (a) pH (b) lactate (c) ATP and (d) NAD for semimembranosus muscles of beef between 1 and 36 hr post mortem. PE muscles (●); AE muscles, Group I (×); Group II (○); Group III (□) and Group IV (△).

Values of pH and lactate levels recorded during the initial 36 hr in the AE and PE muscles are depicted in Fig. 2a and b respectively.

Initial pH values of the PE muscles were within the range 6·80 ± 0·13. The fall in pH was linear and the value of 5·54 observed at 5 hr was subsequently maintained. The initial pH in the AE muscles was similar (6·80 ± 0·16) but then fell more slowly. The spread in pH values recorded at 36 hr (5·60 ± 0·12) appeared to be directly related to the cooling temperatures used.

The rate of lactate accumulation in the AE muscles was appreciably slower than in the PE muscles where lactate had reached a maximum concentration by 5 hr. A high correlation ($r = +0{\cdot}926$ $P < 0{\cdot}001$) was observed between pH and lactate concentration and a buffering capacity of 52 μmol lactate/g/pH was recorded which agrees closely with the value previously reported for beef muscle (Newbold & Scopes, 1967).

pH values and lactate concentrations at 3 and 7 days are shown in Table 1 with the corresponding standard deviations.

TABLE 1. Observed values of pH, lactate, glucose and NAD in the AE and PE muscles at 3 and 7 days

	Time (days)	PE muscles	AE muscles			
			Group I	Group II	Group III	Group IV
pH	3	5·57 (0·22)	5·73 (0·08)	5·75 (0·07)	5·48 (0·03)	5·43 (0·15)
	7	5·53 (0·20)	5·71 (0·10)	5·71 (0·12)	5·46 (0·01)	5·34 (0·04)
Lactate (μmol/g)	3	84·7 (9·7)	78·9 (3·6)	92·6 (8·4)	81·8 (2·0)	82·0 (6·2)
	7	78·9 (7·7)	71·4 (2·5)	88·8 (11·9)	77·6 (2·6)	78·0 (8·6)
Glucose (μmol/g)	3	8·5 (1·9)	10·1 (0·9)	6·6 (2·4)	—	9·3 (2·7)
	7	8·3 (1·7)	9·6 (1·4)	8·2 (3·2)	—	13·2 (4·9)
NAD (μmol/g)	3	0·23 (0·08)	0·30 (0·12)	0·32 (0·12)	0·29 (0·08)	0·45 (0·05)
	7	0·19 (0·07)	0·21 (0·02)	0·23 (0·03)	0 21 (0·05)	0·28 (0·06)

Standard deviations are in parenthesis.

ATP

Initial concentrations of ATP in the PE muscles were in the range 4·82 ± 0·61 μmol/g. These levels were rapidly depleted, negligible ATP remaining at 5 hr. At this time, however, in the AE muscles, approximately 70% of the initial levels (4·81 ± 0·35 μmol/g) were still present (see Fig. 2c). In all groups of AE muscles ATP was completely degraded by 24 hr.

Glucose

Initial concentrations of glucose in both AE and PE muscles were within the range

22·2 ± 6·2 μmol/g. Plateau levels were reached at 5 hr in the PE muscles (8·7 μmol/g) and at 24 hr (8·9 μmol/g) in the AE muscles.

NAD and G-6-P

Mean NAD levels are shown in Fig. 2d. Initial concentrations in the PE muscles amounted to 0·78 ± 0·09 μmol/g and by 8 hr a plateau level equivalent to 35% of the initial values was reached. Degradation in the AE muscles was appreciably slower. In Group I, 54% of the mean initial concentration (0·74 ± 0·07 μmol/g) was present at 36 hr against 66%–75% in Groups II–IV. Further depletion was observed in these muscles up to 7 days (Table 1), when the mean ultimate concentrations amounted to 0·19 μmol/g and 0·23 μmol/g respectively.

G-6-P concentrations recorded in the AE and PE muscles between 1 hr and 7 days are shown in Table 2. In all muscles the initial levels (3–4 μmol/g) subsequently increased two- to three-fold, the rate of increase being most rapid in the PE muscles.

TABLE 2. Concentrations of G-6-P in the AE and PE muscles between 1 hr and 7 days

Time	Mean G-6-P concentration (μmol/g)				
	PE muscles	AE muscles			
		Group I	Group II	Group III	Group IV
1 hr	4·1	3·9	3·9	4·5	2·9
3 hr	4·3	3·0	3·6	4·1	4·1
5 hr	8·7	4·5	4·1	6·7	4·6
8 hr	10·1	6·3	7·5	7·7	4·8
12 hr	10·1	7·4	8·7	8·1	9·8
24 hr	10·2	9·9	11·4	10·4	7·8
36 hr	10·0	10·0	10·0	12·2	7·0
3 days	10·7	10·9	11·1	11·8	8·2
7 days	9·2	9·3	9·8	10·5	6·9

Drip loss

(*i*) *From the vacuum bags.* The maximum mean decrease in drip loss, 85%, was obtained in Group II, whereas in Group IV a mean increase of 37% was observed (see Fig. 3).

(*ii*) *From the consumer packs.* A maximum mean *reduction* in drip (25%) occurred in the Group III retail joints (Fig. 3) whereas a mean *increase* in drip (19%) was observed in those from Group I (cooled at +15°C). Joints prepared from the muscles of Groups II and IV showed only marginal reductions.

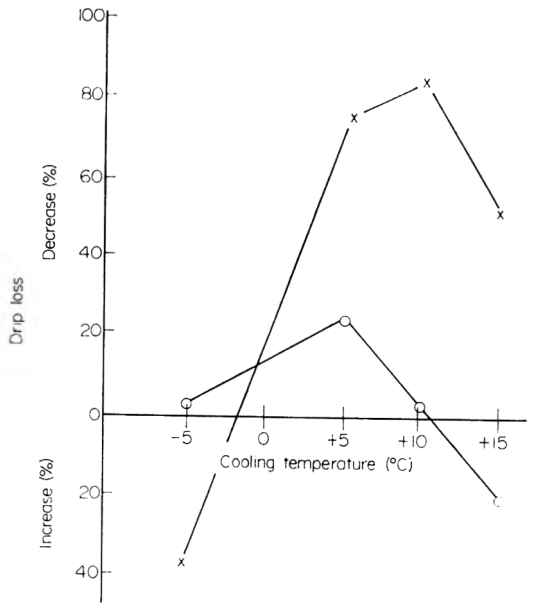

FIG. 3. Mean percentage increase or decrease in loose drip from (i) the vacuum packs (×) and (ii) the retail joints (○) of the AE muscles compared with the PE muscles (represented by zero percentage change).

TABLE 3. Mean cooking losses for the AE and PE muscles at 3, 7 and 13 days

Time (days)	Mean cooking loss (% of original weight of meat)							
	Group I		Group II		Group III		Group IV	
	AE	PE	AE	PE	AE	PE	AE	PE
3	23·9	24·2	19·8	19·0	NT	NT	26·9	28·4
7	24·4	25·4	21·2	20·6	28·2	26·3	34·9	35·0
13	27·0	28·0	19·8	20·0	27·9	26·5	24·9	27·3

Cooking losses and objectively measured tenderness

Differences in mean cooking loss between the AE and PE muscles were small (Table 3).

With the exception of Group IV, the AE muscles were more tender, in terms of shear values determined at 3–13 days, than the PE muscles (Fig. 4).

Sarcomere lengths

Table 4 shows the mean values for each group of muscles. Mean sarcomere length of the PE muscles at 3 days was 2·02 (s.d. 0·08) μm increasing to 2·13 (s.d. 0·07) μm by

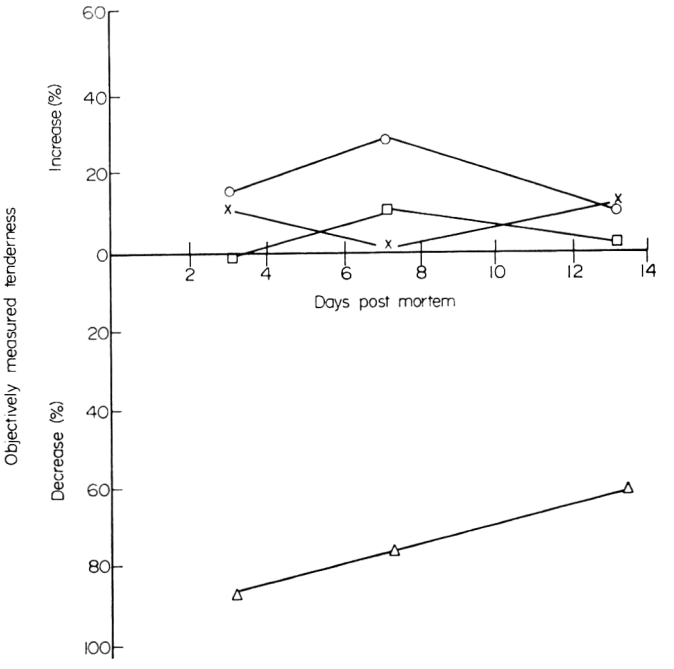

FIG. 4. Mean percentage increase or decrease in objectively measured tenderness for the AE muscles between 3 and 13 days compared with the corresponding PE muscles (represented by zero percentage change). Group 1 (×), Group 2 (○), Group 3 (□), Group 4 (△).

7 days. AE muscles cooled at +5°C had the longest sarcomeres (averaging 2·43 μm at 7 days) whereas those of Group IV were shortest (averaging 2·09 μm at 7 days).

Bacteriological assessment

Mean counts of colonies after incubation at 22°C and 37°C are presented in Fig. 5a and b respectively. Mean counts at 22°C were higher for the AE muscles of Groups I–III than for the corresponding PE muscles. A progressive rise was observed as the cooling temperature was raised, the mean count for Group I being 100-fold greater than that of the PE muscles. Mean counts at 37°C showed growth patterns related similarly to cooling temperature, although differences between AE and PE muscles were smaller.

Oxygen uptake

The oxygen uptake of the AE muscles of Groups I–IV at 7 days is depicted in Table 5. The mean percentage increase observed in Group IV was approximately four fold that recorded in Groups I–III.

Discussion

The wide differences in rate of temperature fall observed at 2 cm and 8 cm depth in the PE muscles exemplify the broad range of cooling rates experienced in the M.semi-

TABLE 4. Mean sarcomere lengths recorded in the AE and PE muscles at 3 and 7 days

Muscle group		Mean sarcomere length (μm)	
		3 days	7 days
I	AE	2·14	2·25
	PE	2·07	2·09
II	AE	2·03	2·23
	PE	2·05	2·05
III	AE	2·33	2·43
	PE	1·90	2·19
IV	AE	1·87	2·09
	PE	2·07	2·17

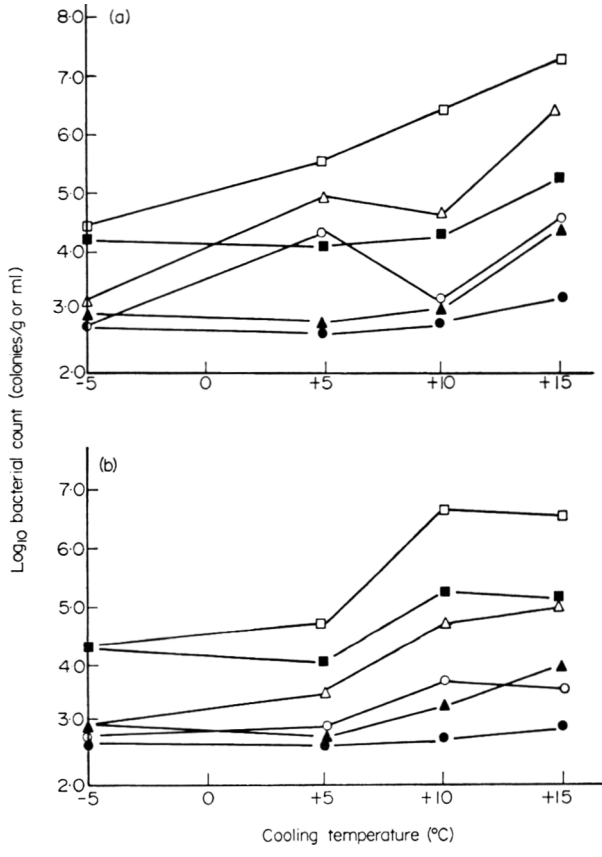

FIG. 5. Bacterial counts as $\log_{10}$ (a) 22°C and (b) at 37°C for AE (oper. symbols) and PE (solid symbols) muscles. (□) Drip; (△) exterior of the meat; (○) interior of the meat.

TABLE 5. Mean percentage increase in oxygen uptake for the AE muscles at 7 days

Group	Cooling temp. °C	Increase in uptake at 7 days (%)
I	+15	24
II	+10	22
III	+5	18
IV	−5	80

membranosus during normal cooling and rigor. Early excision reduced these temperature differences and produced meat of a more consistent quality. Excision reduced the initial temperature of the muscles, measured at 2 cm below the exposed internal face, by approximately 7°C, and equilibration with the cooling air was complete within 18–24 hr. Thus the early excision procedure had the dual effect of lowering the temperature in the deep muscle region at the commencement of cooling, and increasing the overall cooling rate.

Subjecting beef muscles excised prior to rigor to temperatures below 14°C induces toughening of the meat due to the cold contraction phenomenon (Locker & Hagyard 1963; Marsh & Leet, 1966), which cannot be completely resolved by normal maturation (Davey, Kuttel & Gilbert, 1967). These observations were based upon experiments in which small portions of muscle were excised and held at controlled air temperatures between 0°C and 37°C. Under these circumstances temperature equilibration between the meat and the surrounding environment can be very rapid. However, there are very few, if any, regions in a beef carcass where such a rapid rate of cooling can occur. Nevertheless, in commercial practice, temperatures below 14°C may be attained in some regions of the carcass before rigor is complete. In these regions tension is developed within the muscles which are restrained by ligament or bone structure, nodes of contraction occur, and a prolonged maturation period is required for tenderization (Jungk *et al.*, 1967; Busch, Goll & Parrish, 1972). An everyday example of this tension development is seen when beef sides are prematurely quartered and 'shrink-back' is observed on the cut surfaces of the rib eye meat. No such tensions can occur in the excised muscles since they are not held under restraint, but should these muscles attain a temperature where cold contraction can occur, then toughness would undoubtedly ensue.

Cold contraction can only occur in excised, entire muscles under rapid conditions of cooling, such as in the AE muscles of Group IV, where the mean tenderness was adversely affected. However, cooling the excised semimembranosus muscles in air at +5°C, and above, had no detrimental effect on ultimate tenderness. Nevertheless it may be speculated that some other muscles (e.g. in the leg region) which are of relatively

small size and large surface area, may undergo a degree of cold contraction in normal commercial cooling in air at +2·5°C, and are therefore toughened. Early excision of the leg region followed by cooling at the higher temperature +5°C, or above, should result in *slower* rates of cooling and a possible increase in tenderness.

Rates of biochemical change were rapid in the deep layers of the PE muscles, near ultimate levels of all the biochemical intermediates examined being attained within 5–8 hr of death. Rigor was therefore complete within a few hours of death (Bendall, 1960) while the meat temperature was still high (>35°C). Such meat will exhibit poor water binding properties (Disney *et al.*, 1967; Hamm, 1960) and low pigment reducing capacity (Atkinson & Follett, 1973) compared with meat which has been cooled to below 20°C before lactate has accumulated appreciably.

In contrast, biochemical changes in the excised muscles were markedly retarded by the initial faster cooling rates, and a pronounced effect upon ultimate tenderness and drip loss was observed as anticipated (Bendall, 1960; Lawrie, 1966). NAD was maintained at a high concentration throughout the initial 36 hr, and only after 7 days was the level similar to that observed in the PE muscles at 5 hr. Higher concentrations of NAD greatly influence the stability of the reduced form of the meat pigment after exposure to air and prior to vacuum packing (unpublished data). This was borne out visually by the absence of surface discolouration on the excised muscles after 7 days' maturation in vacuum bags, whereas the PE muscles showed large patches of discolouration on the exposed internal faces.

G-6-P degradation and accumulation in the AE and PE muscles was similar. It has been suggested by Schmidt, Zuidam & Sybesma (1971) that high concentrations in pig biopsy samples are a good indicator of the PSE syndrome and it has also been shown that concentrations of this phosphate in postrigor porcine muscle are negatively correlated with ultimate pH, and positively correlated with amounts of drip (Hatton *et al.*, 1972). Scopes (1971) reported that high levels of G-6-P in post-mortem tissue at pH 6·3 characterize PSE meat. It is not clear from the present observations what part, if any, G-6-P plays in the formation of drip in bovine muscle, apart from its role as an intermediate in lactate production, or as a contributor to the lowering of the muscle pH by dissociation of the phosphate group (Scopes, 1971; Hatton *et al.*, 1972).

Drip loss in the vacuum-packed AE muscles was influenced by the different cooling conditions, maximum decreases being observed in Groups II and III. In contrast, cooling the excised muscles at −5°C (Group IV) enhanced the loose drip. Volumes of drip from the excised muscles, cooled at +5°C and +10°C ranged between 2 and 5 ml compared with 20–25 ml normally recorded in topside muscles (weighing 15–20 lb) vacuum-packed under commercial conditions (unpublished data).

Drip loss from the consumer packs was also affected by the cooling conditions. A mean decrease in drip of 20% was observed in the AE muscles of Group III. Cooling at +15°C (Group I) enhanced the drip loss by 37%, indicating that conditions were produced during cooling which encouraged the development of watery tissue. The

apparent advantage in tenderness obtained by holding excised muscles at 15·5°C, (Busch *et al.*, 1967) or beef sides at +15°C (Smith, Arango & Carpenter, 1971; Fields Smith & Carpenter, 1971; Busch *et al.*, 1967) must therefore be equated with the commercial disadvantage of increased drip.

It is clear from Fig. 4 that cooling the early excised muscles at different temperatures influenced the tenderness of the meat. Muscles in Groups I–III were ultimately more tender, whereas those in Group IV showed a substantial decrease in tenderness (60–80%). The latter supports previous data obtained from excised semitendinosus muscles cooled in slush ice, where marked toughening occurred within the *first* day and remained throughout 13 days' storage. It would appear that these changes in tenderness, induced by rapid cooling, are a permanent feature of the resultant meat (Davey *et al.*, 1967; Taylor *et al.*, 1972).

It is well documented that muscle sarcomere length can be related to the ultimate meat tenderness (Marsh & Leet, 1966; Hostetler *et al.*, 1970; Howard & Judge, 1968). Herring *et al.* (1967) reported that small decreases in sarcomere length below 2·0 μm were associated with significant increases in shear value, whereas large increases above 2·0 μm had relatively little effect upon shear values. Present data, although not statistically demonstrated, is in agreement; mean sarcomere lengths of the excised muscles in Groups I–III were all greater than 2·0 μm and in each case greater than those of the corresponding PE muscles. It must therefore be concluded that these cooling temperatures did not lower the temperature of the muscles sufficiently to produce cold shortening. On the other hand, sarcomere lengths recorded for the AE muscles of Group IV were distinctly shorter than those in the corresponding PE muscles, especially at 3 days (mean 1·87 μm) and it was these muscles which showed a very substantial decrease in tenderness throughout maturation.

Raising the cooling temperature progressively increased the mean bacterial counts (both at 22°C and 37°C). However, from a commercial view point the most significant counts were those recorded internally in the meat where only marginal increases were observed ($<$ ten-fold). When butchered it is this meat which will ultimately form the majority of exposed surfaces of the retail joints, where it is important to suppress bacterial growth in order to gain maximum retail display life (Hood, 1971; Taylor & Dant, 1971).

Cooling the excised muscles at the different temperatures led to some interesting observations with respect to the mean oxygen uptake. AE muscles in all Groups showed increased oxygen uptake with the highest percentage change occurring in Group IV.

It has been shown recently by Atkinson & Follett (1973) that the oxygen uptake value of meat has a direct influence upon the retail life. The higher this value the shorter is the display life, because of the rapid discolouration at the meat surface caused by metmyoglobin formation. The PE muscles in the present investigation may be considered comparable to muscles handled in normal commercial practice, when 3–4 days' display life is expected from the retail commodity after prepacking. Any sizeable increase in

uptake such as that recorded in the AE muscles of Group IV will result in a reduction of the display life. The marginal increases in uptake for Groups I–III would have little or no deleterious effect upon display life.

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Book Reviews

Nutritional Problems in a Changing World. Ed. by D. HOLLINGSWORTH and M. RUSSELL.
London: Applied Science Publishers Ltd, 1973. Pp. xvi + 309. £10.

The title well describes the proceedings of a Conference called by the British Nutrition Foundation in Cambridge in 1973. Over 100 scientists from industry and academic institutions heard twenty-eight papers on topics of current interest, several from overseas speakers.

There were four plenary lectures; the first by the former Chief Medical Officer of the Department of Health and Social Security, Sir George Godber, reviewing the nutritional work of his department. The other three dealt with some of the current major problems—obesity, fibre and the Common Market.

The symposium fell into five groups: (1) surveillance of the nutritional status of the population, which included speakers from Czechoslovakia, Canada and USA, as well as UK; (2) infant feeding, which revealed startling variations in manufactured infant foods as well as frightening variations in methods of preparing the feeds; (3) a Report of the National Child Development Study, in which all children born in England, Scotland and Wales in the week 3–9 March 1958 have been followed up, so far, for 15 years (17,000 children); (4) a discussion of the food intakes of the adult population, including the elderly, and a discussion of the need for and risks in fortification of foods; (5) 'implications of modern nutritional thought for the food industry'. The final section of the book summarizes the current research and makes a number of brief recommendations for action.

Clearly, the greater value of such a symposium was the detailed discussion between scientists from industry and the academic world, none of which is printed, but the book itself is an extremely valuable source of information on the subject of its title. Anyone who wants to know what is, and what ought to be going on in the nutrition world will find his answer here.

A. E. BENDER

The Microbiological Safety of Food. Ed. by BETTY C. HOBBS and J. H. B. CHRISTIAN.
London and New York: Academic Press, 1973. Pp. xv + 487. £7.50.

This book is the Proceedings of the Eighth International Symposium on Food Microbiology held at Reading, England, in 1972. There are forty-one papers given by more than sixty contributors together with a record of the discussion which followed each paper. Sir Graham Wilson in his introductory address stated the theme which runs throughout the book and holds the many aspects together in these words 'What we are all really concerned about is the prevention of food poisoning'. The symposium was

divided into four sessions entitled: The bacteriology of various commodities in relation to food poisoning, The epidemiology of food-borne infection in man and animals, Special laboratory techniques, Legislation and non-legal specifications, and Education.

Because of the very wide range of the papers and the diversity of topics which were covered it is difficult, if not impossible, to present a brief, but comprehensive account of the scope of the symposium. However, there are papers in the first section concerned with genera traditionally associated with food poisoning—*Salmonella* and *Staphylococcus*. There is a most interesting paper by Eklund and Poysky on the association of bacteriophage and toxin production by *Cl. botulinum*. There are also papers on *Vibrio parahaemolyticus* and *Bacillus cereus*, organisms which are receiving increasing attention as agents of food poisoning. Other topics discussed included hygiene during meat production and in catering, papers on semi-preserved foods and weaning formulae.

The second section concerned with epidemiology, contains reviews of the situation in England and Wales, the USA and Australia. There are papers on *Salmonella* infections in humans and animals and also consideration of ways in which other infections are transmitted from animals to man. Additionally there are discussions of the significance of marine micro-organisms and of viral infections.

The third section on special laboratory techniques contains papers on staphylococcal enterotoxin detection, bio-assay methods for mycotoxin and a paper by Baillie, Crowther and Baird-Parker on the use of fluorescent antibody for the detection of *Cl. botulinum* and on electro-immunodiffusion techniques for the detection of botulinum toxin.

The fourth section on legislation and non-legal specifications could perhaps better be entitled 'legislation and non-legislative specifications' since microbiological specifications may be incorporated in a contract and thereby acquire some sort of standing in law. There are papers describing stituations and approaches in other countries which make interesting comparison with the position in the UK. A paper by Goldenberg and Elliot on the value of agreed non-legal specifications gives a useful and fairly detailed account of the Marks and Spencer approach to their safe handling of perishable foods. This paper ought to be read along with the paper in the following section by Goldenberg and Greta Edmonds on Education in Microbiological Safety Standards to get the complete picture. In this fourth section there is the second paper by Sakazaki on *Vibrio parahaemolyticus* dealing with control of contamination and the isolation and identification of the *Vibrio*.

The fifth and final section on Education deals not only with academic education and training in the UK, USA, Holland and Germany but also with the training needed by public health workers as well as workers (and management) in the food processing and distributive industries.

The concluding remarks by Sir Graham Wilson emphasized the importance of education including that of educating the housewife and concluded 'The exchange of different views has proved very stimulating and the large gaps in our knowledge have emphasized the need for further research'.

The reader of this book, whether a microbiologist, a food technologist or one concerned with general quality control will find it stimulating, and instructive. He will have a good picture of the 'state of the art' in 1972 and will want to draw the attention of junior colleagues to specific parts of the text.

D. A. SHAPTON

Pollution Control in Meat, Poultry and Seafood Processing. By H. R. JONES. New Jersey: Noyes Data Corporation, Pollution Technical Review No. 6,1974. Pp. xi +261. US$36.

This work is a compilation of data from American sources, mainly government ones, but with examples from US patents. It is divided into four parts and deals with Meat Processing (129 pp.). Poultry Processing (86 pp.), Seafood Processing (38 pp.), and Future Trends and Recommendations (9 pp.).

Under each main heading is described the structure of the industry, the processes in use and the resulting types of polluting by-products. Waste water treatment facilities available and the cost of pollution reduction are given. In the case of Meat Processing air pollution problems and their control are also discussed.

Part I—Meat Processing

Much useful data is given on typical volumes of water used, pounds of BOD, suspended solids and grease produced. Emphasis is placed on blood recovery and by-product utilization. Detailed references are made to water conservation and selection and modification of process equipment. Processes for treating waste waters are described and include trickling filters, rotating biological discs, lagoon systems and the activated sludge process.

Five 'case histories' are given of actual pollution control systems at beef and hog slaughtering and processing plants. The flows treated varied from 315,000 to 3,000,000 gallons (US) per day.

Part II—Poultry Processing

Individual processes are described, and by-product recovery, good housekeeping and water conservation are treated in detail. The chapter on 'In-plant poultry processing waste treatment' deals very concisely with conservation and re-use of water and the reduction of pollution in the processes of killing and bleeding, scalding, de-feathering, evisceration, chilling and cleaning up—finishing with a general list of twenty-two steps for improved water management.

Part III—Seafood Processing

In this section a bibliography is provided and references are made to sixty-five entries.

The seafood dealt with includes tuna, salmon, shrimp, crab, menhaden and bottom fish. The high percentages of waste, on average 30%, can lead to serious disposal problems, and in many instances the fat and grease content cause further complications. Coagulation and air flotation are described, but better utilization of by-products would seem to be required.

Part IV—Future Trends and Recommendations

The items included under this heading could have been added to the previous sections. The only serious contribution being to the future of the seafood industry.

The foreword claims that the Noyes Data Corporation have 'by-passed the delays in the conventional book publishing cycle' and having closed the gap between manuscript and the completed book provide the reader with an up-to-date review of the relevant industrial technology. The latest references in the bibliography on seafood waste treatment are from the year 1970, so there is still a 4 year delay in this instance.

It is disappointing to find considerable repetition of the subject matter in Parts I and II which detracts from the readability of this work.

A proper index would have been of considerable value.

A. H. POTTEN

Shortenings, Margarines and Food Oils. By M. T. GILLES.
New Jersey: Noyes Data Corporation, Food Technology Review No. 10, 1974. Pp. x + 333. US $36.

The volume is an up-dated version of six out of the twelve sections of Review No. 5 'Edible Oils and Fats' dated 1969. Thus only sixty-four out of the 174 US patents here reviewed are new, the descriptions of the others being taken from the older volume with a few minor editorial alterations. The sections covered are fluid, plastic and powdered shortenings, margarines and spreads, salad oils, mayonnaise and dressings, and frying and cooking oils. The publication takes much of the frustration out of the study of patent literature but cannot obviate the need to scan new patents. The essence of each patent is fully described with elegant clarity and patents on related subjects are grouped together.

The book therefore provides a valuable survey of many technical efforts and advances. As one would expect the subject reflects the fashionable research topics of the last two decades, such as low cholesterol and reduced caloric diets, polyunsaturated acid content, functionality of emulsifiers in foods, more efficient materials handling and processing in factories.

The foreword contains a number of forthright claims for the importance of patent literature, some of which are undoubtedly true of the book's contents. 'Patents, unlike periodical literature, are bound by definition to contain new information.' Yes, but the

periodical literature is more likely to be objective, whereas a patent may well deliberately emphasize the less important, or obscure the essential parameters.

Browsing through the book is a stimulating experience. It raises many questions. How many of the emulsifiers described are not permitted in foods? What is the real truth about Beta phase versa Beta prime phase in shortenings? In how many of these patents could infringements be detected?

Out of the 174 patents described 57 are in the name of Procter and Gamble, 28 are Unilevers and 10 Corn Products Co. property. Are there some clearcut corporate patenting policies behind this activity and have they paid off commercially over the 15 years covered in this review?

The format of the book has been reduced to a standard shelf size and it is bound in hard covers. This is a worthwhile improvement. The book is recommended to all those involved in searching for or applying new ideas in the field of oils and fats, with the caveat that 'all is not gold that glisters'.

K. G. BERGER

Recommended Dietary Allowances. COMMITTEE ON DIETARY ALLOWANCES. FOOD AND NUTRITION BOARD, NATIONAL RESEARCH COUNCIL.
Washington: National Academy of Sciences, 1974. 8th revised edition. Pp. 128. US $2.50.

As a food scientist, this is a book I am glad to have on my bookshelf. So often one is called upon for an opinion on such provocative subjects as fluoridation, atherosclerosis, unprotected calories, ascorbic acid and the common cold, and, not being a nutritionist, wish that someone else was there to hold the fort. This compact work is a mine of sensible, quotable opinion on these and many other controversial topics. It shows every sign of careful consideration and meticulous phrasing, with the greatest economy in marshalling evidence on either side of these controversial questions. Moreover the evidence is thoroughly up-to-date, references being mainly from the 1960s and some as recent as 1972.

In an introductory section every care is taken to ensure that the data given (the 'RAD') are not to be regarded as pragmatic. They are 'the levels of intake of essential nutrients considered, in the judgment of the Food and Nutrition Board on the basis of available scientific knowledge, to be adequate to meet the known nutritional needs of practically all healthy persons'. Later, the requirements of special cases are dealt with and the meaning of 'practically' explained.

As in the case of 'Natural Toxicants in Foods', recently reviewed, published by the same authority, the price is surprisingly moderate; the format and typography impeccable. It is a book well worth having.

E. C. BATE-SMITH

Books received

Experimental Work in Food Science. By J. R. SALFIELD.
London: Heinemann Educational Books, 1974. Pp. 113. £0.80.

Elementary laboratory experiments for courses of home economics and catering in schools.

Sausage Casing Technology. By E. KARMAS.
New Jersey: Noyes Data Corporation, 1974. Pp. x + 366. US $36.00.

A guide to the US patent literature relating to all types of sausage casings and their uses.

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CONTENTS

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CONTENTS

Volume 9, Number 1, March 1974

Volume 9, Number 2, June 1974

Volume 9, Number 3, September 1974

Volume 9, Number 4, December 1974

AUTHOR INDEX

SUBJECT INDEX

JOURNAL OF FOOD TECHNOLOGY: NOTICE TO CONTRIBUTORS

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Arrangement. Papers should normally be divided into: (a) Summary, brief, self-contained and embodying the main conclusions; (b) Introduction; (c) Materials and methods; (d) Results, as concise as possible (both tables and figures illustrating the same data will rarely be permitted); (e) Discussion and conclusions; (f) Acknowledgments; (g) References.

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

SI UNITS

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

NON SI UNITS

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

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

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

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Contents

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