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The heating of foodstuffs in a microwave oven

D. KIRK* AND A. W. HOLMES

Summary

A method for calculating the temperature profile in a solid material after a period of microwave heating is described. The calculation incorporates internal heat generation, caused by the microwave radiation, together with other forms of heat transfer. It is shown that the calculated profile, based on the dielectric and thermal properties of water, agrees with experimentally measured temperature profiles in gels of 1% Ionagar in water.

Introduction

One of the main applications of microwave ovens in the catering industry is the re-heating of pre-cooked chilled foods. It is found in practice that this heating is not uniform throughout the food. Values of the loss tangent (tan δ) and half-power depths have been used to describe the temperature distribution. These are not altogether satisfactory because they are temperature-dependant. Also, other modes of heat transfer are taking place. When a food is heated in a microwave oven, the heating effect at any point is a function of the power flux at that point and of the dielectric properties of the food. Once the heat has been generated it is then distributed throughout the food by conduction; heat may also be lost from the surface by convection and radiation.

As microwave radiation is propagated through a medium, it is attenuated according to the relationship:

$$Px = P_0 . \exp(-2 \alpha x)$$

where P_0 is the power flux at the surface, Px is the power flux at a depth of x cm in the direction of propagation and α is the attenuation coefficient. The latter is a property of the propagating material and can be calculated from the real and imaginary relative permitivities, ϵ'_r and ϵ''_r (von Hippel, 1954).

The energy lost from the wave, as a result of this attenuation, is dissipated in the

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propagating medium as heat. Thus, if the dielectric properties of the material are known, the heating effect can be calculated.

Known values of the dielectric properties of foodstuffs at the common microwave frequency (2450 MHz) are summarized by Bengtsson & Risman (1971). The majority of these measurements are at a single temperature and are therefore of limited value for predicting temperature profiles. Ohlsson & Bengtsson (1971) used values of ϵ'_r and ϵ''_r over the temperature range 0–60 °C to calculate temperature profiles for a number of meat products. Unfortunately such detailed information is available for only a small number of foods. An alternative approach, which uses values of ϵ'_r and ϵ''_r calculated from the dielectric properties of water is described in this paper.

It is possible to calculate the real and imaginary relative permittivities for a material from its ionic conductivity, relaxation time τ, static dielectric constant Ko and high frequency dielectric constant K_∞. Since the effect of temperature on all of these constants for water is well documented, it follows that the effect of temperature on the values of ϵ'_r and ϵ''_r can be calculated for water.

ϵ''_r is made up of two parts: $\epsilon''_{r\ ionic}$ is that portion of ϵ''_r caused by ionic conductivity in the water and $\epsilon''_{r\ dipole}$, which constitutes the remainder of the lossiness, is caused by molecular rotation in the electromagnetic field.

$$\epsilon''_r = \epsilon''_{r\ ionic} + \epsilon''_{r\ dipole}.$$

$\epsilon''_{r\ ionic}$ can be calculated from the electrical conductivity of water:

$$\epsilon''_{r\ ionic} = 60.\lambda.\sigma.$$

$\epsilon''_{r\ dipole}$ and ϵ'_r are a function of the dielectric properties of the material τ, Ko and K_∞ (Lane & Saxton, 1952a).

$$\epsilon''_{r\ dipole} = \frac{\omega.\tau\,(Ko - K_\infty)}{1 + \omega^2.\tau^2}$$

$$\epsilon'_r = K_\infty + \frac{(Ko - K_\infty)}{1 + \omega^2.\tau^2}$$

where ω is the frequency of the microwave radiation, in radians/sec. For water, and other foodstuffs with a low ionic conductivity, the contribution of $\epsilon''_{r\ ionic}$ is small and can be neglected. The relationship between temperature, Ko and τ can be obtained from the data of Lane & Saxton (1952a) and von Hippel (1954). From this data, the following equations were developed, which can be used to predict the effect of temperature on Ko and τ, over the temperature range 0–95 °C.

$$\tau_t = [16{\cdot}3 \times \exp(-0{\cdot}033t) + 2{\cdot}4] \times 10^{-12}$$
$$Ko_t = Ko_0 - 0{\cdot}4t$$

where τ_t is the value of τ at a temperature of t°C; Ko_0 is the value of Ko at 0 °C; and

Ko_t is the value at t°C. K_∞ is independent of temperature and for the calculation a value of 5·5 was used (Hasted, 1961).

Experimental

The experimental work described in this paper consists of two parts: a description of the method by which the temperature profile in a slab of material was calculated is followed by an account of a method devised for measuring the temperature distribution in agar gels, with the radiation entering through one or both of the faces of a slab.

The calculated temperature profile

A finite difference method was used to calculate the microwave heat generation and unsteady state heat transfer processes (Gebhart, 1971). The material was assumed to be in the form of an infinite slab, with microwave radiation normal to either one or both faces of the slab. Because of this simplification, heat transfer processes could be treated as being uni-dimensional. To enable comparison with the experimentally measured profiles the surface power flux for the calculation was fixed at the same values as calculated for the experimental profiles (by graphical integration of the heat generation-depth graph).

The calculation was carried out on the computer of the University of Surrey, using Algol 60 (Kirk, 1973). For the calculation the slab depth was divided into a large number of incremental slabs of thickness Δx cm; similarly the heating time was divided into incremental heating periods of duration $\Delta\tau$ sec. The ratio of incremental time and depth was adjusted to give a Fourrier number of $\frac{1}{2}$ in order to simplify the heat transfer calculation. The temperature rise in an incremental slab during a time period was calculated from the difference of the power flux at the front and rear faces of the slab:

$$\Delta t_{n,p} = P_n \left[1 - \exp\left(-2\alpha x\right)\right] \frac{\Delta\tau}{S.\rho.x}$$

where Δt is the temperature rise in the nth slab during the pth time increment and S is the specific heat in Joules g^{-1} °C^{-1}. P_n is the power flux (watts/cm^2) at the front face of the nth slab. The power flux at the surface (P_0) is provided as a parameter in the calculation. Thus starting with the first increment it is possible to calculate the temperature rise in the first slab and the power flux at the face of the second slab. This can be repeated for each of these slabs in turn, for a single time period. A value for α is calculated for each incremental slab and time period based on the temperature in that slab at the end of the preceding time period.

Once the temperature rise in all of the slabs has been calculated for a single time increment, this is added to a store location which represents the temperature for each slab. The effect of internal and surface heat transfer is calculated using standard uni-

dimensional finite difference heat transfer techniques. Physical parameters in the simulation were based on data for water. Surface heat transfer coefficients were measured experimentally for agar gels of the same dimensions as those used for measuring temperature profiles.

When the temperature rise for each slab has been calculated and corrected for heat transfer changes, the procedure is repeated for successive time increments. A simplified flow diagram for the calculation is shown in Fig. 1.

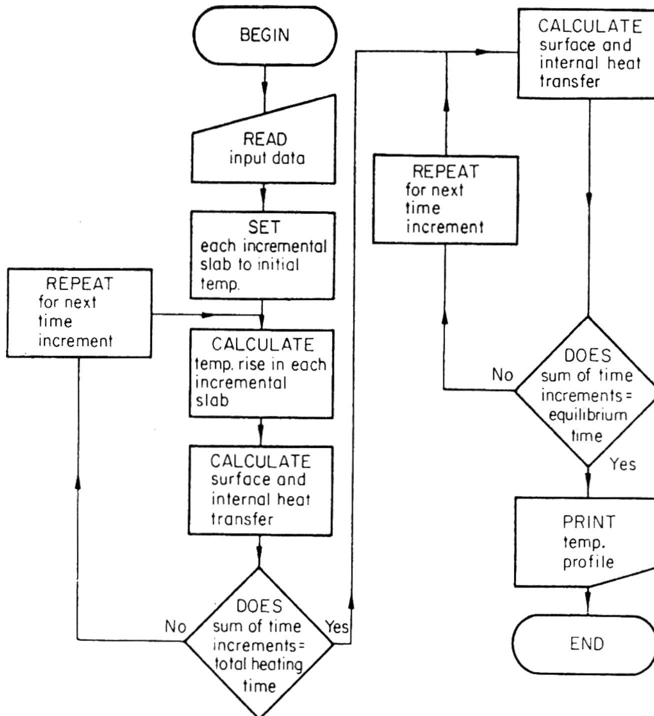

FIG. 1. A simplified flow diagram for the calculation of the temperature profile in a slab of material.

At the end of the heating period an allowance is made for the time delay, in the experimentally measured profiles, between the end of the heating time and the measurement of temperature profiles (equilibrium time). In most cases this corresponded to a time of 30 sec, although it could be varied to study, for example, the redistribution of heat in a food between heating and consumption.

For slabs with radiation incident on both of the surfaces, the total temperature rise in an incremental slab for a single heating period was assumed to be equal to the sum of the heat generation from both of the faces considered individually.

Experimentally measured temperature profiles

Heating profiles were measured experimentally in gels of 1% Ionagar. For the case of microwave radiation on a single surface the gel was in the form of a cylinder of radius 7 cm and depth 10 cm (Fig. 2a). The gel was held in a polythene beaker and surrounded by a metal can which shielded all but the upper surface from the microwaves. To ensure uniform initial temperature the gels were held overnight at constant temperature.

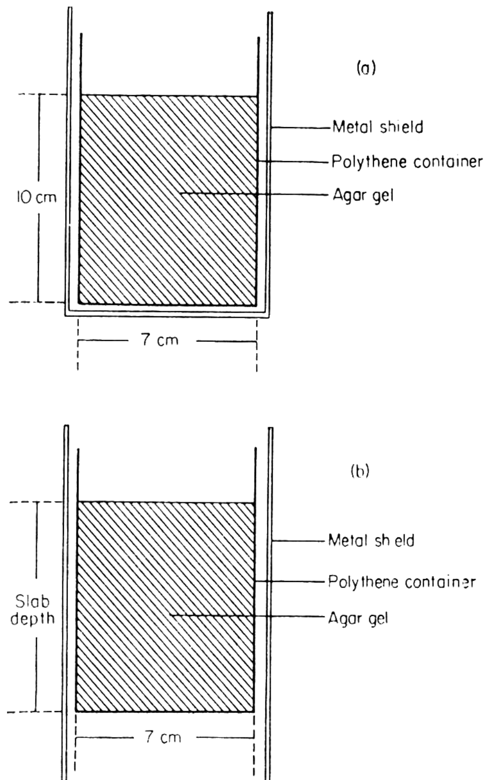

FIG. 2. Arrangement of the agar gel for the experimentally measured profiles. (a) Microwave radiation incident on a single surface; (b) microwave radiation incident on two parallel surfaces.

The oven used for the microwave heating experiments was a commercial catering microwave oven (Philips 1102). After the period of microwave heating temperatures were measured at 1-cm intervals in the depth of the agar, using thermocouples mounted on a plastic strip which was inserted into the agar. Temperatures were measured 30 sec after the end of the heating period. For slabs with radiation incident on both parallel faces, a metal cylinder was used to shield the sides of the slab from the

microwaves. The gels were moulded in a polythene beaker to give the desired slab depth (Fig. 2b).

Graphical integration of the temperature–depth data was used to calculate the surface power flux for use as a comparison in the simulated temperatures profiles.

Results and discussion

The results are given in the form of a direct comparison between the experimental and calculated temperature profiles, under the same conditions (initial temperature, air temperature, heating time, equilibrium time, power flux and depth of slab).

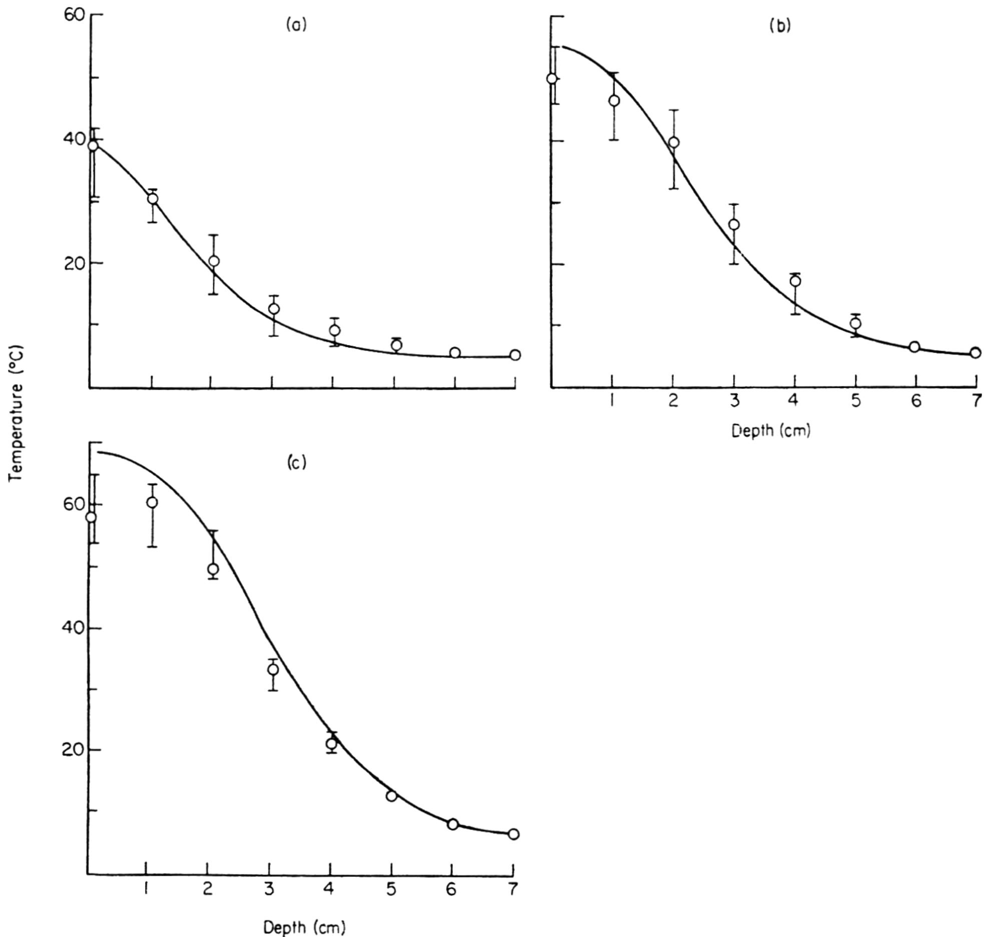

FIG. 3. Temperature profiles for slabs with radiation on a single surface showing the effect of varying heating time. Mean of experimental values, ○; range of experimental values, I; calculated values, (———). (a) 30 sec heating time; (b) 60 sec heating time; (c) 90 sec heating time.

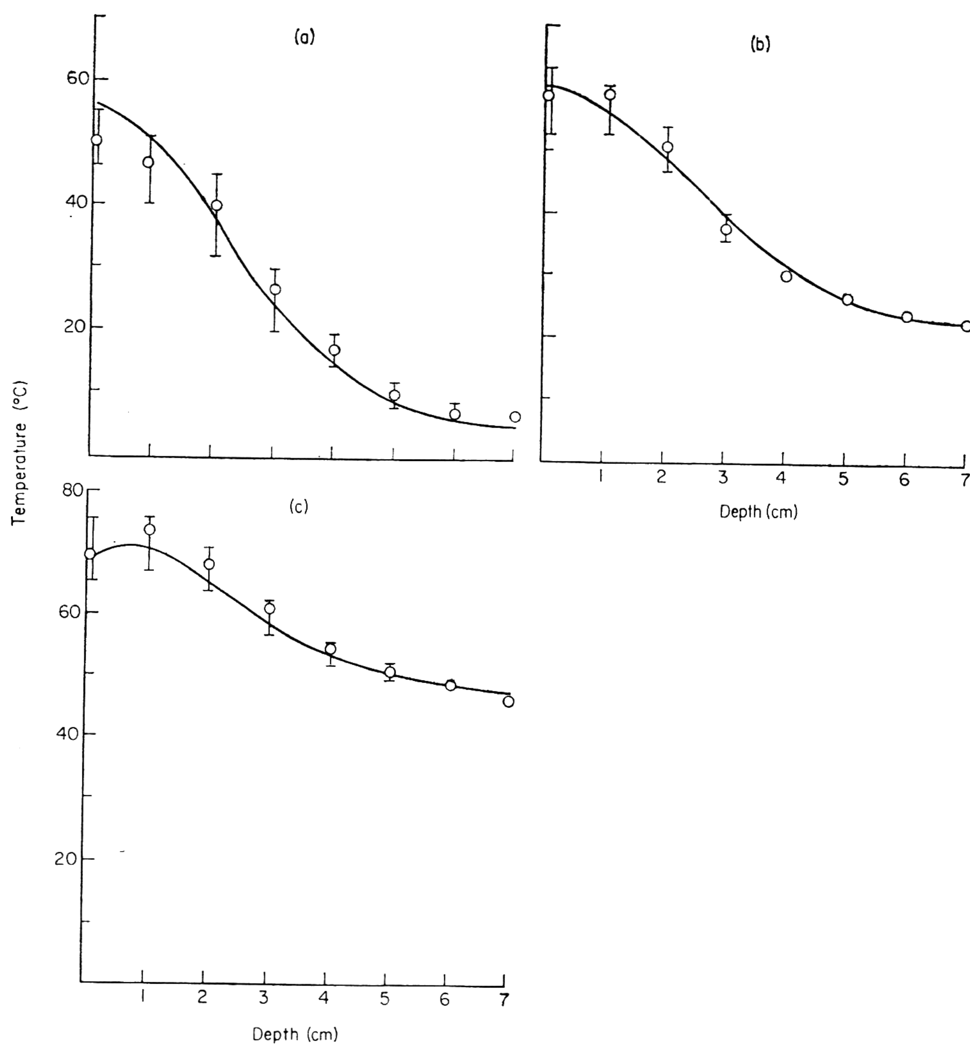

FIG. 4. Temperature profiles for slabs with radiation on a single surface showing the effect of initial slab temperature. Mean of experimental values, ○; range of experimental values, I; calculated values, (———). (a) 5 °C; (b) 20 °C; (c) 40 °C.

Figure 3 shows the effect of varying heating times for gels at an initial temperature of 5 °C, with microwave radiation incident on one surface only. The experimental profiles are based on five experiments and the air temperature in each case was 20 °C. Figure 4 shows the effect of three different initial temperatures (5, 20 and 40 °C). The heating time was 60 sec and the air temperature 20 °C.

The data shown in Fig. 5 is for slabs of varying thickness, with microwave radiation normal to the two parallel faces. These results are for an initial temperature and an air

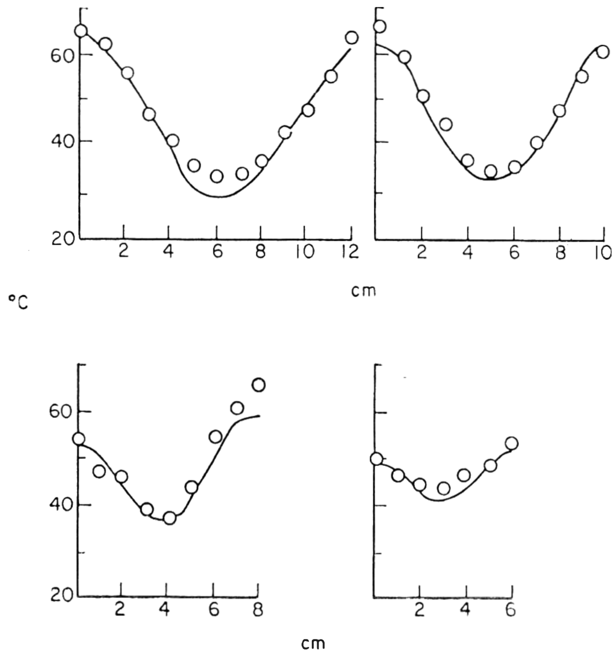

FIG. 5. Temperature profiles for slabs with radiation on two surfaces, at an initial temperature of 20 °C. Mean of experimental values, ○; calculated values, (———).

temperature of 20 °C and for a heating time of 60 sec. The results in Fig. 6 are also for slabs with radiation at two faces, but in these cases the initial temperature is 5 °C.

The results shown in Figs 3–6 show that the procedure for calculating the temperature profile based on values of τ, Ko and K_∞ for water give good agreement with experimentally measured profiles in agar gels containing 99% water. There was a high variability in replicate experimental temperature profiles indicated in Figs 3 and 4. This was thought to be due to errors in locating the thermocouple strip in the agar gel. Because of the high temperature gradient, particularly near to the surface, a small difference in the position of a thermocouple causes a large difference in the measured temperature.

The heat transfer calculation in the simulation contained several simplifications. Thus the finite difference calculation used fixed values for the thermal conductivity, whereas in practice this is temperature dependent. This error is unlikely to be significant during the short time periods involved in these experiments. A more important source of error is the value used for the surface heat transfer coefficients. Four values were used to represent different situations: for both the upper and lower surface the air temperature can be either hotter or colder than the surface temperature of the solid. The values for these constants were obtained from experimental measurements on agar gels, of the same dimensions as used for the heating experiments, over a temperature range 20–40 °C. In practice, during the microwave heating experiments, the agar

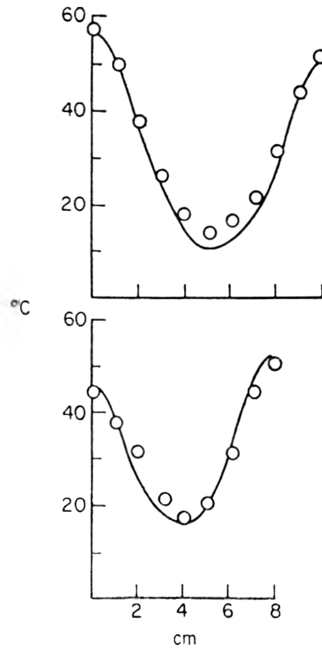

FIG. 6. Temperature profiles for slabs with radiation on two surfaces, at an initial temperature of 5 °C. Mean of experimental values, ○; calculated values, (———).

temperature at the surface rose to a temperature considerably higher than 40 °C. Since, at a higher temperature, the surface heat transfer will be greater, the calculated surface temperature is likely to be higher than that measured experimentally. Another possible source of error in the simulation is that no allowance is made for the 1% solid in the gel, nor for the 0·8 g/g of irrotationally bound water associated with it (de Loor & Meejboom, 1966).

The success of this simple model in predicting the temperature distribution in high water content solids indicates that it might be applicable to more complex materials. Thus it should be possible to modify the model to allow for the presence of non-polar solids, together with any associated bound water. Hasted (1961) gives formulae for calculating the effect of these materials on the value of Ko for water. De Loor & Meejboom (1966) have indicated that, for a range of foodstuffs, the presence of solid material has no effect on the value of τ. Preliminary experimental work has indicated that this approach can be used satisfactorily to calculate the effect of up to 10% sucrose (10% non-polar solid) and 10% cooked corn starch (10% non-polar solid plus 1 g/g bound water). For materials with a higher solid content the simulation was found to be unsuitable (Kirk, 1973).

Similarly the effect of electrolytes can be incorporated into the model: these cause changes in the values of Ko, τ and $\epsilon''_{r\ \mathrm{ionic}}$. One interesting feature of an increase in the

value of the ionic conductivity is that this, unlike $\epsilon''_{r\ dipole}$, has a positive temperature coefficient. This means that the initial heating just below the surface of the material leads to an increase in the value of ϵ''_r and hence α. This in turn causes 'runaway' heating at this point in the material. The calculation of the effect of elecrolytes on the value of Ko and τ can be obtained from the data of Lane & Saxton (1952b).

Conclusion

A model for determining the temperature distribution in a slab of material having a high water content, after a period of microwave heating, has been developed based on the relaxation time and low and high frequency dielectric constants for water. This model, when incorporated in a finite difference heat transfer simulation for semi-infinite solids and infinite slabs, can be used to predict the temperature distribution in slabs of agar gel for which the surface power flux is known. The model was found to work under a variety of heating times and initial temperatures.

Acknowledgments

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References

Bengtsson, N. & Risman, P. (1971) Dielectric properties of foodstuffs at 3 GHz as determined by cavity perturbation techniques. *J. Microwave Power*, 6 (2), 107.

Gebhart, B. (1971) *Heat Transfer*, 2nd edn. McGraw-Hill, New York.

Hasted, J. (1961) The dielectric properties of water. *Prog. Dielect.* **3,** 101.

Hippel, A. von (1954) *Dielectric Materials and Applications*. M.I.T., Cambridge, Massachusetts.

Kirk, D. (1973) M.Phil thesis, University of Surrey.

Lane, J. & Saxton, J. (1952a) Dielectric dispersion in pure polar solvents at very high frequencies. I. Water, ethyl alcohol and methyl alcohol. *Proc. R. Soc.* **A213,** 400.

Lane, J. & Saxton, J. (1952b) Dielectric dispersion in pure polar solvents at very high frequencies. II. Effect of electrolytes in solution. *Proc. R. Soc.* **A214,** 531.

Loor, G. de & Meejboom, F. (1966) The dielectric constants of foods and other materials with high water contents at microwave frequencies. *J. Fd Technol.* **1** (4), 313.

Ohlsson, T. & Bengtsson, N. (1971) Microwave heating profiles in foods. *Microwave Energy Applications Newsletter*, **4** (6), 2.

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Comparison of water vapour sorption by sugar beet root components

HÉCTOR A. IGLESIAS, JORGE CHIRIFE AND JOSÉ L. LOMBARDI

Summary

The purpose of the present study is to verify if the water sorption isotherms of raw sugar beet root could be predicted from knowledge of sorption behaviour of its main components, namely sucrose and the water insoluble fraction.

For this purpose, a study of water sorption in amorphous sucrose at 35 and 47 °C was undertaken. These results were used along with previously reported isotherms of raw sugar beet root and its water insoluble fraction.

Good agreement between the predicted and experimental isotherms was observed at low relative humidities (up to 25% RH); at higher relative humidities poor agreement was obtained, probably because phase transitions of the sucrose.

Introduction

The effect of the composition of a food on the shape of the water sorption isotherms has not been investigated in detail. According to Labuza (1968), in most cases it may be assumed that the amount of water sorbed at any relative humidity is derived by the weight percentage of each component, times the amount it would absorb alone. Berlin, Anderson & Pallansch (1968) for milk powder and Palnitkar & Heldman (1971) for freeze dried beef tried to predict product isotherms from knowledge of component isotherms.

In a previous paper (Iglesias, Chirife & Lombardi, 1975) the water sorption isotherms of raw sugar beet root and its water insoluble fraction, at different temperatures, were reported. In the present study an attempt is made to predict sugar beet root isotherms from knowledge of sorption behaviour of its main components, namely sucrose and the water insoluble fraction. For this purpose this investigation includes the measurement of water sorption by amorphous sucrose at 35 and 47 °C.

Material and methods

Water vapour sorption studies were conducted on amorphous samples of sucrose at 35 and 47 °C. Amorphous sucrose was obtained by freeze drying a 20% aqueous sucrose

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solution; it is well-known that this procedure yields amorphous cakes (White & Cakebread, 1966; Flink & Karel, 1972).

Amorphous sucrose dispersed in a solid support was also prepared in order to simulate the structure of a food. Porous ceramic cylinders were chosen as solid supports because this kind of material does not adsorb water in the conditions at which the experiments were performed. The cylinders had 1·5 cm nominal size and a void fraction of 42%; they were soaked for several hours in a 20% aqueous sucrose solution and then freeze dried at 37 °C and 100 μ.

Vacuum desiccators were prepared with saturated salt solutions or aqueous sulphuric acid solutions of known concentration to give different constant relative humidities. The desiccators were placed in constant temperature baths ($\pm 0 \cdot 1$ °C). The freeze dried sucrose cakes or solid-dispersed sucrose, were weighed in weighing bottles with outside ground stoppers and were used in triplicate at each humidity. The samples were placed in the desiccators at the various relative humidities and a vacuum was drawn in order to speed up the equilibrium.

Results and discussion

Water uptake by the samples of amorphous sucrose was determined periodically from changes in weight of samples kept at 35 and 47 °C in the vacuum desiccators which maintained constant relative humidities. The course of the moisture uptake with time is shown in Figs 1 and 2.

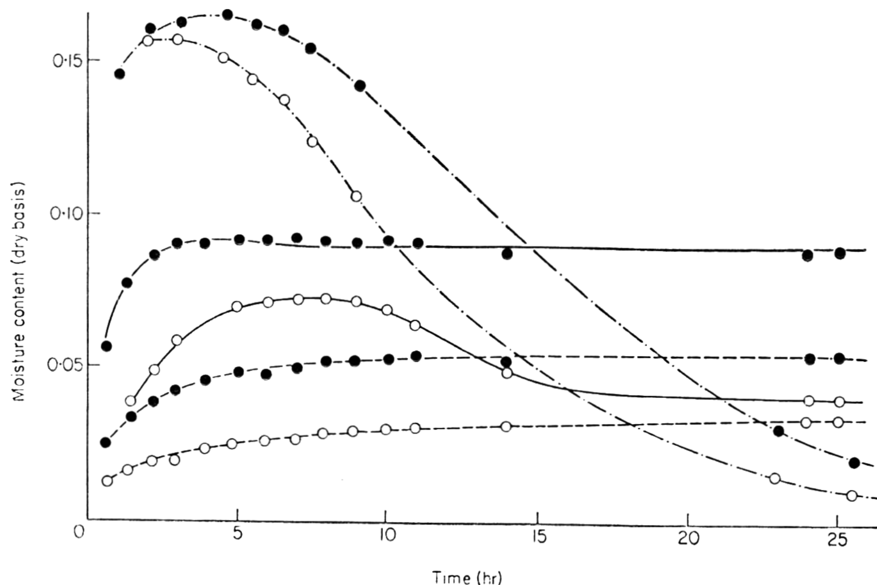


FIG. 1. Water uptake by amorphous sucrose at several relative humidities at 35 °C. ●, Supported; ○, 'free'. RH: (– – –), 25·5%; (———), 37·5%; (—·—·), 54%

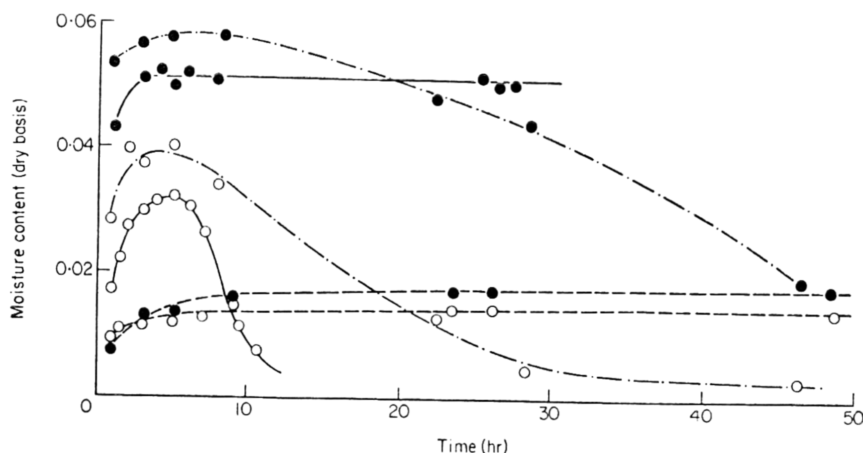


Fig. 2. Water uptake by amorphous sucrose at several relative humidities at 47 °C. ●, Supported; ○, 'free'. RH: (– – –), 11%; (———), 24·5%; (—·—·), 31·5%.

It can be seen that at low relative humidity an equilibrium moisture level was reached, this equilibrium value being higher for the solid-dispersed sucrose. This difference may be attributed to the higher internal area available for water sorption in the dispersed sample.

As the relative humidity was increased (37·5% RH at 35 °C and 24·5% RH at 47 °C) both types of samples gained moisture. After a maximum was reached the cakes lost moisture indicating that crystallization occurred (Makower & Dye, 1956; Karel, 1973), but there was no apparent crystallization in the solid-dispersed samples. It seems that the solid frame prevents crystallization, at least over the time it was investigated (60 hr).

At higher relative humidity both types of samples reached a maximum and then lost moisture, but at a different rate; the process of crystallization was always delayed in the solid-dispersed samples.

Amorphous sucrose is theoretically unstable. However, the very high viscosity of the medium prevents a molecular rearrangement and stabilizes the amorphous state for a relatively long time (White & Cakebread, 1966; Chevalley, Rostagno & Egli, 1970). The sorption of water imparts mobility to the sucrose molecules, and this mobility results in the transformation of sucrose from the metastable amorphous state to the more stable crystalline state (Karel, 1973). In this process the sucrose loses water as indicated by the experiments shown in Figs 1 and 2. Probably, the delay in the begining of crystallization in solid-dispersed samples was due to the difficulties for the rearrangement of sucrose molecules as a result of the porous structure of the solid support.

It is worth mentioning that the relative humidity at which crystallization is observed is decreased as temperature is increased. This is in accordance with the fact that

temperature favours crystallization because the viscosity is reduced (Chevalley *et al.*, 1970).

The maximum values for the sorbed water in samples that did not crystallize represent the equilibrium moisture content; additional values were estimated from the maxima observed in the sorption experiments (Figs 1 and 2). All the values considered were those obtained with the solid-dispersed samples. These 'equilibrium' values (Makower & Dye, 1956) were used to draw the sorption isotherms of amorphous sucrose at 35 and 47 °C. The results are shown in Fig. 3; values reported by Makower & Dye (1956) were also plotted for the purpose of comparison. The isotherms obtained may be considered as a Type III isotherm according to B.E.T.'s classification (Labuza, 1968). Three regions may be distinguished in these isotherms: the first one from 0% RH up to 37·5% RH for 35 °C, and 24·5% RH for 47 °C, where no crystallization occurred and 'true' equilibrium was found; a second one up to the saturated solution point characterized by a metastable behaviour; and a third one where solution effects prevails.

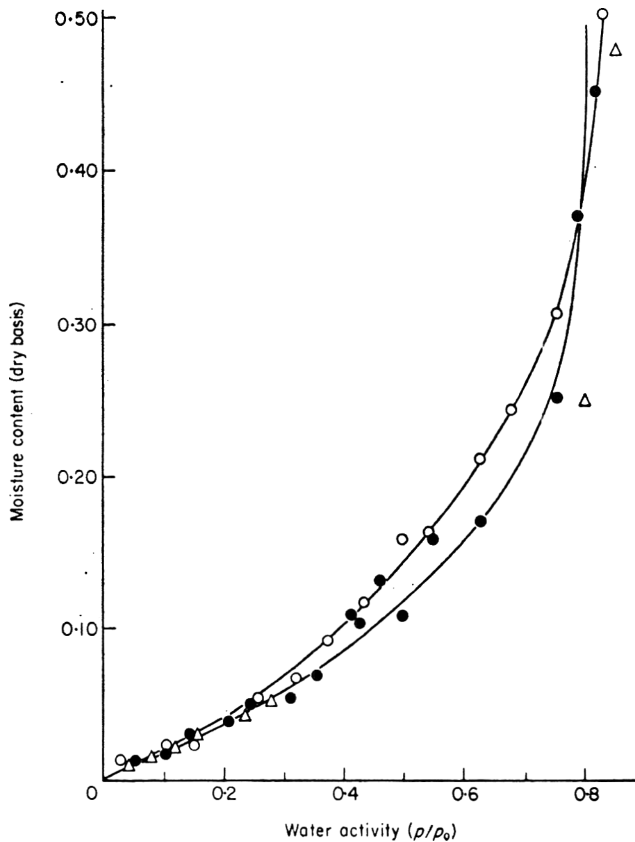


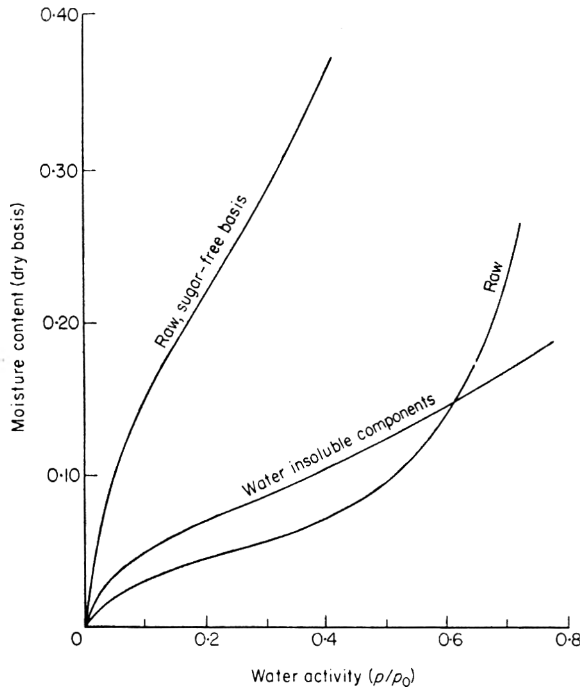
FIG. 3. Water sorption isotherms of sucrose at several temperatures. △, 25 °C (Makower & Dye, 1956); ○, 35 °C; ●, 47 °C.

It is seen that at high relative humidity the equilibrium moisture content values increase as the temperature increases in accordance with the solution theory (Loncin, Bimbenet & Lenges, 1968).

It is known that some food polymers adsorb more water at low relative humidities than crystalline substances like sugars (Labuza, 1968). For instance, Berlin *et al.* (1968) found that in milk powder the water vapour is preferentially bound by casein at low relative humidity. As the relative humidity increases towards about 50%, lactose becomes more active in water binding.

Figure 4 was drawn with data reported in a previous paper (Iglesias *et al.*, 1975); it shows the desorption isotherms of raw sugar beet root and its water insoluble fraction at 35 °C. It can be seen that at low and intermediate relative humidities the water insoluble fraction binds more water than the raw material; however, at high relative humidities the sucrose becomes more active and there is a noticeable water sorption.

In order to verify if the sucrose contributes significantly to water sorption at low relative humidities, the isotherm for raw sugar beet root was recalculated on a sucrose-free basis. The results are shown in the same Fig. 4. The lack of agreement with the isotherm for the water insoluble fraction, for the whole range of relative humidity, indicates that even at low relative humidity sucrose contributes to the total water sorption.



Fɪɢ. 4. Desorption isotherms of raw sugar beet root and its water insoluble fraction at 35 °C.

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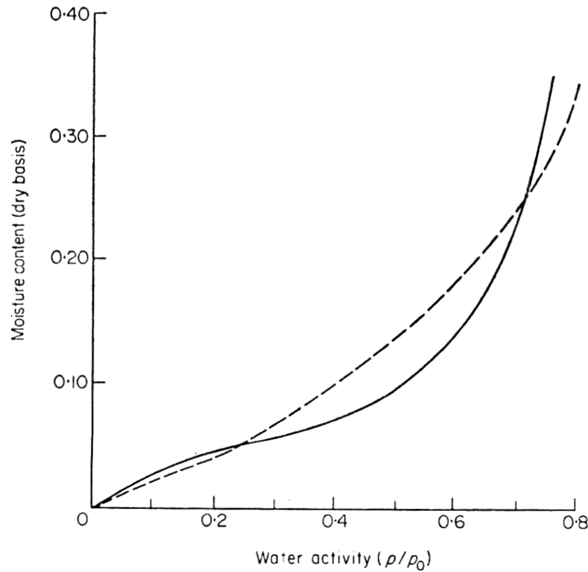

FIG. 5. Comparison of calculated water sorption isotherm for raw sugar beet root with experimental isotherm at 35 °C. (———), Raw; (- - -), composite.

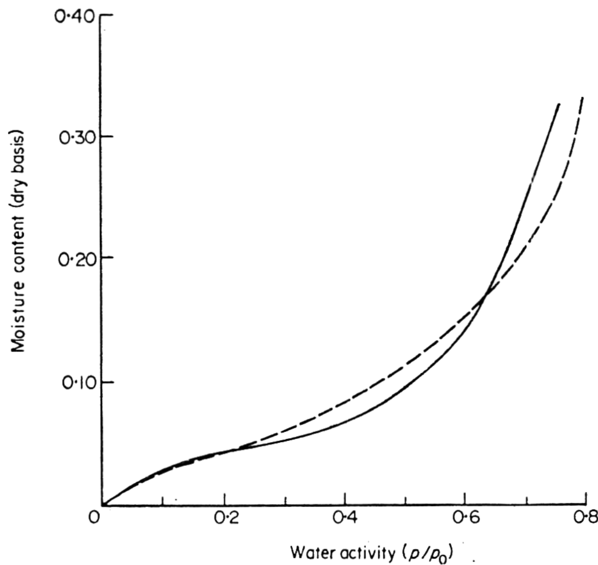

FIG. 6. Comparison of calculated water sorption isotherm for raw sugar beet root with experimental isotherm at 47 °C. (———), Raw; (- - -), composite.

As it was mentioned, some authors (Labuza, 1968; Berlin *et al.*, 1968) have suggested the possibility of predicting moisture sorption isotherms for food products from knowledge of the product component isotherms. From sorption isotherms of sucrose and of the water insoluble fraction of raw sugar beet root, along with the relative amounts of

each in the product (about 80% sucrose and 20% water insoluble material, on a dry basis), the composites sorption isotherms at 35 and 47 °C were predicted. The results are shown in Figs 5 and 6 and compared with the experimental desorption isotherms for raw sugar beet root.

Good agreement between the actual isotherms and the constructed ones is observed at low relative humidities (up to 25% RH). At higher relative humidities poor agreement is observed, probably because phase transitions occurring in the sucrose. Similar difficulties were found by Berlin *et al.* (1968) in predicting the sorption isotherm of milk powder from the sorption behaviour of its main components, casein, lactose and mineral salts.

It has been stated (Karel, 1973) that sucrose may be present in one of the several states: crystalline solid, amorphous solid (bound to other food components) and aqueous solution. The phase transformations of the sucrose may strongly influence the water sorption behaviour of sugar beet root. However, according to the composite isotherms constructed (Figs 5 and 6) it may be considered that up to 25% RH the sucrose contained in the sugar beet root is in amorphous state. The phase transitions which may occur at higher relative humidities may be also influenced by the structural elements of the sugar beet root, as it is suggested by the crystallization experiments on solid-dispersed sucrose.

References

Berlin, E., Anderson, B.A. & Pallansch, M.J. (1968) *J. Dairy Sci.* **51,** 912.
Chevalley, J., Rostagno, W. & Egli, R.H. (1970) *Int. Choc. Rev.* **25,** 3.
Flink, J.M. & Karel, M. (1972) *J. Fd Technol.* **7,** 199.
Iglesias, H.A., Chirife, J. & Lombardi, J.L. (1975) *J. Fd Technol.* **10,** 299.
Karel, M. (1973) *C.R.C. Crit. Rev. Fd Technol.* **4,** 329.
Labuza, T.P. (1968) *Food Technol., Champaign,* **22,** 263.
Loncin, M., Bimbenet, J.J. & Lenges, J. (1968) *J. Fd Technol.* **3,** 131.
Makower, B. & Dye, W.B. (1956) *J. agric. Fd Chem.* **4,** 72.
Palnitkar, M.P. & Heldman, D.R. (1971) *J. Fd Sci.* **36,** 1015.
White, G.W. & Cakebread, S.H. (1966) *J. Fd Technol.* **1,** 73.

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J. Fd Technol. (1975) **10,** 393–405.

Further studies on ginger storage in salt brine

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Summary

Green ginger rhizome storage in acidified salt brines with and without yeast fermentation, and in sodium metabisulphite/acid preservative solutions, was investigated. In unpreserved salt brines (7–9%) short vigorous yeast fermentations were obtained within the first sixteen days of storage.

After a commercial manual grading and dicing procedure, it was found that mean ginger drained weights from vats containing sulphuric acid were significantly lower than those from either citric or acetic acid vats; and further, diced first grade ginger from sulphuric acid vats had a lower mean recovery after syruping than that from citric or acetic acid vats.

Panel evaluations revealed a preference for a sulphur dioxide-bleached colour in syruped ginger. Judges generally unfamiliar with ginger products could not detect any significant differences in flavour and texture; however, judges from a commercial ginger factory showed a preference in flavour and texture for syruped ginger which came from sulphuric or acetic acid storage vats.

Introduction

Investigations were carried out during 1969 on the characteristics of green ginger stored in salt brines with and without fermentation (Brown & Lloyd, 1972) and comparisons were made with green ginger stored in preservative solutions (sodium metabisulphite/acid). Vigorous natural and acid-controlled yeast fermentations were obtained in salt brines, but brined ginger did not support a lactic acid fermentation because of lack of sufficient nutrients.

It was thought that a further study of yeast fermentations in acidified brines might provide useful information on the textural changes, flavour development and drained weight recovery of ginger stored in salt brine, in comparison with ginger stored in metabisulphite/acid preservative solutions, as well as the subsequent processing characteristics of the first-grade syruped ginger. The study also provided an opportunity to obtain basic information, not available in the literature, on the types and characteristics of yeast occurring in such storage media for ginger (Lloyd, 1975).

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Experimental: technological aspects

Ginger rhizomes were first washed in a rotary washer to remove skin, adhering soil and other extraneous matter. Five brining treatments were studied, each comprising three vats as shown in Table 1.

TABLE 1. Experimental details of green ginger rhizome storage

Chemical treatment	Vat. no.	Initial pH	Adjusted pH after 16 days	Acid used	0·2% SO_2 added initially	0·2% SO_2 added and maintained after 16 days
Salt brines						
A0	1	3·5	3·0	Acetic	No	No
	2	3·5	3·0	Citric	No	No
	3	3·5	3·0	Sulphuric	No	No
A1	4	3·5	3·0	Acetic	No	Yes
	5	3·5	3·0	Citric	No	Yes
	6	3·5	3·0	Sulphuric	No	Yes
A2	7	3·0	3·0	Acetic	No	No
	8	3·0	3·0	Citric	No	No
	9	3·0	3·0	Sulphuric	No	No
A3	10	3·0	3·0	Acetic	Yes	Yes
	11	3·0	3·0	Citric	Yes	Yes
	12	3·0	3·0	Sulphuric	Yes	Yes
Sodium metabisulphite acid solutions						
A4	13	3·0	3·0	Acetic	Yes	Yes
	14	3·0	3·0	Citric	Yes	Yes
	15	3·0	3·0	Sulphuric	Yes	Yes

Treatments A0, A1, and A2 (salt brine) were designed to allow yeast fermentation in the storage brines, whereas treatment A3 (salt brine) and A4 (metabisulphite/acid) were formulated with sulphur dioxide (SO_2) initially present to prevent fermentation.

For each of the salt brine treatments, 30 lb of washed ginger was immersed in 40 lb of 10% salt solution (Brown & Lloyd, 1972) acidified with acetic, citric or sulphuric acid as shown in Table 1 and allowed to equilibrate for two days. Treatments A0, A1 and A2, A3 were initially acidified to pH 3·5 and pH 3·0 respectively. After equilibration, salt concentration was adjusted to 7% and maintained at that concentration for one week. Salt concentration was then increased at the rate of 1% per week. After sixteen days yeast fermentation was complete, and the pH and SO_2 concentration in the brines were adjusted as shown in Table 1. After nine weeks, salt concentration had

been increased to 16% and was maintained thereafter by fortnightly analysis and adjustment for the ten-month storage period. Treatment A4 (vats 13, 14 and 15), which contained no salt, was formulated with the appropriate acid and SO_2 (0·2%) as reported previously (Brown & Lloyd, 1972).

Chemical and physical analyses

The following chemical and physical determinations were made: pH, total sulphur dioxide (SO_2) by the method of Shipton (1954) and per cent salt (NaCl) by the A.O.A.C. method (1970).

Mean penetrometer values on drained syruped ginger were determined from twenty readings by Magness penetrometer with a 4·00–mm diameter tip.

Drained weight was determined by draining each batch of ginger on ¼–inch wire mesh screens for 5 min, and weighing the ginger.

Post-storage treatments

Grading. After approximately ten months' storage, ginger from each vat was transferred to a ginger processing factory, where skilled factory personnel diced and sorted each batch of ginger by normal commercial methods into the following grades*: 1st (commercially fibre-free); 2nd (including fibrous or otherwise downgraded 1st grade); 4th (including fibrous necks and stems); tips; waste. Each grade was then weighed.

Leaching. Because confectionery ginger is confined to 1st grade, only the individual batches of 1st grade diced ginger were leached in several changes of cold acidified water (citric acid, 4·00 pH) over a period of 24 hr, and the drained weight of each batch determined in preparation for boiling and syruping.

Boiling and syruping: commercial factory. Duplicate 8-lb batches of 1st grade leached, diced ginger from each vat were boiled for 1 hr in water in a commercial vat. The ginger batches were then quickly immersed in cold water for 20 min, drained, weighed and placed in a commercial vat for syruping. They were syruped by the commercial process in acidified (4·00 pH) sucrose/invert syrup over a period of twelve days to a final syrup concentration of 72% soluble solids. Drained weight was determined on each batch of ginger.

Technological results were analysed statistically, treatment identification being as follows. A: SO_2/pH treatments (A0 vats: 1–3; A1 4–6; A2: 7–9; A3: 10–12; A4: 13–15); B: acid type (B0: acetic acid vats; B1: citric acid vats; B2: sulphuric acid vats).

Experimental: taste panel evaluations

Drained syruped ginger from each of the fifteen vats was presented on two separate occasions to a panel of nineteen tasters at the Sandy Trout Food Preservation Research

* Improved commercial grading techniques now incorporate 3rd grade ginger into 2nd grade.

Laboratory, Hamilton and to a panel of twenty-one tasters at a ginger factory. The tasters were asked to rate each sample for colour, flavour and texture on an hedonic scale from 0 (dislike extremely) to 9 (like extremely). The results were analysed statistically, treatment identification being as described previously.

Results and discussion

Visual observations

In acetic acid vats 1 and 7, a distinct pink discolouration was produced soon after the ginger was immersed in the brine, and this colour remained throughout storage. The pink discolouration was present in vat 4 until 0·2% sulphur dioxide was added and thereafter, as in vat 10, the brine cleared and the ginger became bleached.

A similar mild pink discolouration was noted also in citric acid vats 2, 5 and 8, although with prolonged storage, both the ginger and brine in vats 2, 5 and 8 became a dark murky grey colour

The sulphuric acid vats 3, 6 and 9 developed a murky grey discolouration soon after the ginger was immersed, and this became more pronounced with storage. In all vats where sulphur dioxide was introduced (Table 1), the discolouration in both ginger and brine was removed, and yeast and mould activity was completely inhibited (Lloyd, 1975).

Ginger from treatment A0 (vats 1–3) and A2 (vats 7–9) in which no sulphur dioxide was used, was dark and discoloured. Ginger in treatment A1 (vats 4–6) where sulphur dioxide was added after 16% salt concentration was reached, had an acceptable colour, but was darker than ginger from treatment A3 (vats 10–12) which had a bleached translucent appearance; whereas ginger from preservative solution treatment A4 (vats 13–15) had an opaque bleached appearance which was typical of that treatment.

Microbiological aspects

Vigorous yeast fermentation developed within the first sixteen days of storage in eight of the vats 1–9, the exception being vat 7 (acetic acid to pH 3·0 from commencement), which showed much less fermentation throughout the first sixteen days presumably due to the high percentage of the weaker acetic acid necessary to achieve 3·0 pH in the brine. No notable differences were observed in yeast fermentations in other vats up to sixteen days (Lloyd, 1975).

After three months storage, vats 1, 4, 5, 6 and 7 were clear and apparently stabilized microbiologically, whereas vats 2, 3, 8 and 9 showed greenish grey mould covering the entire surface of the brine. The citric acid vats 2 and 8 had much heavier surface mould growth than the sulphuric acid vats 3 and 9. This, together with the fact that the pH

of the citric acid brines was frequently above 3·0 at fortnightly adjustments, indicated that citric acid was being metabolized by the mould.

Technological aspects

Commercial manual grading. In considering the recovery of first grade ginger after leaching (Table 2), treatments A3 (vats 10–12) and A4 (13–15) with no fermentation had significantly higher recoveries than treatments A0, A1 and A2 in which yeast fermentations occurred. The poor recovery of first grade ginger from treatments A0, A1 and A2 (i.e. vats 1–9) is reflected in the higher percentages of second grade ginger for these three treatments. Fermentation during ginger storage in these vats had a detrimental effect on recovery of first grade ginger, the best result being obtained from treatment A1 (vats 4–6).

It was evident (Table 2) that overall the use of sulphuric acid (treatment B2) was detrimental to recovery of first grade ginger, particularly in comparison with the use of acetic acid (treatment B0).

No significant differences between treatments or between acid types were established for tips and waste. This indicated that in the case of tips, treatment was severe enough to result in any excessive degradation of the ginger rhizomes to produce an abnormal percentage of waste in any vat.

TABLE 2. Recovery (as percentage of original weight) after commercial manual grading of stored ginger

Vat. no.	Grade of ginger					
	1st		2nd	4th	Tips	Waste
	After grading	After leaching				
1	74·3	65·4	11·3	6·3	4·7	4·7
2	67·0	61·7	17·6	6·0	4·3	4·7
3	66·0	57·9	14·7	7·0	5·3	6·3
4	81·0	73·8	11·3	3·7	4·7	5·3
5	76·3	70·0	11·7	5·7	5·3	5·3
6	74·0	68·3	16·3	4·3	4·3	4·7
7	76·0	70·0	11·3	5·7	4·7	5·7
8	70·3	65·0	13·3	7·0	5·7	7·0
9	69·0	63·3	17·6	6·3	4·3	4·3
10	81·3	74·0	12·0	4·0	4·3	5·3
11	83·7	76·3	6·3	4·7	6·3	5·7
12	82·7	75·2	11·3	4·0	4·3	4·7
13	79·6	78·5	7·3	3·3	5·0	4·3
14	78·3	76·9	9·7	4·3	4·7	4·3
15	76·3	76·0	8·7	3·7	4·3	4·3

Table 2 (*cont.*)

First grade ginger after leaching

A means*

A0	A1	A2	A3	A4
61·67	70·70	66·10	75·17	77·13

SE = 1·11 LSD: 3·63 ($P = 0{\cdot}05$), 5·29 ($P = 0{\cdot}01$).

Sig. diffs: $1 \gg 0$; $2 > 0$; $3 \gg 0, 2$; $4 \gg 0, 1, 2$.
> 2 > 1

B means**

B0	B1	B2
72·34	69·98	68·14

SE = 0·86 LSD: 2·81 ($P = 0{\cdot}05$), 4·09 ($P = 0{\cdot}01$).

Sig. diffs: $0 \gg 2$.

Second grade ginger

A means

A0	A1	A2	A3	A4
14·53	13·10	14·07	9·87	8·57

SE = 1·50 LSD: 4·89 ($P = 0{\cdot}05$), 7·11 ($P = 0{\cdot}01$).

Sig. diffs: $0 > 4$; $2 > 4$.

B means

B0	B1	B2
10·64	11·72	13·72

SE = 1·16 LSD: 3·78 ($P = 0{\cdot}05$), 5·51 ($P = 0{\cdot}01$).

No. sig. diffs.

Fourth grade ginger

A means

A0	A1	A2	A3	A4
6·43	4·57	6·33	4·23	3·77

SE = 0·30 LSD: 0·97 ($P = 0{\cdot}05$), 1·40 ($P = 0{\cdot}01$).

Sig. diffs: $0 \gg 1, 3, 4$; $2 \gg 1, 3, 4$.

B means

B0	B1	B2
4·60	5·54	5·06

SE = 0·23 LSD: 0·75 ($P = 0{\cdot}05$), 1·09 ($P = 0{\cdot}01$).

Sig. diffs: $1 > 0$.

Tips

No significant differences between A means or B means.

Waste

No significant differences between A means or B means.

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* A0–A4: Five treatments applied to vats 1–3, 4–6, 7–9, 10–12, 13–15.

** B0–B2: Use of acetic (vats 1, 4, 7, 10 and 13), citric (vats 2, 5, 8, 11 and 14) and sulphuric acid (vats 3, 6, 9, 12 and 15) respectively.

Weight gain/loss of 1st grade ginger compared with original green ginger weight. Overall percentage weight gain/loss results are shown in Table 3. Within treatments A0–A3, where salt was used, the drained weight of ginger from treatment A3 (vats 10–12, with no fermentation) was significantly higher than that from all other salt treatments. Treatment A1 (vats 4–6) had a higher ginger drained weight than either A0 or A2 in which drained weights were not significantly different. Therefore a brief yeast fermentation controlled by subsequent addition of sulphur dioxide resulted in higher drained weight of ginger than the prolonged yeast fermentations. The lower initial pH in vats 7–9 compared with vats 1–3 did not have a significant effect on ginger drained weight. Treatment A3 was not significantly different from treatment A4 (preservative solutions).

On considering the effect of the three acids (B0, B1 and B2) (Table 3) it was apparent that overall the mean ginger drained weight from sulphuric acid vats 3, 6, 9, 12 and 15 was significantly lower than that from either citric acid (1, 4, 7, 10 and 13) or acetic acid (2, 5, 8, 11 and 14) vats, the means of which were not significantly different. Therefore, as previously noted (Brown, 1972; Brown & Lloyd, 1972) the use of sulphuric acid was detrimental to recovery of ginger after brining.

The overall trend was that the order of preference of use, based on drained weight recovery of first grade ginger, should be acetic > citric > sulphuric, although no significant differences between acetic and citric acid was recorded. The use of sulphuric

TABLE 3. Overall percentage weight gain/loss of ginger (as percentage of original weight) after storage for approximately ten months

Vat no.	After brining	After grading	After leaching	After syruping
1	+4·6	−25·7	−34·7	−21·0
2	+3·3	−33·0	−38·3	−25·3
3	+2·1	−34·0	−42·0	−32·7
4	+7·5	−19·0	−26·3	−10·0
5	+5·8	−23·7	−30·0	−14·3
6	+5·2	−26·0	−31·7	−17·3
7	+5·0	−24·0	−30·0	−14·7
8	+4·2	−29·7	−35·0	−21·0
9	+2·9	−31·0	−36·7	−23·3
10	+7·9	−18·7	−26·0	−10·3
11	+7·9	−16·3	−23·7	−6·0
12	+7·9	−17·3	−24·7	−8·7
13	+1·3	−20·3	−21·3	−7·3
14	+2·1	−21·7	−23·0	−4·0
15	−1·3	−23·7	−24·0	−8·3

Table 3 (*cont.*)

After brining (total ginger weight)

A means

A0	A1	A2	A3	A4
+3·33	+6·17	+4·03	+7·90	+0·70

SE = 0·46 LSD: 1·50 (P=0·05), 2·19 (P=0·01).

Sig. diffs: 0≫4; 1≫0, 4; 2≫4; 3≫0, 2, 4.
 >2 >1

B means

B0	B1	B2
+5·26	+4·66	+3·36

SE = 0·36 LSD: 1·17 (P=0·05), 1·70 (P=0·01).

Sig. diffs: 0≫2; 1 > 2.

After grading (1st grade ginger only)

A means

A0	A1	A2	A3	A4
−30·90	−22·90	−28·23	−17·43	−21·90

SE = 1·31 LSD: 4·27 (P=0·05), 6·22 (P=0·01).

Sig. diffs: 1≫0; 3≫0, 2; 4≫0, 2.
 >2 >1, 4

B means

B0	B1	B2
−21·54	−24·88	−26·40

SE = 1·01, LSD: 3·31 (P=0·05), 4·81 (P=0·01).

Sig. diffs: 0≫2.
 >1

After leaching (1st grade ginger only)

A means

A0	A1	A2	A3	A4
−38·33	−29·33	−33·90	−24·80	−22·77

SE = 1·10 LSD: 3·59 (P=0·05), 5·22 (P=0·01).

Sig. diffs: 1≫0; 2 > 0; 3≫0, 2; 4≫0, 1, 2.
 >2 >1

B means

B0	B1	B2
−27·66	−30·00	−31·82

SE = 0·85 LSD: 2·78 (P=0·05), 4·04 (P=0·01).

Sig. diffs: 0≫2.

After syruping (1st grade ginger only)

A means

A0	A1	A2	A3	A4
−26·33	−13·87	−19·68	−8·33	−6·53

SE = 1·80 LSD: 5·89 (P=0·05), 8·56 (P=0·01).

Sig. diffs: 1≫0; 2 > 0; 3≫0, 2; 4≫0, 2.
 >1

B means

B0	B1	B2
−12·66	−14·12	−18·06

SE = 1·40 LSD: 4·56 (P=0·05), 6·63 (P=0·01).

Sig. diffs: 0 > 2.

acid has been consistently shown to have detrimental effects on drained weight recovery of ginger after storage.

After syruping, the same effects were evident for both treatment (A means) and acid type (B means).

Syruping characteristics. On considering the weight gain of first grade ginger after syruping as a percentage of its pre-syruping weight (Table 4), no significant differences

TABLE 4. Percentage weight gain by syruping (as percentage of pre-syruping weight of first grade ginger only)

Vat no.	% Weight gain
1	20·8
2	21·1
3	16·4
4	22·0
5	22·5
6	20·8
7	21·7
8	21·6
9	20·7
10	21·1
11	23·3
12	21·3
13	19·9
14	24·7
15	20·4

A means

A0	A1	A2	A3	A4
19·43	21·77	21·33	21·90	21·67

SE=0·77; LSD: 2·51 ($P=0\cdot05$), 3·66 ($P=0\cdot01$).
No sig. diff.

B means

B0	B1	B2
21·10	22·64	19·92

SE=0·60; LSD: 1·95 ($P=0\cdot05$) 2·83 ($P=0\cdot01$).
Sig. diffs: $1>2$.

were established among the five treatments A0–A4. Thus, it appeared that leached first grade ginger was unaffected by the presence or absence of yeast fermentation in its capacity to absorb syrup during processing. However, on considering the different types of acid similarly, B2 (sulphuric acid vats) had significantly lower weight gain than B1 (citric acid vats). It would appear that textural changes occurring in the

sulphuric acid vats during storage, adversely affected the capacity of leached first grade ginger to absorb syrup.

No statistical differences between treatments or acid types were apparent from the penetrometer values of syruped first grade ginger (mean values 2·2–2·5 lb/sq. in 'with' fibre and 2·7–3·0 lb/sq. in 'against' fibre) and no correlation could be established with taste panel evaluations of texture.

Taste panel evaluations

Syruped ginger (or any other ginger confection) is a difficult food to evaluate organoleptically because of the overwhelming sensory effect of 'hotness', which might easily have a masking effect on the palate of a taster, particularly one unfamiliar with the product. It was anticipated that an interesting comparison might have become evident between the laboratory tasters (generally unfamiliar organoleptically with syruped ginger) and the experienced staff at a ginger factory.

Colour. This was simply a visual rating of each sample and from Table 5 it was evident that the results from the laboratory and the factory were very similar. Treatments A1, A3 and A4, in which sulphur dioxide produced a bleached appearance, and the use of acetic acid (B0) in lieu of citric or sulphuric acids, were preferred.

Flavour and texture. Results of the flavour and texture evaluations (Tables 6 and 7 respectively) were very similar, with the factory tasters detecting quite significant

TABLE 5. Statistical analyses—subjective colour evaluation

(a) Means of colour rating with respect to chemical treatments A0–A4 (vats 1–3, 4–6, 7–9, 10–12, 13–15, respectively)

	A0	A1	A2	A3	A4
Laboratory	4·69	7·24	4·78	7·66	7·61
	SE = 0·20; LSD: 0·62 (P=0·05), 0·86 (P=0·01).				
	Sig. diffs: 1≫0, 2; 3≫0, 2; 4≫0, 2.				
Factory	2·83	5·69	2·57	7·29	7·69
	SE = 0·22; LSD: 0·67 (P=0·05), 0·92 (P=0·01).				
	Sig. diffs: 1≫0, 2; 4≫0, 1, 2.				

(b) Means of colour rating with respect to acid type (B0: acetic; B1: citric; B2: sulphuric)

	B0	B1	B2
Laboratory	6·88	5·98	6·33
	SE = 0·16; LSD: 0·48 (P=0·05), 0·67 (P=0·01).		
	Sig. diffs: 0≫1, 2.		
Factory	5·80	4·75	5·09
	SE = 0·17; LSD: 0·52 (P=0·05), 0·72 (P=0·01).		
	Sig. diffs: 0≫1.		
	>2.		

TABLE 6. Statistical analyses—subjective flavour evaluation

(a) Means of flavour rating with respect to chemical treatments A0–A4 (vats 1–3, 4–6, 7–9, 10–12, 13–15, respectively)

	A0	A1	A2	A3	A4
Laboratory	7·26	7·45	7·22	7·53	7·52
	SE = 0·10; LSD: 0·30 (P = 0·05), 0·42 (P = 0·01).				
	Sig. diffs: 3 > 2.				
Factory	5·51	6·20	5·78	6·36	6·47
	SE = 0·18; LSD: 0·56 (P = 0·05), 0·79 (P = 0·01).				
	Sig. diffs: 1 > 0; 3 ≫ 0; 4 ≫ 0.				
	> 2.				

(b) Means of flavour rating with respect to acid type (B0: acetic; B1: citric; B2: sulphuric)

	B0	B1	B2
Laboratory	7·45	7·38	7·35
	SE = 0·08; LSD: 0·24 (P = 0·05), 0·33 (P = 0·01)		
	No sig. diffs.		
Factory	6·31	5·74	6·13
	SE = 0·14; LSD: 0·44 (P = 0·05), 0·61 (P = 0·01).		
	Sig. diffs: 0 > 1.		

TABLE 7. Statistical analyses—subjective texture evaluation

(a) Means of texture rating with respect to chemical treatments A0–A4 (vats 1–3, 4–6, 7–9, 10–12, 13–15, respectively)

	A0	A1	A2	A3	A4
Laboratory	6·64	6·88	6·64	6·81	6·82
	SE = 0·13; LSD: 0·38 (P = 0·05), 0·53 (P = 0·01).				
	No sig. diffs.				
Factory	5·52	6·21	5·79	6·37	6·43
	SE = 0·15; LSD: 0·46 (P = 0·05), 0·63 (P = 0·01).				
	Sig. diffs: 1 ≫ 0; 3 ≫ 0; 4 ≫ 0, 2.				
	> 2.				

(b) Means of texture rating with respect to acid type (B0: acetic; B1: citric; B2: sulphuric)

	B0	B1	B2
Laboratory	6·67	6·70	6·90
	SE = 0·10; LSD: 0·29 (P = 0·05), 0·41 (P = 0·01).		
	No sig. diffs.		
Factory	6·29	5·78	6·12
	SE = 0·12; LSD: 0·35 (P = 0·05), 0·49 (P = 0·01).		
	Sig. diffs: 0 ≫ 1.		

differences. The most preferred chemical treatments were A1, A3 and A4, in which sulphur dioxide was used to totally inhibit yeast and mould growth, which, according to the results for A0 and A2, appear to adversely affect syruped ginger flavour and texture. This is further emphasized by the factory tasters' relative dislike for the use of citric acid (B1), including vats 2 and 8 which had the heaviest surface mould growth during storage. In considering texture, however, it has been observed previously (Brown, 1972; Brown & Lloyd, 1972) that sulphuric acid in particular causes tissue softening, while the use of citric acid and salt brine results in firm crisp ginger. In commercial practice, it has been observed that tissue softening occurs whenever ginger is stored in sulphuric-acidified preservative solutions. The factory tasters' relative dislike for citric acid usage (B1) may reflect a preference for a soft-textured product (analogous to the commercial syruped ginger) over a crisper and firmer product. From a commercial viewpoint, however, the softer-textured product may, of course, be preferred.

In considering the laboratory evaluations of flavour and texture, there was again a trend in preference for treatments A1, A3 and A4 over treatments A0 and A2, although no significant differences were established. The most apparent trend in the results of acid type was also a relative dislike for the use of citric acid, possible for the same reasons as mentioned previously, viz. the effect of mould on flavour, and the relative firmness of ginger from citric acid vats.

Conclusions

There appear to be no obvious advantages in yeast fermentation during storage of green ginger rhizomes in salt brines. In fact it has serious disadvantages in promoting lower recoveries of sound ginger, and darker, less attractive ginger with relatively poor flavour and texture in comparison with ginger from smiliar salt brines with added sulphur dioxide, or from sodium metabisulphite/acid preservative solutions.

Microbiologically, ginger storage in acidified salt brines without sulphur dioxide is unusual technologically in that the yeast fermentation is primary and little if any lactic fermentation occurs. In unpreservatized brines the initial yeast fermentation was followed by heavy mould growth.

Mean ginger drained weights from vats containing sulphuric acid were significantly lower than those from either citric or acetic acid vats; further, ginger from sulphuric acid vats had a lower mean drained weight gain of syruped ginger than that from citric or acetic acid vats.

Overall, in terms of recovery of 1st grade ginger, the order of preference of the three acids used was acetic > citric > sulphuric; ginger can be stored in either salt brines or preservative solutions, provided sulphur dioxide is added to totally inhibit yeast and mould growth.

Taste panel evaluations of flavour and texture revealed that familiarity with ginger products was necessary to detect significant organoleptic differences among the different

treatments. Experienced and inexperienced judges demonstrated a visual preference for syruped ginger which had been stored in brines containing sulphur dioxide and acetic acid; however, only the experienced judges from a ginger factory were able to determine significant differences in flavour and texture, with a preference for syruped ginger from sulphuric or acetic acid storage vats.

Acknowledgments

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References

Association of Official Agricultural Chemists (1970) *Official Methods of Analysis of the Association of Official Agricultural Chemists*, 11th edn, p. 296.
Brown, B.I. (1972) *J. Fd Technol.* **7,** 153.
Brown, B.I. & Lloyd, A.C. (1972) *J. Fd Technol.* **7,** 309.
Lloyd, A.C. (1975) *J. Fd Technol.* **10,** 407.
Shipton, J. (1954) *Fd Preserv. Q.* **14,** 54.

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Yeasts in ginger storage brines

ANNICE C. LLOYD

Summary

When green ginger rhizomes were stored in acidified salt brines (7–9%) a short vigorous yeast fermentation was obtained. The yeasts present and the changes in yeast flora during the fermentation period were investigated.

Fifty-seven yeast cultures isolated from the brines were classified in the following genera: *Candida* (24 isolates), *Pichia* (21 isolates), *Hansenula* (11 isolates) and *Rhodotorula* (1 isolate). During the early part of the fermentation, *Candida* species (mainly *C. tropicalis*) predominated, whereas towards the end of fermentation the more salt tolerant *Pichia* species (e.g. *P. ohmeri*) predominated.

Introduction

Comparisons of green ginger storage in salt brines and in preservative solutions (sodium metabisulphite/acid) have previously been reported (Brown & Lloyd, 1972). In acidified and non-acidified salt brines vigorous yeast fermentations were observed, but the brined ginger would not support a lactic acid fermentation due to lack of sufficient nutrients. A further study of ginger storage in acidified salt brines (Brown, 1975) provided an opportunity to investigate the types and characteristics of yeasts present.

Experimental

Brine formulation and sampling

Green ginger rhizomes were stored in acidified salt brines and in preservative (sodium metabisulphite/acid) solutions as described in the preceding paper (Brown, 1975). Active yeast fermentations occurred in nine salt brine vats (set A0 (vats 1–3), set A1 (vats 4–6), set A2 (vats 7–9)) over a period of sixteen days. During this time brine pH was maintained at 3·0 (set A2) or 3·5 (set A0 and A1) and salt concentration was increased incrementally from 7 to 9% in all vats at the rate of 1% per week. The first, second and third vats in each set were acidified to the required pH with acetic, citric and sulphuric acids respectively.

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Brines (1–9) were stirred and sampled after 1, 2, 3, 6, 8, 13, 16, 18 and 25 days. No attempt was made to distinguish between surface and subsurface growth. At the end of active fermentation (sixteen days) brines in all nine vats were adjusted to 10% salt and pH 3·0 and metabisulphite (0·2%) was added to vats 4, 5 and 6. In the remaining six brines, which contained no metabisulphite, yeast counts were discontinued after twenty-five days due to heavy mould growth.

Yeast isolation and identification

Total yeast counts were carried out with Wickerham's glucose—yeast extract—malt extract agar (YM agar) (Wickerham 1951) acidified with sterile 6% tartaric acid at the rate of 5 ml/100 ml to give a final pH 3·5–3·7.

At each sampling time, brines were also streaked on Wickerham's media, with and without 7% salt, to determine if any obligate halophilic yeasts were present. After three days at 30°C well isolated colonies of Wickerham's media were examined with a stereo-micrsocope and cells from representative colonies were examined in wet mounts. As this investigation was only intended to be of a qualitative nature, no attempt was made to identify every different colony from every brine plate at each sampling time. Instead, the above preliminary examination of colonies served to demonstrate the approximate proportions of different yeasts present in each brine at each sampling date. A limited number of representative colonies from each set of plates was subcultured for later identification and classification according to the primary tests of Lodder (1970).

TABLE 1. Yeasts in ginger storage brines

Yeast type	No. representative isolates identified	Days on which most isolations were made	Growth in salt media (%)		
			10	15	20
Rhodotorula rubra (Demme) Lodder	1	1–2	1+	—	—
Candida tropicalis (Cast.) Berkhout	15	2–8	3+	—	—
Candida krusei (Cast.) Berkhout	3	8–16	3+	—	—
Candida rugosa (Anderson) Diddens et Lodder	6	8–16 (mainly vat 7)	3+	—	—
Hansenula anomola (Hansen H. et P. Sydow)	11	8–16	3+	3+/—	—
Pichia membranaefaciens (Hansen)	2	8–16	—	—	—
Pichia ohmeri (Etchells & Bell) v. Rij.	19 (including 15 haploid strains)	13—16	3+	3+	—

Confirmation of classification to the species level of a single typical isolate from each group shown in Table 1 was provided by the Centraalbureau voor Schimmelcultures, Delft.

Salt tolerance was estimated by growth in YM broth containing 0, 10, 15 and 20% salt. After six days turbidity was read against a white card on which black lines approximately $\frac{3}{4}$ mm wide were drawn. Growth was rated according to the following scale (Wickerham, 1951):

3 + : lines completely obscured;
2 + : lines visible as diffuse bands;
1 + : lines distinguished but with indistinct edges;
– : clear lines and no growth.

A qualitative check on the presence or absence of Lactobacillus species was made by streaking undiluted brines on to the agar of de Man, Rogosa & Sharpe (1960) with bromocresol green indicator and observing for acid-forming colonies (Brown & Lloyd, 1972).

Results

Small numbers of lactobacilli were present in all brines up to three days, which is in agreement with previous results (Brown & Lloyd, 1972) where it was found the lactobacilli, although present in salt brined ginger, are unable to produce a lactic acid fermentation due to lack of suitable nutrients.

Vigorous yeast fermentation developed in eight out of nine ginger brines, the exception being vat 7 (adjusted to pH 3·0 with acetic acid) in which fermentation was greatly reduced probably due to the high percentage (approx. 1%) of acetic acid necessary to achieve pH 3·0 in the brine. Brines adjusted to pH 3·0 with citric (approx. 0·15%) and sulphuric (approx. 0·08%) showed no reduction in fermentation compared with brines at pH 3·5. Total yeast counts in all vats reached a maximum after about eight days. (Figs 1, 2 and 3).

At the end of active fermentation (sixteen days), all brines were adjusted to pH 3·0 and 10% salt, and 0·2% SO_2 was added to vats 4, 5 and 6, the SO_2 producing an immediate inhibitory effect on fermentation. Reduction in pH of brines 1, 2 and 3 from 3·5 to 3·0 had a slight retarding effect on yeast growth. Total counts in vats 8 and 9 remained relatively unaltered up to twenty-five days when all counts were discontinued due to excessive mould growth on the surface of these brines. The effects of ten months' storage, following initial fermentation, on the recovery, texture, flavour and colour of the brined ginger are described elsewhere (Brown, 1975).

The numbers and types of brine yeasts identified, together with their salt tolerances and the days on which they were isolated are shown in Table 1. None of the fifty-seven isolates was found to have an obligate requirement for salt in the media. Twenty-four of the ginger brine isolates were classified in the genus *Candida*, the majority (fifteen) of

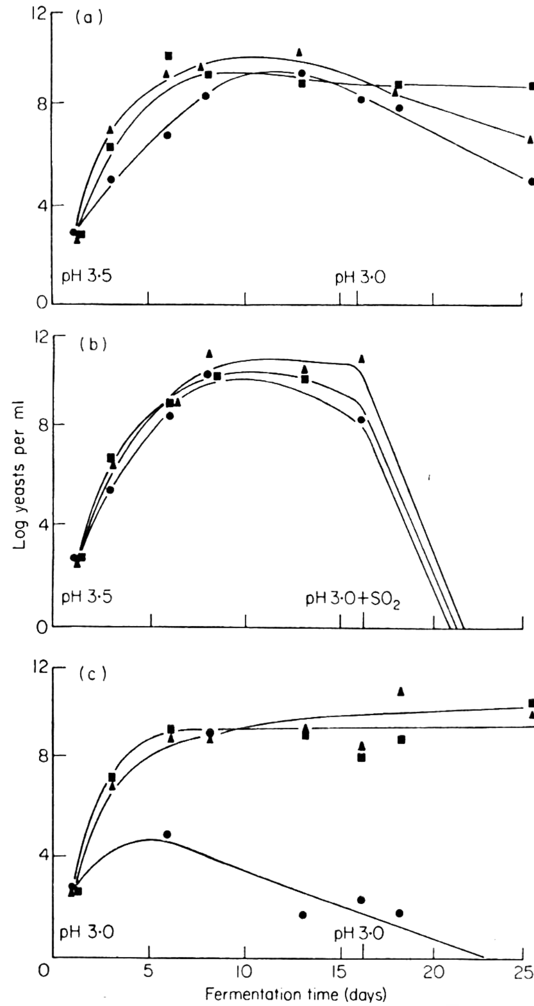

FIG. 1. Yeast growth in ginger brines. (a) Set A0. ●: acetic, vat 1; ■: citric, vat 2; ▲: sulphuric, vat 3. (b) Set A1. ●: acetic, vat 4; ■: citric, vat 5; ▲: sulphuric, vat 6. (c) Set A2. ●: acetic, vat 7; ■: citric, vat 8; ▲: sulphuric, vat 9.

these belonging to the species *C. tropicalis*. Six of the isolates appeared to be closely related to *C. rugosa* except for their ability to ferment glucose, which is reportedly absent in this species (Lodder, 1970). The remaining three *Candida* isolates which produced distinctive curved mycelium on Dalmau plates of corn meal agar were classified as *C. krusei*. The second largest group of isolates belonged to the genus *Pichia*; *P. membranaefaciens* (two) and *P. ohmeri* (nineteen). Of the nineteen cultures of *P. ohmeri*, only four were observed to sporulate, the other isolates being confirmed by Centraalbureau voor Schimmelcultures as haploid strains of the species. Eleven nitrate

assimilating isolates placed in the genus *Hansenula* were the most varied in morphological and biochemical properties. Nine of these isolates were classified as *H. anomala*, var *anomala* although only four of these were observed to spore giving rise to one or two smooth hat shaped or round spores per ascus. The five non-sporing cultures differed further from the sporing isolates in that they failed to produce even a primitive pseudomycelium on Dalmau plates. Two other sporing isolates also classified as *H. anomala* differed slightly from the other strains in their fermentative ability.

The proportional distribution of different yeasts present during the fermentation was remarkably similar in all brines studied, with the exception of vat 7. This brine had the highest level of acid, fermentation was retarded and there appeared to be a marked predominance of *C. rugosa* towards the end of fermentation.

Only one *Rhodotorula* culture was subjected to detailed identification, but similar pink isolates were present in large numbers in all brines early in the fermentation (1–2 days): *Rhodotorula* species, however, quickly disappeared as would be expected in view of the low salt tolerance of *R. rubra* which grew poorly in 10% salt media and which would consequently have little significance in the flora of long-term storage brines. *Candida* species predominated during the greater part of the fermentations. In broth media all three species of *Candida* grew well in 10% salt but not in 15% or 20% salt. *C. tropicalis* predominated at the beginning of fermentation (2–8 days) and *C. krusei* and *C. rugosa* appeared later in the fermentation period (8–16 days). All isolates of *P. ohmeri* grew well in both 10% and 15% salt and formed a characteristic heavy crinkly film in brine or liquid salt media. This yeast was present in large numbers in all brines except vat 7 on the last two sampling days (13–16 days). *H. anomala* isolates grew well in 10% salt but varied in their ability to grow in 15% salt. The two isolates of *P. membranaefaciens* were the only species isolated from the brines which failed to grow in 10% salt media. *Hansenula* species and *P. membranaefaciens* did not appear to predominate in the brines at any stage but were isolated throughout the latter half of the fermentation period.

Discussion

The yeasts occurring in various brined vegetable products during fermentation and storage have been extensively investigated, but yeasts present in salt brined green ginger have not previously been reported. The salt brining treatments studied here differ from the normal commercial treatments for cucumbers and olives because the brined ginger will not support an active lactic acid fermentation. (Brown & Lloyd, 1972). The ginger brines are, however, acidified initially which enables them to be compared microbiologically with other vegetable brines, in which the lactic fermentation and not the yeast fermentation is of primary importance. Although the active fermentation period (sixteen days) for acidified brined ginger was very short compared with normal commercial operations for other products, a definite similarity can be demonstrated between yeasts present and those reported for other brines of comparable

salt concentration. The ginger brines studied here covered the intermediate salt concentration. range (7–9%), and contained some yeasts present in both higher and lower salt brines reported elsewhere.

Etchells & Bell (1950a), in examining high strength (10–18%) commercial cucumber brines found that *Torulopsis* and *Brettanomyces* species constituted approximately 90% of the subsurface yeast isolates. The predominant film forming yeasts in such brine belonged to the genus *Debaryomyces* (Etchells & Bell, 1950b). However, no representatives of these genera were isolated from ginger brines. Etchells & Bell (1950a, b) also obtained a number of isolates of *Endomycopsis* from brine films and these were classified as a new species, *E. ohmeri*. These yeasts which formed a heavy wrinkled film in 15% salt media were later found to be heterothalic and were reclassified as *Pichia ohmeri* (Kreger van Rij, 1963). Fifteen of the isolates from brined ginger were found to be haploid strains of the same species. An additional four sporing strains of *P. ohmeri* were also isolated. The ginger brine isolates also included eleven strains of *Hansenula anomala* which have previously been reported as normally present in high strength brines (Etchells & Bell, 1950a).

Candida tropicalis and *C. krusei* which predominated early in the ginger fermentation have also been reported as the predominate subsurface isolates from low salt (5%) cucumber brines. (Etchells, Borg & Bell, 1961). In green olive brines of comparable strength (6·5%), Mrak *et al.* (1956) found that during a sixteen week fermentation period, fermentative yeasts, (mainly *C. krusei*), predominated in the first seven weeks, followed by more aerobic types (mainly *P. membranaefaciens*) during the last part of fermentation. Both these species were present in small numbers, but in a similar pattern in ginger brines.

Conclusion

When green ginger is stored in acidified brines (7–9% salt), a short yeast fermentation occurs with *Candida* species predominating early in the fermentation followed mainly by *Pichia* species towards the end of fermentation.

Hence the general yeast flora of salt brined ginger does not differ greatly from that reported for other low salt brine vegetable products, but the value of allowing such fermentation to occur during storage must be assessed in terms of recovery, and acceptability of the final processed product.

Acknowledgments

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References

Brown, B.I. (1975) *J. Fd Technol.* **10,** 393.
Brown, B.I. & Lloyd, A.C. (1972) *J. Fd Technol.* **7,** 309.
Etchells, J.L. & Bell, T.A. (1950a) *Farlowia,* **4,** 87.
Etchells, J.L. & Bell, T.A. (1950b) *Fd Technol., Champaign,* **4,** 77.
Etchells, J.L. Borg, A.F. & Bell, T.A. (1961) *Appl. Microbiol.* **9,** 139.
Kreger van Rij, N.J.W. (1963) *A taxonomic study of the yeast genera Endomycopsis, Pichia & Debaromyces.* Thesis, University of Leiden.
Lodder, J. (1970) *The Yeasts—a Taxonomic Study,* 2nd edn. North-Holland. Amsterdam.
Man, J.C. de, Rogosa M. & Sharpe, M.E. (1960) *J. appl. Bact.* **23,** 130.
Mrak, E.M., Vaughn, R.H., Miller, M.W. & Phaff, H.J. (1956) *Fd. Technol., Champaign,* **16,** 416.
Wickerham, L.J. (1951) *Tech. Bull. U.S. Dep. Agric.* **1029.**

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Effects of γ-irradiation on quality and enzyme activities of prepacked cut chicory

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Summary

The effects of γ-irradiation on quality and enzyme activities of prepacked cut chicory were investigated. Freshly cut slices of chicory were washed, centrifuged and packed in polythene bags. These samples were γ-irradiated and stored in the dark at 10 °C. The samples were tested organoleptically and the peroxidase and polyphenoloxidase activities were determined. The chlorogenic acid content was also measured.

The results showed that, as far as the keeping quality of the prepacked chicory was concerned, 100 krad irradiation was superior to both the 0 krad and the 300 krad treatment, but irradiation in perforated bags (high O_2 content) increased discolouration (browning). Peroxidase and polyphenoloxidase activities did not change for about two days after irradiation in the perforated or non-perforated bags. These activities increased strongly in the non-irradiated series. Some chicory was treated with cystein and NaCl in order to inhibit some of the discolouration, but rotting of this sample occurred sooner than in non-irradiated samples. No difference in the chlorogenic acid content was found as a result of irradiation.

Introduction

Discolouration which appears as browning of the injured surface of plants such as cut fruits and vegetables, has been studied earlier (Stadtman, 1948; Joslyn, 1951; Joslyn & Braverman, 1954; Romani, 1966). It is generally assumed that the discolouration is due to chemical reactions of polyphenols. These reactions can be divided into two types of mechanism: firstly, enzymatic oxidation or polymerization of phenolic substances by enzymes such as peroxidase or polyphenoloxidase and secondly, a non-enzymatic oxidation or polymerization (Maxie & Abel-Kader, 1966; Mathew & Parpia, 1971).

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Many discolouration properties present problems in food irradiation technology. Examples are, the browning of potatoes (Ogawa *et al.*, 1968; Ogawa & Uritani, 1970), discolouration of cut vegetables (Langerak *et al.*, 1972), internal browning of mushrooms (Langerak, 1973), discolouration of the growth point of onions (van Kooij, Heins & Langerak, 1973), skin browning of bananas (Thomas & Nair, 1971), and surface browning of citrus fruits (Riov, Monselise & Kahan, 1970).

It is generally considered that discolouration following irradiation is caused mainly by non-enzymatic reactions (free radical reactions) (Maxie & Abel-Kader, 1966; Mathew & Parpia, 1971). However, it is not excluded that the discolouration following irradiation may also be caused by enzymatic reactions, because the discolouration does not occur after irradiation when the peroxidase and polyphenoloxidase have been affected by the irradiation (Maxie & Abel-Kader, 1966). It is important to understand the mechanisms of the discolouration phenomena not only from the physiological aspect but also from the food technology aspect.

The present study deals with the extension of shelflife of cut prepacked chicory by γ-irradiation, since it has been found that the leaves of the irradiated cut chicory discolour, therefore decreasing its trade value. To investigate the nature of discolouration in irradiated chicory the factors mentioned above (peroxidase, polyphenoloxidase and polyphenol) were studied. Concerning the polyphenol content, chlorogenic acid was chosen since this compound is supposed to be one of the substrates involved in enzymatic browning (Zucker & Ahrens, 1958).

Material and methods

Material

Commercial chicory was used and the leaves were machine-cut into strips of 5–10 mm thickness. The cut chicory was washed with water for 5 min and centrifuged for 0·5 min ($900 \times g$) before being packed into 0·02-mm-thick polythene bags. The bags used were either with or without two perforations of 2 mm diameter and they were sealed with packaging sealers.

Irradiation and storage

The product was irradiated with γ-rays at 0, 100 and 300 krad (dose rate: about 200 krad/hr) in the pilot plant for food irradiation of the Association Euratom-ITAL. Irradiated samples were stored in the dark for 4–7 days at 10 °C (80% R.H.).

Quality evaluation

The odour, flavour, colour and state of the leaves (state of desiccation and microbiological decay) were evaluated with scores of 1–5, with 1 being very bad and 5 excellent. The trade value was expressed as 0–100 points, with the fresh product having 100 points and the decayed one 0 points (Langerak *et al.*, 1972).

Measurement of enzyme activities (Ponting & Joslyn, 1948; Maehly & Chance, 1954; Chance & Maehly, 1955; Maier & Schiller, 1961; Monselise & Halevy, 1962; Goren & Monselise, 1965)

Preparation of crude enzyme solution. The activities of peroxidase and polyphenoloxidase were measured in crude and appropriately diluted extracts of the chicory. Ten grams of the cut leaves were taken at random and homogenized with 50 ml ice-cooled 0·1 M citrate buffer (pH 6·5) for 2 min in a Turmix homogenizer. Two to three drops of octanol were then added to the homogenate to prevent excessive foaming, and made up to 100 ml with the cold buffer. The homogenate was centrifuged for 5 min at 30 000 × ***g*** with a 5 °C conditioned centrifuge OMEGA-II. After centrifugation, a slightly red supernatant was obtained and kept in an ice bath until required. The peroxidase activity remained stable overnight in this state, but the polyphenoloxidase activity was not stable for longer than 2–3 hr (Kalinov, 1972).

Measurement of peroxidase activity. For the determination of peroxidase activity, the guaiacol test, described by Chance & Maehly (1955) and Maehly & Chance (1954), modified and used. The oxidation of guaiacol, to a brown coloured product catalysed by peroxidase in the presence of H_2O_2 was recorded spectrophotometrically. The reaction mixture contained 1 ml of 3 mM H_2O_2 (prepared each time from 60 m stock solution, stored in the dark and kept cool), 1 ml of 20 mM guaiacol dissolved in 0·1 M citrate buffer (pH 6·5) and 1 ml of the appropriately diluted crude enzyme solution. After vigorously stirring the reaction mixture, the changes in absorbance at 470 nm were measured with a Zeiss spectrophotometer at 25 °C. The increase in absorption at 470 nm was recorded for 1 min and a straight line was obtained. Each spectrophotometric measurement was performed two or three times. A blank test was also run but no colouring occurred.

Measurement of polyphenoloxidase activity. Polyphenoloxidase activity was measured by means of a catechol test (Ponting & Joslyn, 1948; Maehly & Chance, 1954; Chance & Maehly, 1955; Maier & Schiller, 1961), similar to the guaiacol test. The reaction mixture contained 2 ml of 50 mM pyrocatechol in 0·1 M citrate buffer (pH 6·5). To start the enzymatic reaction, 1 ml of the five times diluted enzyme extract was rapidly pipetted into the cell and the line recorded. The first 20–40 sec gave a straight line after which it curved slightly. The change in absorbance was recorded at 420 nm. This test was also repeated two or three times. A blank test was also carried out but no colour formation was detected. The enzymatic activities of peroxidase were expressed as ΔA470/min/ml and those of polyphenoloxidase as ΔA420/40 sec/ml.

Determination of chlorogenic acid

Chlorogenic acid was measured by the method described by Zucker & Ahrens (1958). Twenty grams of sample were crushed with 20 ml of −20 °C cooled acetone and 10 g sea sand in a mortar. After rapid and thorough crushing the mixture was extracted by aspiration filter, washed with cooled acetone and made up to 200 ml. Five millilitres

of the sample extract with acetone was passed through a column, size 1×3 cm of chromatographic Al_2O_3, previously washed with water, and the acetone soluble chlorogenic acid was absorbed. The column was washed with a mixture of 5 ml of 80% ethanol and 10 ml water. Then a mixture of 2 ml of 0·5% $NaNO_2$ and 2 ml of 5% CH_3COOH (prepared just before extraction) was added and the column was again washed with 10 ml of water. Subsequently, 10 ml of 5N NaOH was added and the red coloured substance was dissolved. The column was washed and then filled with 25 ml water. The alkali red solution was measured at 525 nm by the spectrophotometer.

Results and discussion

The chicory irradiated at 100 krad in the perforated polythene bags discoloured more than non-irradiated samples after being stored for two days; its quality was lower than that of non-irradiated samples. This discolouration did not increase after three days, but the control discoloured very badly due to rotting, and its trade value decreased. The 300 krad sample became more discoloured during storage and the trade value became less than the control (Table 1).

In order to compare the discolouration by irradiation and the activities of peroxidase and polyphenoloxidase which are concerned in polyphenol metabolism, the chlorogenic

TABLE 1. Quality evaluation of cut chicory packed in the perforated polythene bags, irradiated and stored in the dark at 10°C.

Dose (krads)	Days				
	0	1	2	3	4
Colour					
0	5	4	3	2	2
100	4–5	3	3	3	2–3
300	3–4	2	2	1	1
Odour					
0	5	5	5	3	2
100	3–4	3–4	3	3	4
300	2–3	2–3	2	2	2
State					
0	5	5	3–4	2	1–2 (rot)
100	5	5	4–5	4	4
300	5	5	5	3	3
Trade value					
0	100	90	75	45	40
100	90	75	70	65	65
300	75	60	55	35	35

acid content and the enzyme activities were measured. The chlorogenic acid content increased between one and four days at each dose, but no significant difference in the effects caused by the dose was found (Fig. 1). On the other hand, the enzyme activities were highly susceptible to irradiation. The peroxidase activity of the control increased acutely after cutting and during storage but in the case of irradiated samples, increases in activity were inhibited during the first two days, after which time they increased

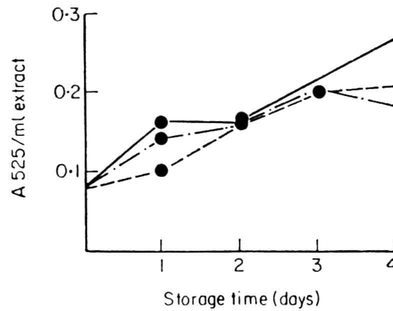

FIG. 1. The influence of γ-irradiation on the chlorogenic acid content of cut chicory. The chicory was packed in perforated polythene bags of 0·02 mm thickness and stored in dark at 10 °C. ——, 0 krad; —·—, 100 krad, - - -, 300 krad.

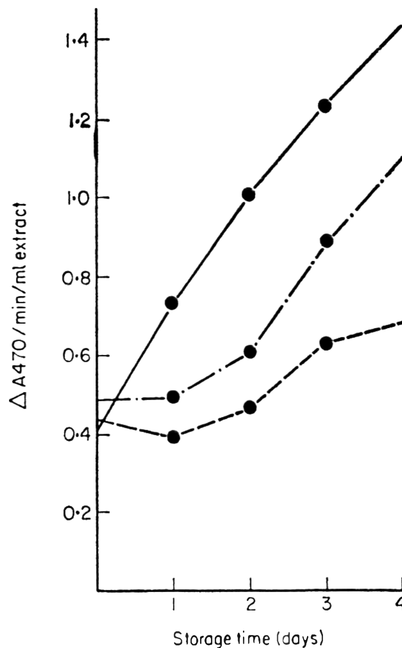

FIG. 2. The influence of γ-irradiation on the peroxidase activity of cut chicory. The chicory was packed in perforated polythene bags of 0·02 mm thickness and stored in dark at 10 °C. ——, 0 krad; —·—, 100 krad; - - -, 300 krad.

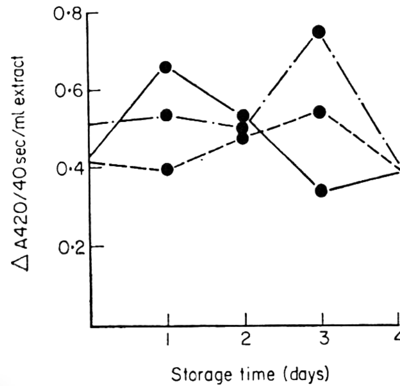

FIG 3. The influence of γ-irradiation on the polyphenoloxidase activity of cut chicory. The chicory was packed in perforated polythene bags of 0·02 mm thickness and stored in dark at 10 °C. ——, 0 krad; —·—, 100 krad; – – –, 300 krad.

gradually (Fig. 2). Polyphenoloxidase of the control had a peak of activity after storage of one day, but the irradiated samples did not reach a peak until they had been stored for three days (Fig. 3). In other words, the increase in activities of the two enzymes was slowed down for two days by irradiation. It was assumed therefore that physiological activity was reduced during this time by irradiation.

As the discolouration of cut chicory in perforated bags increased in spite of the inhibition of enzyme activities by irradiation, it is suggested that irradiation discolouration was non-enzymatic.

In parallel experiments (Langerak & Hovestad, 1973) it was observed that the number of micro-organisms increased during storage of chicory and knowing that infection by micro-organisms caused an increase in peroxidase activities of the host (Hislop & Stahmann, 1971) it might be possible that the observed increase in peroxidase activities in the control, is related to the increase in the number of micro-organisms. In the irradiated series with a low peroxidase activity the number of micro-organisms was greatly reduced from 10^7 to 10^2/g. However, further studies are necessary to prove a causal relationship.

As far as the effects on the shelflife of prepacked fruit and vegetables are concerned, a deficiency of oxygen might cause the reduction of physiological activity and consequently also reduce the discolouration (browning). With a sufficient supply of oxygen (e.g. perforation) the opposite of the above phenomena could occur.

The prepacked cut chicory discoloured more in the perforated bags than in the non-perforated bags, because in the non-perforated bags the O_2 content was lower and the CO_2 content was higher (Langerak & Hovestad, 1973). As far as irradiation in the bags was concerned, the perforated and irradiated bags were the most discoloured, followed by the perforated and non-irradiated bags. The non-perforated and irradiated

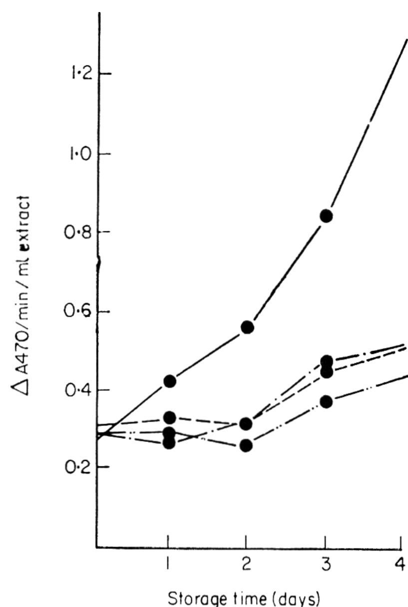

Fig. 4. The influence of γ-irradiation and packaging on the peroxidase activity of cut chicory. The chicory was packed in perforated and non-perforated polythene bags of 0·02 mm thickness and stored in dark at 10 °C. ——, 0 krad (perf.); —·—, 100 krad (perf.); – – –, 0 krad (non-perf.); —··—, 100 krad (non-perf.).

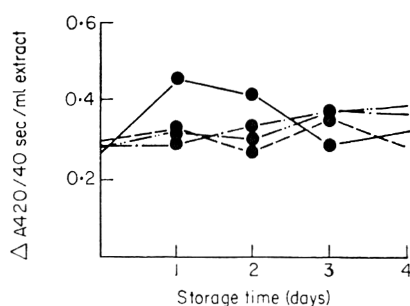

Fig. 5. The influence of γ-irradiation and packaging on the polyphenol activity of cut chicory. The chicory was packed in perforated and non-perforated polythene bags of 0·02 mm thickness and stored in dark at 10 °C. ——, 0 krad (perf.); —·—, 100 krad (perf.); – – –, 0 krad (non-perf.); —··—, 100 krad (non-perf.).

bags were the least discoloured. Thus, discolouration was induced more by the presence of oxygen than by irradiation. The differences observed in peroxidase activities between perforated and non-perforated bags are probably also due to differences in oxygen content (Fig. 4). The polyphenoloxidase activity of the sample in the perforated and in non-irradiated bags had a peak of activity after one to two days of storage, but other

samples were hardly influenced either by the perforation or irradiation during storage except that there was a tendency for the activity to increase after three to four days (Fig. 5).

In the next experiment, which was carried out to try and prevent the discolouration, cut chicory was treated with either $4 \cdot 0 \times 10^{-3}$ M cystein solution or $0 \cdot 1$ M sodium chloride solution for 5 min instead of washing with water. It was then dried, packed and irradiated immediately. The cystein treatment was expected to prevent any radiation damage (Langerak & Hovestad, 1973), and the sodium chloride treatment was expected to prevent any physiological discolouration (Joslyn, 1951; Sharon &

TABLE 2. Quality evaluation of cut chicory washed with some chemicals, packed in the perforated polythene bags, γ-irradiated and stored in the dark at 10 °C

Treatment*	Dose (krad)	Days				
		0	1	2	4	7
Colour						
Water	0	5	4	3+	1–2	1–2
Water	100	5	3	2–3	2	2
Cystein	0	5	4–5	4	1–2	1–2
Cystein	100	5	4	3	3	2
NaCl	0	5	4	3	3	2
NaCl	100	5	2–3	2	1–2	1
Odour						
Water	0	5	5	5	3	1
Water	100	5–	5–	4–5	4	3
Cystein	0	5	5	5	3–4	1
Cystein	100	4	4–5	4	4	3
NaCl	0	5	5	5	4	1
NaCl	100	4	4–5	3–4	3	2–3
State						
Water	0	5	5	4–5	2	1 (rot)
Water	100	5	5	4–5	4	3
Cystein	0	5	5	4–5	3	1 (rot)
Cystein	100	5	5	4–5	4	3 (rot)
NaCl	0	5	5	4–5	3	2 (rot)
NaCl	100	5	4–5	4	3	2–3 (rot)
Trade value						
Water	0	100	90	70	40	0
Water	100	100	80	70	60	20
Cystein	0	100	95	80	20	0
Cystein	100	95	85	70	60	20
NaCl	0	100	95	70	50	0
NaCl	100	95	70	50	40	10

*Concentration of the chemical: cystein, $4 \cdot 0 \times 10^{-3}$ M; NaCl, $0 \cdot 1$ M.

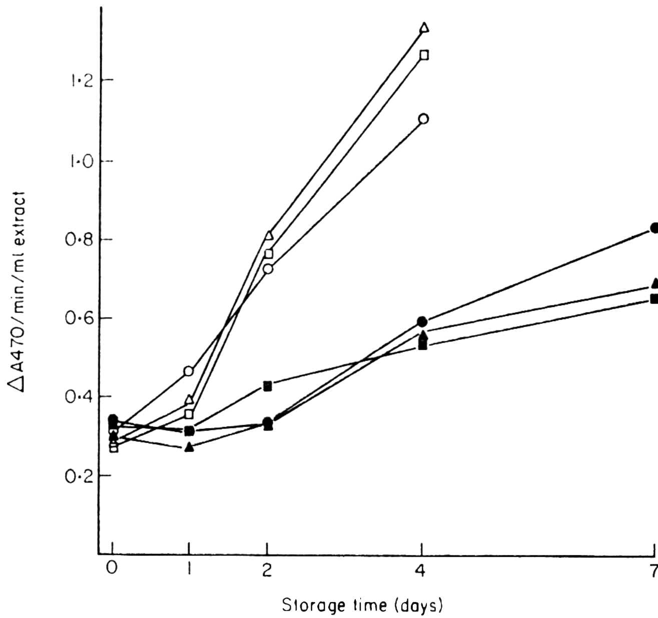

FIG. 6. The influence of γ-irradiation and chemicals on the peroxidase activity of cut chicory. The chicory was packed in perforated polythene bags of 0·02 mm thickness and stored in dark at 10°C. —○—, 0 krad (water); —△—, 0 krad (cystein); —□—, 0 krad (NaCl); —●—, 100 krad (water), —▲— 100 krad (cystein); —■—, 100 krad (NaCl).

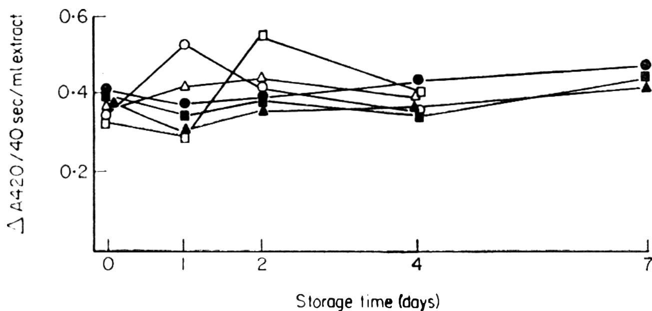

FIG. 7. The influence of γ-irradiation and chemicals on the polyphenoloxidase activity of cut chicory. The chicory was packed in perforated polythene bags of 0·02 mm thickness and stored in dark at 10°C. —○—, 0 krad (water); —△—, 0 krad (cystein); —□—, 0 krad (NaCl); —●—, 100 krad (water); —▲—, 100 krad (cystein); —■—, 100 krad (NaCl).

Mayer, 1967). Table 2 gives the results of the quality evaluation. The cystein treated and irradiated chicory showed some discolouration inhibition, but the odour and the state of the leaves (i.e. the trade value) were not affected. The quality of the chicory which was treated with sodium chloride and also irradiated was lower than the control which was washed with water and was not irradiated. Chemical treatment with NaCl and cystein solution had only little influence on peroxidase activities (Fig. 6). The polyphenoloxidase activity was hardly affected by irradiation except for a little unexplainable irregularity of the activity (Fig. 7).

Conclusions

(1) In the quality evaluation test, 100 krad increased the shelflife of prepacked cut chicory in non-perforated bags more than the 0 krad or 300 krad. However, irradiated samples in perforated bags discoloured after one or two days of storage and their trade value decreased.

(2) Discolouration increased in perforated bags [high O_2 content] and decreased in non-perforated bags [low O_2 content].

(3) Treatment with cystein or sodium chloride solution before irradiation did not have an obvious effect on the extension of shelflife or on discolouration.

(4) Because the peroxidase and polyphenoloxidase activities remained at the same level for two days by irradiation, it is assumed that the physiological metabolism was decreased for the same time.

(5) In the non-irradiated samples the peroxidase activities were related to the discolouration. In the irradiated samples this relationship was not obvious in the presence of a high O_2 content (perforated bags).

(6) Chlorogenic acid content was not clearly affected by irradiation. A relationship between chlorogenic acid content and discolouration was not found; probably this compound is not characteristic for the browning in chicory.

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References

Chance, B. & Maehly, A.C. (1955) Assay of catalases and peroxidases. *Meth. Enzym.* **2,** 764.

Goren, R. & Monselise, S.P. (1965) Inter-relations of Hesperidin, some other natural components and certain enzyme systems in developing Shamouti orange fruits. *J. Hort. Sci.* **40,** 83.

Hislop, E.C. & Stahmann, M.A. (1971) Peroxidase and ethylene production by barley leaves infected with *Erysiphe gramines* f. sp. hordes. *Physiol. Pl. Path.* **1,** 297.

Joslyn, M.A. (1951) Enzyme-catalyzed oxidative browning of fruit products. *Adv. Fd Res.* **3,** 1.

Joslyn, M.A. & Braverman, J.B.S. (1954) The chemistry and technology of the pretreatment and preservation of fruits and vegetable products with sulfur dioxide and sulfites. *Adv. Fd Res.* **5,** 97.

Kalinov, V. (1972) Comparative study on enzyme activities in prepacked cut endive. Association Euratom-ITAL, Annual Report 1971, 51.

Kooij, J.G. van, Heins, H.G. & Langerak, D.I. (1973) Food preservation by irradiation. Association Euratom-ITAL, Annual Report 1972, 150.

Langerak, D.I. (1973) Ionizing radiation in food technology. Association Euratom-ITAL, Annual Report 1972, 29.

Langerak, D.I., Hovestad, R. & van Duyvendyk, K. E. (1972) Influence of irradiation and packaging the keeping quality of prepacked endive. Association Euratom-ITAL, External Report no. 8.

Langerak, D.I. & Hovestad, R. (1973) Influence of handling, packaging, and irradiation on the keeping quality of prepacked cut chicory. Association Euratom-ITAL, External Report no. 13.

Maehly, A.C. & Chance, B. (1954) The assay of catalases and peroxidase. *Meth. biochem. Analysis,* **1,** 357.

Maier, V.P. & Schiller, F.H. (1961) Studies on domestic dates. II. Some chemical changes associated with deterioration. *J. Fd Sci.* **26,** 322.

Mathew, A.G. & Parpia, H.A.B. (1971) Food browning as a polyphenol reaction. *Adv. Fd Res.* **19,** 75.

Maxie, E.C. & Abel-Kader, A. (1966) Food irradiation-physiology of fruits. *Adv. Fd Res.* **15,** 105.

Monselise, S.P. & Halevy, A.H. (1962) Effects of Gibberellin and AMO-1618 on growth, dry matter accumulation, chlorophyll contact and peroxidase activity of citrus seedlings. *Am. J. Bot.* **49,** 405.

Ogawa, M., Majima, R., Uritani, I. & Namiki, M. (1968) Effects of gamma-ray irradiation on metabolic changes in potato tubers in response to cutting. *Pl. Cell Physiol., Tokyo,* **9,** 511.

Ogawa, M. & Uritani, I. (1970) Tissue browning of potato tubers induced by gamma irradiation. *Agric. Biol. Chem.* **34,** 870.

Ponting, J.D. & Joslyn, M.A. (1948) Ascorbic acid oxidation and browning in apple tissue extracts. *Archs Biochem.* **19,** 47.

Riov, J., Monselise, S.P. & Kahan, R.S. (1970) Radiation damage to grapefruit in relation to ethylene production and phenylalanine ammonia-lyase activity. *Radiat. Bot.* **10,** 281.

Romani, R.J. (1966) Radiobiological parameters in the irradiation of fruits and vegetables. *Adv. Fd Res.* **15,** 57.

Sharon, M. & Mayer, A.M. (1967) The effect of NaCl on catechol oxidase from apples. *Israel J. Chem.* **5,** 275.

Stadtman, E.R. (1948) Non-enzymatic browning in fruit products. *Adv. Fd Res.* **1,** 325.

Thomas, P. & Nair, P.M. (1971) Effect of gamma irradiation on polyphenoloxidase activity and its relation to skin browning in bananas. *Phytochem.* **10,** 771.

Zucker, M. & Ahrens, J.F. (1958) Quantitative assay of chlorogenic acid and its pattern of distribution within tobacco leaves. *Pl. Physiol.* **33,** 246.

(*Received* 2 *December* 1974)

J. Fd Technol. (1975) **10**, 427–435.

The relationship between respiration, atmosphere and quality in intact and perforated mushroom pre-packs

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Summary

Fresh 'button' mushrooms (*Agaricus bisporus*) were stored at 18 °C in pre-packs overwrapped with one of six polylvinyl chloride films. The effect of piercing the film with one to four pinholes (punctures) on the concentrations of CO_2 and O_2 in the pre-pack atmosphere and the internal browning and CO_2 production of the packed mushrooms were measured.

The respiration, as measured by CO_2 production, of mushrooms in intact packs was depressed by over 50% from the values observed in air, and varied considerably between the films used. There appeared to be an inverse relationship between respiration in the pre-pack and internal browning. Carbon dioxide output of the packed mushrooms increased as a result of perforating the overwrapping film. The effect of perforations on the CO_2 and O_2 concentrations in the pre-pack atmosphere and on the internal browning of packed mushrooms depended on the type of film used. It appeared that one or two pinholes in films which permitted low respiration rates when intact, caused a rise of CO_2 concentration and an increase in internal browning, while little change was seen in O_2 level; more pinholes led to a decrease in CO_2 concentration, an increase in O_2 concentration and some decrease in internal browning. Pre-packed mushrooms overwrapped with a film which permitted a high level of respiration when intact showed decreased CO_2 and increased O_2 concentrations and some decrease in internal browning at all levels of perforation.

The significance of the results is discussed in relation to previous work and to commercial practice.

Introduction

Controlled atmosphere storage is used extensively for increasing the storage life of apples and pears but it seems unlikely that it will be useful for short-lived products like mushrooms in conventional stores. The time taken to develop a suitable atmosphere

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would be too long or the cost prohibitive. An alternative approach is to make use of the modified atmosphere that is developed inside a sealed package as a result of respiration of the produce and this has been tried with some produce (Karel & Go, 1964).

In a previous paper it was reported that internal atmospheres in sealed overwrapped pre-packs of mushrooms rapidly attained equilibrium values of 8–15% CO_2 and 1–2% O_2 at 18 °C (Nichols & Hammond, 1973); the actual values attained depended on the type of film that was used. These results are in general agreement with those reported for other horticultural produce (Tomkins, 1962; Hardenberg, 1971). Deterioration of the mushrooms, expressed by loss of fresh weight, browning and sporophore development, was retarded in the overwrapped pre-packs, although the extent to which this occurred depended upon the film type used. No direct relationship between the package atmosphere and quality could be discerned. However, it seemed possible that films could be selected and modified to give the optimum conditions for a form of controlled atmosphere storage using the natural generation of a suitable atmosphere inside the pre-pack.

Film overwrapping has been found to reduce the respiratory rate of produce by up to one half of its normal value (Scott & Tewfik, 1947) and storage life of horticultural produce has been related to the respiratory rate of the material; high respiratory rates lead to a shorter storage life (Ryall & Lipton, 1972). If the extension of storage life in pre-packed mushrooms is due to suppression of respiration, it is possible that the variation seen between different films is due to the differential effects of the films on the suppression of respiration.

Therefore these investigations were started to find out what effects various films, selected for their reportedly different gas permeability properties, would have on the respiration of overwrapped mushrooms. During the course of the investigations it was found that small leaks in the films had large effects on respiration and the carbon dioxide concentrations inside the packs. Since it was evident from personal observation of commercial overwrapped prepacks, and from published results (Scott & Tewfik, 1947) that leaks were commonplace as a result of inadequate sealing or accidental damage, measurement of the respiration of mushrooms in perforated packs was undertaken; it was anticipated that some of the anomalous results obtained in storage of mushrooms might be attributed to leaks in the film. From the results it has been possible to describe the effects of such leaks on the storage behaviour and respiration of the mushrooms which have been overwrapped.

Methods and materials

Harvesting and packing

Cultivated mushrooms (*Agaricus bisporus*, strain 'Darlingtons 621'), grown at the Institute Mushroom Unit, were picked at the button stage. The mushrooms were packed in styrene punnets (200 g per punnet) and overwrapped with one of six PVC

films. The relative gas permeabilities of these films, according to the manufacturer's data, which were given in full by Nichols & Hammond (1973), were RMT 68H > RMT 67 > VF 71 > AFT 50 > TPF 84 > VF 70; the permeability to O_2 was lower than that to CO_2 for all films. Packing was completed within 3 hr of harvest. The over-wrapped pre-packs were stored at a constant temperature of 18 °C.

The film overwraps were perforated after packing by inserting a 1-mm diameter needle through a strip of adhesive tape stuck on the top surface of the film; where more than one puncture was made they were evenly spaced along the strip.

Four replicates (pre-packs) were used in experiments, except in those where CO_2 production was monitored, when two packs were individually measured for each treatment.

Quality assessment

The quality of the mushrooms after storage was assessed by measurement of the internal reflectance (R_I); this is the reflectance of the cut surface of the pileus after slicing the mushroom in half longitudinally. As described previously (Nichols & Hammond, 1973), R_I is a measure of the autolysis of the tissue of the mushrooms which may occur in a pre-pack, and which may not be evident from their external appearance. The measurement was made with a reflectometer (Evans Electro Selenium Ltd), and the values obtained were expressed as a percentage of the reflectance of a magnesium carbonate block. Six mushrooms from each pack were measured immediately after removing the overwrap. A change from high to low values of R_I indicates a change from white to brown and a deterioration in quality.

Sampling and analysis of the pre-pack atmosphere

The pre-pack atmosphere was sampled for CO_2 and O_2 concentration by with-drawing 1-ml samples with a hypodermic syringe. The samples were analysed by gas chromatography. Each pack was sampled 72 hr after packing, by which time an equilibrium had been established between the pack and external atmosphere. Further details of the above procedures are given by Nichols & Hammond (1973).

Measurement of the CO_2 production of pre-packed mushrooms

Measurement of CO_2 production was carried out as follows. Compressed air from a cylinder was regulated to a flow of approximately 10 l/hr using a flow control valve. Carbon dioxide was removed from the air by passage through self-indicating soda lime. The CO_2-free air was humidified by bubbling through water, and passed into the sample chamber through a calibrated manometer-type flowmeter. The sample chamber was made from a 240-mm length of 150-mm diameter ABS plastic pipe ('Durapipe'), the ends of which were sealed with greased ABS bungs. Air from the sample chamber was passed through a cold finger to remove water vapour, and the CO_2 content was measured using a Type SB2 infra-red gas analyser (Grubb-Parsons Ltd) fitted with

optical edge filters to eliminate interference due to residual water vapour. The air was exhausted to the atmosphere. The CO_2 concentration was monitored continuously using a chart recorder.

The pre-pack was placed in the sample chamber intact. The rate of diffusion of CO_2 out of the pack was considered to be equal to the rate of CO_2 production of the mushrooms in the pack assuming that the pre-pack atmosphere had attained equilibrium. Measurements were made 72 hr after packing. The rate of CO_2 production was calculated from the flow rate and the measured CO_2 concentration once the apparatus had reached equilibrium.

CO_2 production of individual mushrooms

The post-harvest respiration of individual mushrooms was measured using the method and apparatus described in the previous section, except that the 'Durapipe' sample chamber was replaced by a glass chamber of approximately 100 ml volume, and an air flow of 3–5 l/hr was used.

Results

The effect of film type on CO_2 production and quality

The rate of CO_2 production by overwrapped pre-packs of mushrooms varied according to the type of film overwrap used. Although there was some variation in values between experiments, the relative differences in rates of CO_2 production between film types were generally maintained (Fig. 1). Despite this there was no evidence for a simple relationship between the known film characteristics and CO_2 production; for example, VF 70 and TPF 84 which were found at two extremes of the range both have low permeabilities to O_2 and CO_2.

Rates of CO_2 production for button mushrooms in air after 72 hr at 18 °C were in the range 31–35 ml/200 g fresh wt^{-1} hr^{-1}. The values observed in pre-packed mushrooms (7–20 ml/200 g fresh wt^{-1} hr^{-1}) were well below these figures and it is evident that there was a considerable depression of CO_2 production in overwrapped pre-packs.

From the data shown in Fig. 1 the films which allowed high, low and intermediate rates of respiration of the overwrapped mushrooms could be selected (TPF 84, VF 70 and AFT 50) and, for clarity, only these are shown in the figures which follow; in general, for the others (VF 71, RMT 68H, RMT 67) the data fell within the ranges that are illustrated for TPF 84, VF 70 and AFT 50.

Using R_I as a measurement of mushroom quality it was found that variation of values between packs overwrapped with different films was reflected in the variation of CO_2 production (Fig. 1). The correlation coefficient between R_I and CO_2 production was $-0{\cdot}70$ ($P=0{\cdot}05$). One film, RMT 67, showed wide variation in results between experiments (Fig. 1) but the data have been included in the regression analysis. Thus, films which permitted high rates of respiration were associated with lower quality mushrooms after storage.

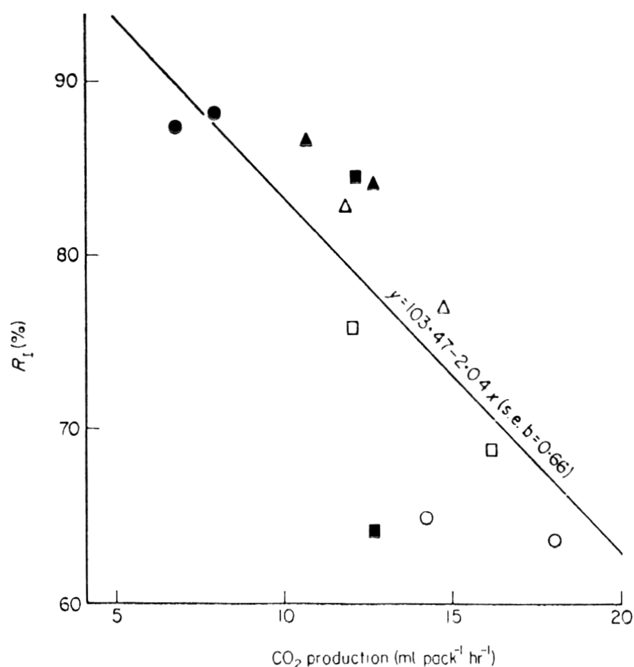

FIG. 1. The variation of internal reflectance (R_I) with CO_2 production of mushrooms in pre-packs overwrapped with intact film. ●, VF70; ▲, VF71; △, RMT68H; □, AFT50; ■, RMT67; ○, TPF84.

The effect of perforation on CO_2 production and the pre-pack atmosphere

The CO_2 production of mushrooms packed under any film increased with increasing levels of perforation until a maximum was attained at which no further perforation gave an increase in CO_2 production, within the limits of the experiment (Fig. 2). The number of punctures required to achieve the maximum production depended on the type of film used in overwrapping. For example, using VF 70 the maximum rate was reached at two to three punctures; whilst with TPF 84, which permits high rates of CO_2 production when intact (Fig. 1), the maximum rate was achieved with one or more punctures.

The result of perforating the overwrapping film on CO_2 concentration in the pre-pack atmosphere varied according to the type of film employed (Table 1). In those packs in which the intact film gave low values for CO_2 production, e.g. VF 70 (Fig. 1), perforation initially led to an increase in CO_2 levels, whereas films permitting higher levels of CO_2 production when intact showed decreases in accumulated CO_2 when punctured, presumably because no great increase in CO_2 production occurred. The effect of a single puncture in film RMT 67 appears to be an exception, but the critical ratio was only just significant at $P = 0·05$.

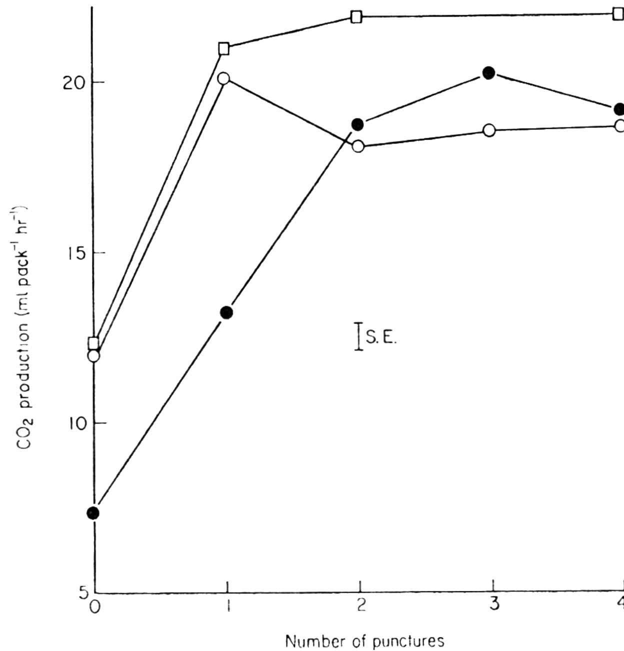

FIG. 2. The change in CO_2 production of pre-packed mushrooms with perforation of the film. ●, VF70; □, AFT50; ○, TPF84. Each point is the mean for two values from different experiments, and the mean standard error (S.E.) for all points is given.

Oxygen levels remained low and the CO_2 concentration increased as the numbers of punctures increased; but levels of O_2 were higher when CO_2 levels showed only a small increase or a decrease compared with packs with fewer perforations (Table 1). It seems, therefore, that increases in CO_2 production, and hence CO_2 concentration, are linked with increases in aerobic respiration.

The effect of perforation on mushroom quality

The R_I values of mushrooms stored in intact and perforated pre-packs are shown in Fig. 3.

Perforating the film increased or decreased the R_I value depending on the number of punctures that were made and the film type. Increasing the number of punctures in film TPF 84 caused an increase in R_I, whereas the same treatment applied to VF 70 caused an initial decline in R_I and then an increase. The values of R_I under AFT 50 exhibited a similar pattern to VF 70 but the effect of a single puncture was much less; these results are best considered together with those from the previous experiments concerned with the effects of perforations on the respiration of mushrooms and the internal gas concentrations of the pre-packs. It was noted that the mushroom caps developed when there were three to four punctures in film TPF 84 and to a lesser extent

TABLE 1. The percentage of CO_2 and O_2 in pre-packs overwrapped with punctured PVC films

Film type	Number of punctures									
	0		1		2		3		4	
	a	b	a	b	a	b	a	b	a	b
VF70	9·8	1·4	12·6	1·4	13·4	4·2	13·1	5·3	10·2	11·2
VF71	6·9	1·5	8·5	2·3	6·8	9·8	5·8	13·8	5·9	14·6
RMT68H	8·8	1·5	7·7	6·9	6·7	10·6	5·4	15·3	4·8	18·2
AFT50	11·6	1·4	12·8	4·4	9·4	9·0	8·0	13·1	6·5	15·0
RMT67	11·0	1·3	13·7	3·2	9·6	10·4	8·3	14·2	6·5	16·9
TPF84	15·1	1·4	13·9	3·1	9·9	11·4	9·2	13·9	7·0	16·3

Critical ratio ($P=0·05$): a, CO_2 level (%) 1·11; b, O_2 level (%) 1·66. There is a significant difference between values ($P=0·05$) when the ratio of the larger value to the smaller one is greater than the critical ratio.

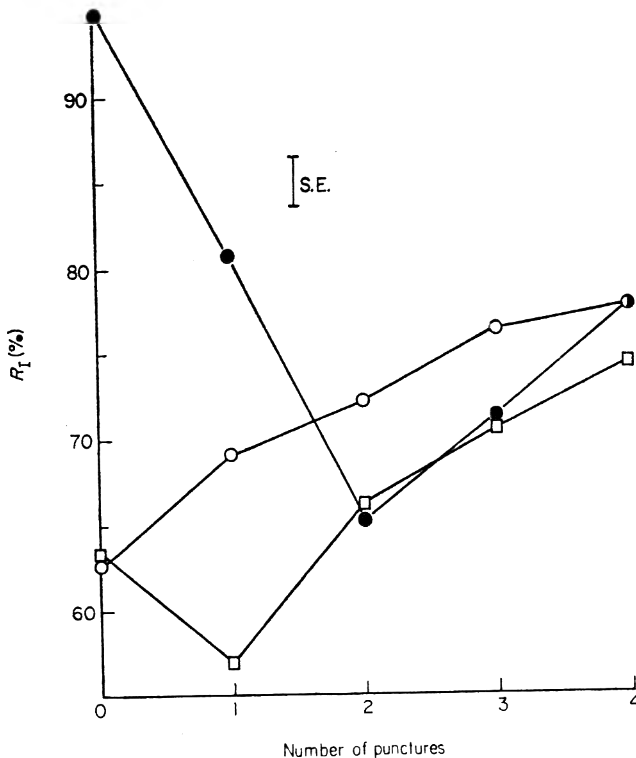

FIG. 3. The changes in internal reflectance (R_I) of pre-packed mushrooms after perforation of the film. ●, VF70; □, AFT50; ○, TPF84. Each point is the mean of four observations and the mean standard error (S.E.) for all points is given.

in other films. In unperforated films of all types development of the mushroom cap and stalk is effectively inhibited (Nichols & Hammond, 1973).

Discussion

It was evident that the rate of respiration of pre-packed mushrooms, as measured by the rate of CO_2 production of the whole pack, was considerably lower than the normal value for mushrooms respiring in air; it appears that this was due to the effect of both high CO_2 and low O_2 concentrations that are developed inside the packs under the film overwrap. It was not possible to find a simple correlation between the gas permeabilities of the films and the observed rates of CO_2 production. It is evident that a complex relationship exists between them and work is being undertaken to clarify this.

When the film was perforated with a single puncture, in four out of six films the CO_2 concentration inside the pack increased (Table 1). This was unexpected to some extent because it was anticipated that the concentration should fall as a result of increased gas leakage, but this was the case for only one (TPF 84) of the six films tested; increasing the number of punctures to three or four did cause a fall in gas concentration within all films. It was observed that a single film puncture caused a rise in CO_2 production (Fig. 2) and since, in general, this was accompanied by an increase in CO_2 concentration in the pack, it seems probable that accumulation of CO_2 was not the only factor which depressed the rate of respiration of mushrooms in an intact pack. The intact films are all less permeable to O_2 than they are to CO_2 and perforating the film will have a relatively larger effect on the influx of O_2 than on the efflux of CO_2. The increased availability of O_2 was reflected in the increased production of CO_2 but the level of O_2 in the pack did not rise substantially until two or more punctures were made in the film (Table 1); at this level of perforation the CO_2 concentration might be the factor retarding respiration. The increased CO_2 concentration observed in packs overwrapped with certain films which were then punctured is perhaps a reflection of the high rate of respiration of mushrooms under normal atmospheric conditions. It might be expected that a relatively small change in the rate of influx of O_2 would have a proportionally larger effect on CO_2 production so that higher levels of CO_2 accumulate; the implication is that the respiratory quotient of the mushrooms changes as a result of perforating the film.

The results reported here seem to indicate that the quality of mushrooms as expressed by their R_I in a pre-pack is determined, at least in part, by their respiration rate (Fig. 1) as well as the gas concentrations in the packs. Increases in respiration due to a change of film type, or to a small number of punctures in the films (with the exception of TPF 84 and RMT 67) lead to a loss in quality (Fig. 3). However, it appears that once respiration has reached a stable level, packs with greater numbers of perforations, which have higher O_2 and lower CO_2 levels, allow higher R_I values. This increase in R_I is accompanied by some increased development of the mushroom cap, and it is possible

that this is associated with the change in quality. These results are consistent with the observations made previously (Nichols & Hammond, 1973) in which it was found that the R_I of packed mushrooms did not depend only on the atmosphere inside the pre-pack. Although there was an inverse relationship between R_I and CO_2 concentration within a film type, no such relationship was evident between films. It seems likely that the reason for this was that the spread of CO_2 values found in packs of the same film type was due to small leaks being present. The presence of leaks, as we have now shown, would lead to increased CO_2 production, to a corresponding increase in CO_2 concentration and to a decrease in quality of mushrooms in each pack. The variation between films may then be explained in terms of differing rates of CO_2 production under different film types and corresponding variations in R_I.

In commercial practice it may be expected that some of the pre-packs produced will have minor leaks as a result of handling or inefficient sealing (Scott & Tewfik, 1947). The effect of these leaks on the quality of the packed mushrooms may be important. From the results reported here it seems that those films which give best mushroom quality after storage when the seal is intact will allow a greater drop in quality when perforated than films which give a lower quality when perfectly sealed. Thus perfection of the seal in pre-packed mushrooms is of great importance; if this is impracticable, then films with appropriate gas permeabilities should be selected although they may not give the best mushroom quality.

The results reported here are for mushrooms stored in pre-packs at 18 °C. It is quite possible that the effects of film type and puncturing of the overwrap on mushroom respiration and quality would be different at other temperatures, because the rates of respiration of mushrooms and gas diffusion are temperature dependent.

References

Hardenburg, R.E. (1971) *Hort. Sci.* **6,** 198.

Karel, M. & Go, J. (1964) *Mod. Packag.* **37,** 123.

Nichols, R. & Hammond, J.B.W. (1973) *J. Sci. Fd Agric.* **24,** 1371.

Ryall, A.L. & Lipton, W.J. (1972) In: *Handling, Transportation and Storage of Fruits and Vegetables,* Vol. 1, p. 9. Avi Publishing Co., Connecticut.

Scott, L.E. & Tewfik, S. (1947) *Proc. Am. Soc. hort. Sci.* **49,** 130.

Tomkins, R.G. (1962) *J. appl. Bact.* **25,** 290.

(*Received* 29 *November* 1974)

J. Fd Technol. (1975) **10**, 437–452.

Ultrastructural changes during frozen storage of cod (*Gadus morhua* L.)

III. Effects of linoleic acid and linoleic acid hydroperoxides on myofibrillar proteins

L. JARENBÄCK AND A. LILJEMARK

Summary

Myofibrils and solutions of myofibrillar protein from post-rigor cod muscle were studied with electron microscopy after incubation with linoleic acid and linoleic acid hydroperoxides.

Linoleic acid hydroperoxides were ten times more effective than linoleic acid in reducing the amount of protein in KCl-extracts from incubated myofibrils. Small quantities of hydroperoxides caused precipitation of isolated myofibrillar proteins.

Differences in the structure and aggregation pattern of extracted proteins suggest that linoleic acid and hydroperoxides exert their effects by different mechanisms. According to the structure of extracted myofibrils, linoleic acid seemed to prevent the dissolution of the myofibril framework but appeared not to impair the extraction of myosin. Hydroperoxides appeared to cause a retention of A-bands (myosin) in the myofibrils and in this respect showed similarities to the effect of frozen storage previously observed.

Introduction

A progressive hydrolysis of phospholipids by endogenous enzymes in frozen and stored fish is followed by an accumulation of unsaturated free fatty acids (FFA) in the muscle (Olley & Lovern, 1960; Olley, Pirie & Watson, 1962; Bligh & Scott, 1966). In cod and other lean fish, the FFA are mainly derived from membrane phospholipids (Hanson & Olley, 1965; Addison, Ackman & Hingley, 1968).

Dyer and co-workers (Dyer & Morton, 1956; Dyer & Fraser, 1959) suggested a relationship between lipid hydrolysis and the loss in protein extractability which accompanies the development of toughness in stored frozen fish muscle. It was assumed that the FFA liberated could form *in situ* complexes with the myofibrillar proteins, thus reducing their solubility. Such interactions between proteins and FFA, based on the

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simultaneously occurring liberation of fatty acids and decrease in extractable protein, particularly during frozen storage of cod, have been suggested in many papers, as reviewed by Ackman (1967), Connell (1968) and Powrie (1973). However, when a large number of different species are taken into account, doubt has been raised whether a relationship between lipid breakdown and protein inextractability exists (Connell, 1968). Formaldehyde, formed from trimethyloxide during frozen storage, may also be implicated (Castell, 1971; Babbit, Crawford & Law, 1972), acting perhaps in conjunction with free fatty acids (Childs, 1974).

Model experimentation has aimed at an understanding of the proposed influence of FFA on fish muscle proteins. The exposure of lingcod (*Ophiodon elongatus*) muscle to FFA was recently found to reduce the suspensibility of whole myofibrils (Childs, 1974). In earlier experiments, C_{18} unsaturated fatty acids or their sodium salts, caused a reduction in the quantity of salt-extractable myofibrillar protein when added to cod muscle homogenates (Anderson, King & Steinberg, 1963; Anderson & Steinberg, 1964; Anderson, Steinberg & King, 1965; Castell, Smith & Dyer, 1973). The same acids added to solutions of cod myofibrillar protein brought about a gradual precipitation of protein (King, Anderson & Steinberg, 1962; Anderson *et al.*, 1965; Hanson & Olley, 1965). Sodium linolenate was also effective in aggregating fish myosin (Menzel & Olcott, 1964).

It has occasionally been suggested that insolubilization of protein during frozen storage of fish muscle could be linked to lipid oxidation (Dyer & Morton, 1956; Castell, Bishop & Neal, 1968). As indicated by Roubal (1971), free radical intermediates of oxidized lipids may cause polymerization of fish myofibrillar proteins. However, Connell (1968) pointed out that total insolubilization of myofibrillar proteins may take place in cod muscle even though the lipids seem to remain unoxidized during frozen storage. According to Castell (1971) the apparent absence of oxidation of cod muscle lipids and FFA could be explained by an immediate consumption of the oxidation products in reactions with proteins to form insoluble polymers. This reaction should then be analogous with the general damage to proteins and enzymes caused by lipid peroxides (Desai & Tappel, 1963; Roubal & Tappel, 1966a, b; Tappel, 1973).

Oxidized fatty acids were found to be more effective than unoxidized acids in decreasing the solubility of mackerel myofibrillar proteins (Ota & Nishimoto, 1963) and in aggregating cod myosin (Connell, 1968). In mixtures of fish myofibrillar protein and linoleic acid allowed to autoxidize for several days, increasing precipitation of protein took place (Ikeda & Taguchi, 1967).

Fluorescent compounds, indicative of C=N functional groups, were isolated from an oxidizing system consisting of Coho salmon myosin and methyl linoleate (Braddock & Dugan, 1973). Similar compounds were also present in extracts from frozen and stored salmon. Furthermore, specific destruction of the ϵ-amino groups of myosin was observed, possibly a sign of cross-linking reactions between amino groups and fatty acid oxidation products.

Reaction between tuna myosin and malonaldehyde, an oxidation product of polyunsaturated fatty acids, has been reported (Kwon, Menzel & Olcott, 1965). Buttkus (1967) showed that reactions between trout myosin and malonaldehyde, involving ϵ-amino groups of the protein, take place in solutions at 20 °C and 0 °C and that the reaction is extensive also at -20 °C.

No report on the effect of fatty acids or their oxidation products on the ultrastructure of fish muscle seems to exist. The objective of this investigation was, therefore, to study possible ultrastructural changes in protein-extracted residues of cod myofibrils and in solutions of myofibrillar protein following *in vitro* incubation with linoleic acid and its primary oxidation products, linoleic acid hydroperoxides*. To some extent the observations were compared to the changes taking place during frozen storage of cod muscle previously observed (Jarenbäck & Liljemark, 1975a, b).

Materials and methods

Fish

Cod (*Gadus morhua* L.), 60–80 cm in length, were obtained commercially throughout the study period. They were gutted and had been stored in ice for 3–5 days.

Preparation of myofibrils

All preparative operations were carried out at temperatures between 0 and 4 °C. Myotomes were dissected from the largest muscle bundle adjacent to the backbone and below the first dorsal fin. Myofibrils free from sarcoplasmic proteins were obtained by homogenization of 10 g cod muscle in 200 ml 18·9 mM phosphate buffer, $I = 0 \cdot 05$, pH 7·6 (Connell, 1958) followed by centrifugation at 5900 *g* for 10 min. The procedure was repeated three times on the resulting pellet.

Preparation of linoleic acid emulsions

For the incubation of myofibrils with linoleic acid a stock solution containing 300 μl linoleic acid (>99%, Nu-Chek-Prep, Elysian, Minnesota) dissolved in 3·0 ml 95% ethanol was prepared. Various amounts (6–50 μl) of the ethanolic solution were injected with a Hamilton syringe into incubation flasks containing 20 ml ice cold 18·9 mM phosphate buffer, $I = 0 \cdot 05$, pH 7·6. The linoleic acid (1·7–14·5 μmol) in phosphate buffer was further emulsified with a sonicator (Branson Sonogen Model D-50, Loosdrecht, The Netherlands) for 1 hr at 0 °C. Occasionally, N_2 was blown over the surfaces of the buffers. The amount of hydroperoxides initially present, or formed during the sonication process, was less than 0·5% as measured by the absorption at 234 nm. The size of the emulsion droplets, checked by light microscopy, never exceeded

* Linoleic acid hydroperoxides, abbreviated LAHPO are composed of 9-hydroperoxy-octadecadienoic and 13-hydroperoxy-octadecadienoic acid which are denoted C-9-LAHPO and C-13-LAHPO respectively.

1 μm. The maximum ethanol concentration in the linoleic acid experimental systems was 40 mM. The same concentration of ethanol in control incubation media had no discernible effect on the myofibrils.

Preparation of linoleic acid hydroperoxides

LAHPO were prepared from linoleic acid by oxidation in the presence of soybean lipoxygenase (Worthington, U.S.A.), as outlined by Svensson & Eriksson (1972). The LAHPO and enyzme mixtures were kept frozen in 2·5 ml aliquots at −40 °C. Before use the LAHPO were freed from lipoxygenase by gel chromatography through a 1·25 × 26 cm Sephadex G25 column (Pharmacia Fine Chemicals, Sweden) eluted with 0·05 M K_2HPO_4, pH 9·0. The amounts of C-9-LAHPO, C-13-LAHPO and remaining linoleic acid in pooled eluted fractions were determined by the gas chromatographic method described by Eriksson & Leu (1971). LAHPO concentration was estimated by spectrophotometry at 234 nm by use of $\epsilon_M = 27\,300$ litre mol^{-1} cm^{-1} (Siddiqi & Tappel, 1956).

The incubation media for myofibrils with LAHPO were as follows: 20 ml 18·9 mM phosphate buffer, $I = 0{\cdot}05$, pH 7·6, 1–6 ml (0·25–1·70 μmol) of a solution of LAHPO in 0·05 M K_2HPO_4, pH 7·6 and additional 0·05 M K_2HPO_4, pH 7·6 to keep the volume constant.

Incubation of myofibrils with linoleic acid and LAHPO

Washed myofibrils, corresponding with initially 1 g cod muscle, were gently resuspended in the sonicated linoleic acid or the LAHPO incubation media. The control systems (control myofibrils) consisted of respective incubation medium minus linoleic acid or LAHPO. Both the experimental and the control systems were then gently stirred for 2·5 hr at 2 °C with magnetic stirrers followed by centrifugation at 5900 *g* for 10 min. The supernatants were saved for titration of fatty acids or determination of remaining LAHPO.

Extraction of myofibrillar proteins

Myofibrillar proteins were obtained from previously incubated myofibril pellets by resuspension in 20 ml extractant (0·45 M KCl, 18·9 mM phosphate buffer, $I = 0{\cdot}5$, pH 7·2) using a Torry Brown Homogenizer (A. G. Brown Electronics Ltd, Glasgow) at 3000 rpm for 30 sec. Extraction was then continued without stirring at 2 °C for 20 hr. Following centrifugation at 5900 × g for 15 min, the supernatant protein solution was decanted and the muscle residue weighed. Larger amounts of myofibrillar protein for further experimentation were obtained as previously described (Jarenbäck & Liljemark, 1975b).

Incubation of myofibrillar proteins with linoleic acid and LAHPO

Mixtures of myofibrillar protein and linoleic acid were prepared solely for electron microscopy. One ml aliquots of myofibrillar protein extracts (4–5 mg protein/ml)

were added to separate flasks containing 0·5–55 μmol linoleic acid in 19 ml 0·45 M KCl, 0·05 M Tris-HCl buffer, pH 7·2. Before adding the protein, the linoleic acid was emulsified in the buffered salt solutions by sonication. The protein-linoleic acid mixtures were stirred for 10 min at 2 °C prior to preparation for electron microscopy.

Myofibrillar proteins were incubated with LAHPO in a system similar to that used for myofibrils. 1–6 ml (0·25–1·70 μmol LAHPO) of a solution of LAHPO in 0·05 M K_2HPO_4, pH 7·2 were added to 20 ml aliquots of myofibrillar protein (4–5 mg protein/ml in 0·45 M KCl, 18·9 mM phosphate buffer, $I = 0·5$, pH 7·2). To keep volume and ionic strength constant, 0·05 M K_2HPO_4, pH 7·2, and solid KCl were also added. The mixtures were incubated for 2·5 hr at 2 °C and centrifuged at 10 000 *g* for 30 min.

Protein concentration

Protein concentrations were measured by the Lowry procedure (Lowry *et al.*, 1951), standardized against crystalline bovine serum albumin. The small amounts of LAHPO present in some solutions did not affect the protein estimates which can happen in the presence of lionoleate hydroperoxides (Matsushita *et al.*, 1969).

Titration of fatty acids

Extraction of fatty acids with isopropanol-heptane from incubation media and myofibrillar extracts, and titration with 0·018 M CO_2-free NaOH was carried out by the method of Dole (1956) as modified by Trout, Estes & Freidberg (1960).

Electron microscopy

Negative staining of protein solutions with 1% uranyl acetate and fixation, embedding, thin sectioning and staining of specimens from extracted myofibrils were carried out as previously described by Jarenbäck & Liljemark (1975b). Occasionally, freeze-etching of myofibrils was performed using the method outlined by Jarenbäck & Liljemark (1975a). Electron micrographs were obtained with a JEM-7 electron microscope operated at 80 kV.

Results

Incubation of myofibrils with linoleic acid

A detailed time course for the effect of incubating myofibrils with linoleic acid was not established. However, during the incubation of myofibrils with LAHPO a maximal effect on the amount of protein extractable was found after 2·5 hr while shorter times gave more scattered data. For comparison, the incubation time with linoleic acid was also set to 2·5 hr. A rapid decrease in the amount of protein in KCl-extracts from myofibrils incubated with linoleic acid was found (Fig. 1). The volume of the muscle residue (expressed as 'wet weight') of course increased with decreasing protein concentration.

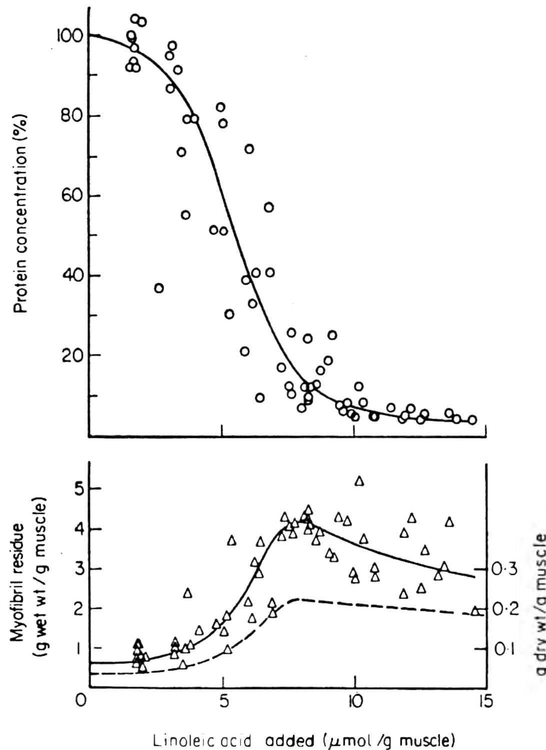

Fig. 1. Effect of incubation of cod myofibrils with linoleic acid for 2·5 hr at 2 °C. ○, protein concentration (% of a control) of centrifuged KCl-extracts from washed myofibrils; △, wet weight of myofibril residues; – – – –, weight of residues freeze dried for 24 hr. The figure represents an average of nine experiments.

The presence of about 8 μmol linoleic acid/g muscle brought about a 90% reduction of protein content in KCl extracts and maximal volume of myofibril residues. The maximum in the 'wet weight' curve (Fig. 1) is caused, as revealed microscopically, by a simultaneous sedimentation of both aggregated myofibrillar proteins and myofibril residues. This heterogeneous pellet has a high capacity to bind moisture which was not effectively removed by freeze drying for 24 hr as shown by the slight maximum in the 'dry weight' curve in Fig. 1. At still higher linoleic acid concentrations the pellets were composed mainly of partially affected myofibrils occupying a smaller volume.

Inclusion of an antioxidant (0·04 mol propylgallate/mol linoleic acid) or performing the incubation under N_2-atmosphere did not significantly change the effect of linoleic acid.

Figure 2 summarizes the results on titration of fatty acids from incubation media and myofibrillar protein extracts. The recovery of fatty acid from incubation media, to which no myofibrils were added, demonstrates the accuracy of the method. The presence of only minor amounts of fatty acid in incubation media, after 2·5 hr mixing

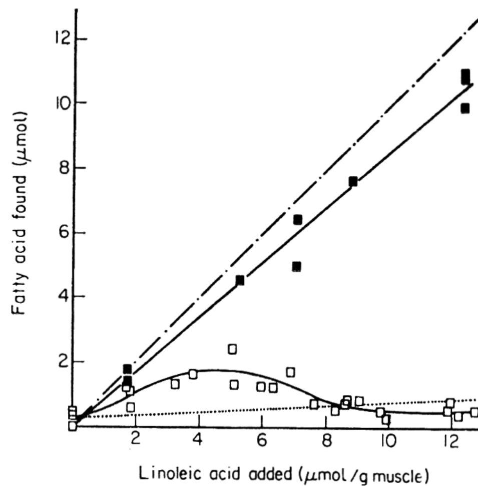

FIG. 2. Titration of fatty acid in incubation media and myofibrillar protein extracts. –·–·–, initial linoleic acid concentration in incubation media; ■, recovery of linoleic acid from incubation media; □, fatty acid content of myofibrillar protein extracts; ......, fatty acid content of incubation media after 2·5 hr mixing with myofibrils and centrifugation.

with myofibrils, indirectly demonstrates that the majority of added fatty acid has been taken up by the myofibrils. The proportion of added linoleic acid found in KCl-extracts from incubated myofibrils is related to the protein concentration. This simply demonstrates that with increasing addition of linoleic acid a higher proportion of this is associated with the myofibril pellet following protein extraction.

Incubation of myofibrils with LAHPO

Pooled fractions of LAHPO, obtained by gel-chromatography of LAHPO and enzyme mixtures, contained 19% unreacted linoleic acid, 9% C-9-LAHPO and 72% C-13-LAHPO. Incubating previously washed myofibrils with 0·25–1·70 μmol LAHPO resulted in a decreased protein concentration in centrifuged KCl-extracts of myofibrillar proteins (Fig. 3). In this respect the effect of LAHPO was very similar to that of linoleic acid (cf. Fig. 1), but occurred at a ten times lower concentration level. On analysis of the media remaining after 2·5 hr incubation with myofibrils, by centrifugation and passage through a gel chromatography column, no LAHPO were found.

The tissue residues, obtained by centrifugation of KCl-extracts from LAHPO-incubated myofibrils (Fig. 3), occupied about the same volume as those from incubation with linoleic acid (cf. Fig. 1) but differed in appearance. At the lower levels of added LAHPO (0·25–0·55 μmol/g muscle) a transparent gel was found on the bottom of the centrifuge tubes while at higher levels of added LAHPO both myofibril residues and gel sedimented upon centrifugation.

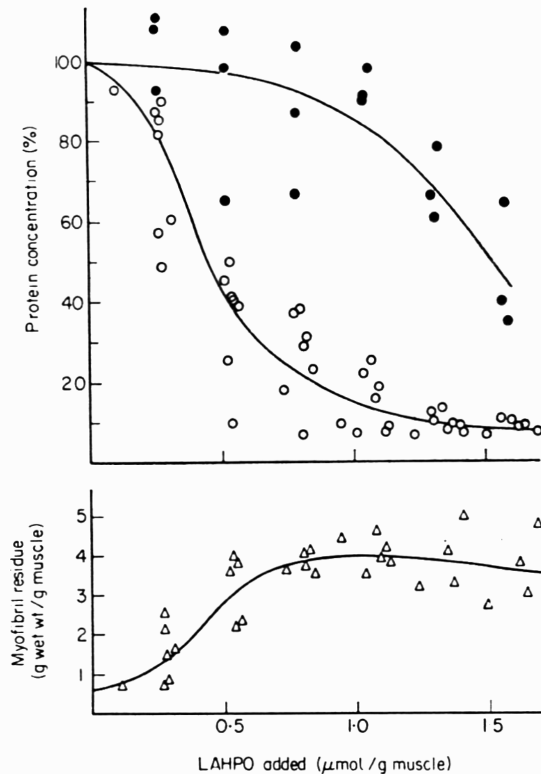

FIG. 3. Effect of incubation of cod myofibrils and whole muscle with LAHPO for 2·5 hr at 2 °C. ●, Protein concentration (% of a control) of centrifuged KCl-extracts from whole muscle (three experiments); ○, protein concentration of KCl-extracts from washed myofibrils (seven experiments); △, wet weight of myofibril residues.

When 1-g portions of cod muscle were incubated for 2·5 hr with increasing amounts of LAHPO, before washing out of sarcoplasmic protein, higher concentrations of LAHPO were needed to affect the protein content in corresponding KCl-extracts (Fig. 3). The scatter in experimental points is explained by the great number of manipulations involved. LAHPO either interact with sarcoplasmic proteins, or, not being strongly bound to myofibrils, are partly removed in subsequent washings which reduces the effective concentration of LAHPO.

Incubation of extracted myofibrillar proteins with LAHPO

The effect of incubating extracted myofibrillar proteins with LAHPO is shown in Fig. 4, where the curve is based on the general shape of curves from each individual experiment. Addition of about 8 nmol LAHPO/mg protein starts a gradual precipitation of myofibrillar protein which levels off at about 15 nmol LAHPO/mg protein.

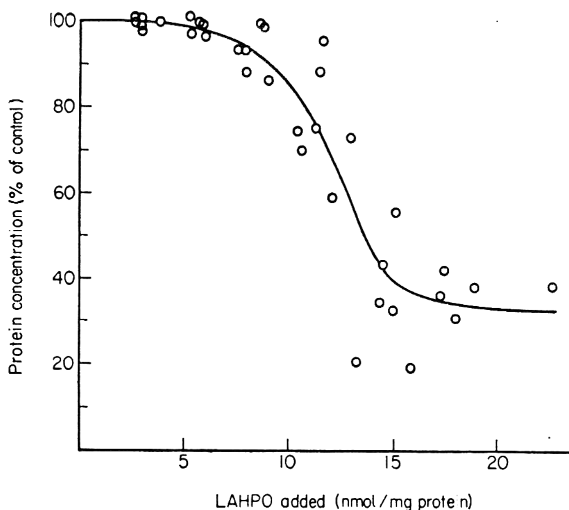

Fig. 4. Protein concentration changes in supernatants from mixtures of myofibrillar protein and LAHPO incubated for 2·5 hr at 2 °C. The curve is based on the general shape of curves from five experiments.

Following centrifugation at 10 000 *g* for 30 min, the precipitated protein formed a loose gel-like pellet visually similar to that obtained from LAHPO-incubated myofibrils as described above.

Structure of myofibril residues and extracted myofibrillar proteins

For comparison, a thin section from KCl-extracted myofibrils not treated with linoleic acid (control myofibrils) is shown in Plate 1a. In this case, consecutive 'I-Z-I-brushes' (thin filaments connected by remnants of the Z-disc) interrupted by precipitated myosin, were found. In general, extraction of control myofibrils yielded very few residual myofibrils, and in those the thick myofilaments were solubilized with part of their myosin content bound to thin filaments. The *in situ* actomyosin filaments thus formed have been previously described by Jarenbäck & Liljemark (1975b).

Myofibrils incubated with linoleic acid. With increasing concentration of linoleic acid the myofibril residues occupied larger and larger volumes. Compared with residues from control myofibrils, those sampled before the maximal volume was reached (cf. Fig. 1) were found to retain a greater degree of the original shape of myofibrils as shown in Plate 1b. Consecutive I-Z-I-brushes meet at the level of the original H-zones where precipitated material, possibly representing myosin, is also observable.

In the range 8–14·5 μmol linoleic acid/g muscle, myofibril residues were composed of large amounts of myofibrils with the appearance shown in Plate 1c. These residual myofibrils appeared to contain more protein but an internal rearrangement of structural

components is obvious. Most thick filaments appeared to be dissolved, as only few structures passed the level of the original H-zones. In these regions, interruptions (as indicated in Plate 1c) were also frequently seen. This interpretation of structural details was supported by parallel preparation of freeze-etch replicas, which showed identical features (Plate 2a).

In freeze-etch replicas from myofibrils incubated with 15 μmol linoleic acid/g muscle but not extracted, no structures representing deposited linoleic acid micelles could be found. This may indicate an even spread of acid over the entire myofibrils.

Negatively stained specimens of myofibrillar protein, obtained from myofibrils incubated with linoleic acid, showed mainly large aggregates and solitary actomyosin filaments. Dense aggregates with obscure structure are probably formed by aggregation of filaments caused by the linoleic acid present in the extracts (cf. Fig. 2). Solitary actomyosin filaments, at all levels of linoleic acid added, showed a fairly regular myosin decoration (Plate 3a) as earlier described for post-rigor cod muscle actomyosin (Jarenbäck & Liljemark, 1975b). With increasing additions of linoleic acid, fewer actomyosin filaments were found in the extracts but they still showed a regular myosin decoration pattern, which indirectly indicates that the extraction of myosin from thick myofilaments is not seriously affected.

Myofibrils incubated with LAHPO. The tissue residue from KCl-extracted myofibrils incubated with small amounts of LAHPO was composed of a gel which at higher amounts of LAHPO also contained myofibril residues, as mentioned earlier.

Thin sections of the myofibril pellet from the bottom of centrifuge tubes and at concentrations of LAHPO less than 1 μmol/g muscle revealed residues essentially similar to those described for myofibrils incubated with up to 8 μmol linoleic acid/g muscle (cf. Plate 1b). However, at concentrations of LAHPO exceeding 1 μmol/g muscle, the myofibril residues demonstrated an incipient retention of material in the original A-bands.

With the highest level of LAHPO used (1·7 μmol/g muscle) most residual myofibrils appeared as shown in Plate 2b indicating an obvious retention of A-bands. At higher magnification of a myofibril from the same specimen (Plate 2c) the density of the original A-bands is more prominent which may be ascribed to remaining thick filaments not effectively solubilized by the extraction. A further indication of thick filament retention is that no interruptions were found at the level of the original H-zones (cf. Plates 1c and 2a).

Negatively stained KCl-extracts from myofibrils incubated with LAHPO showed, similar to the case with linoleic acid, large aggregates and fibrous structures. Some solitary actomyosin filaments were found at all levels of added LAHPO but they decreased markedly in number with increased concentrations of LAHPO. At the highest concentration of LAHPO most single filaments found showed the appearance of F-actin (Plate 3b). This may be a further indication of impaired extraction of myosin.

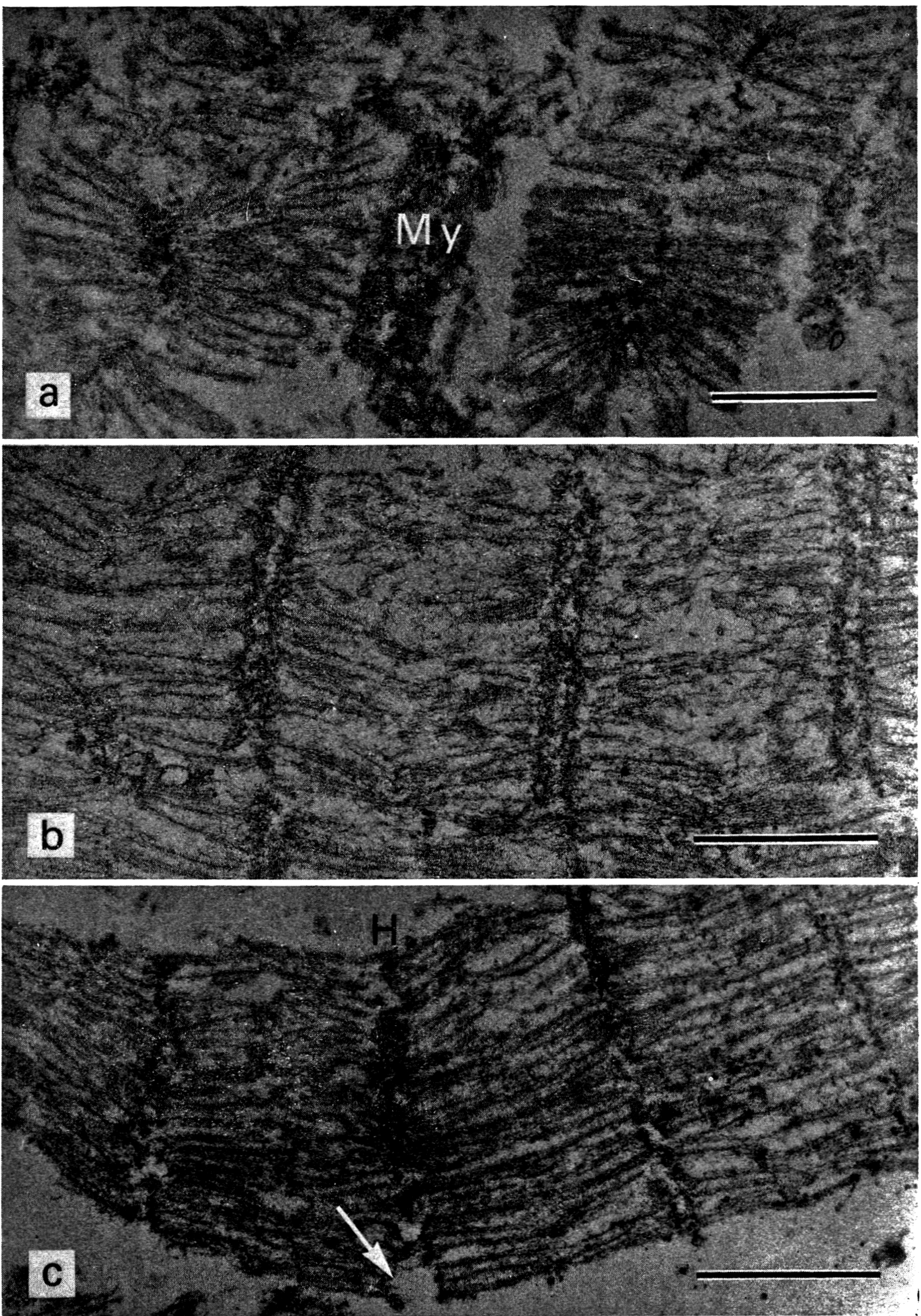

PLATE 1. (a) Longitudinal section of a residue from KCl-extracted control myofibrils. Note consecutive I-Z-I-brushes. Precipitated myosin is indicated (My). ×27 000. (b) Myofibril residue from KCl-extracted myofibrils incubated with 5 μmol linoleic acid/g muscle. ×27 000. (c) As (b) but incubated with 10 μmol linoleic acid/g muscle. Note distinct myofibril outlines. Thick myofilaments appear to be dissolved. Interruption at the level of the original H-zone is indicated. ×27 000. Scales represent 1 μm.

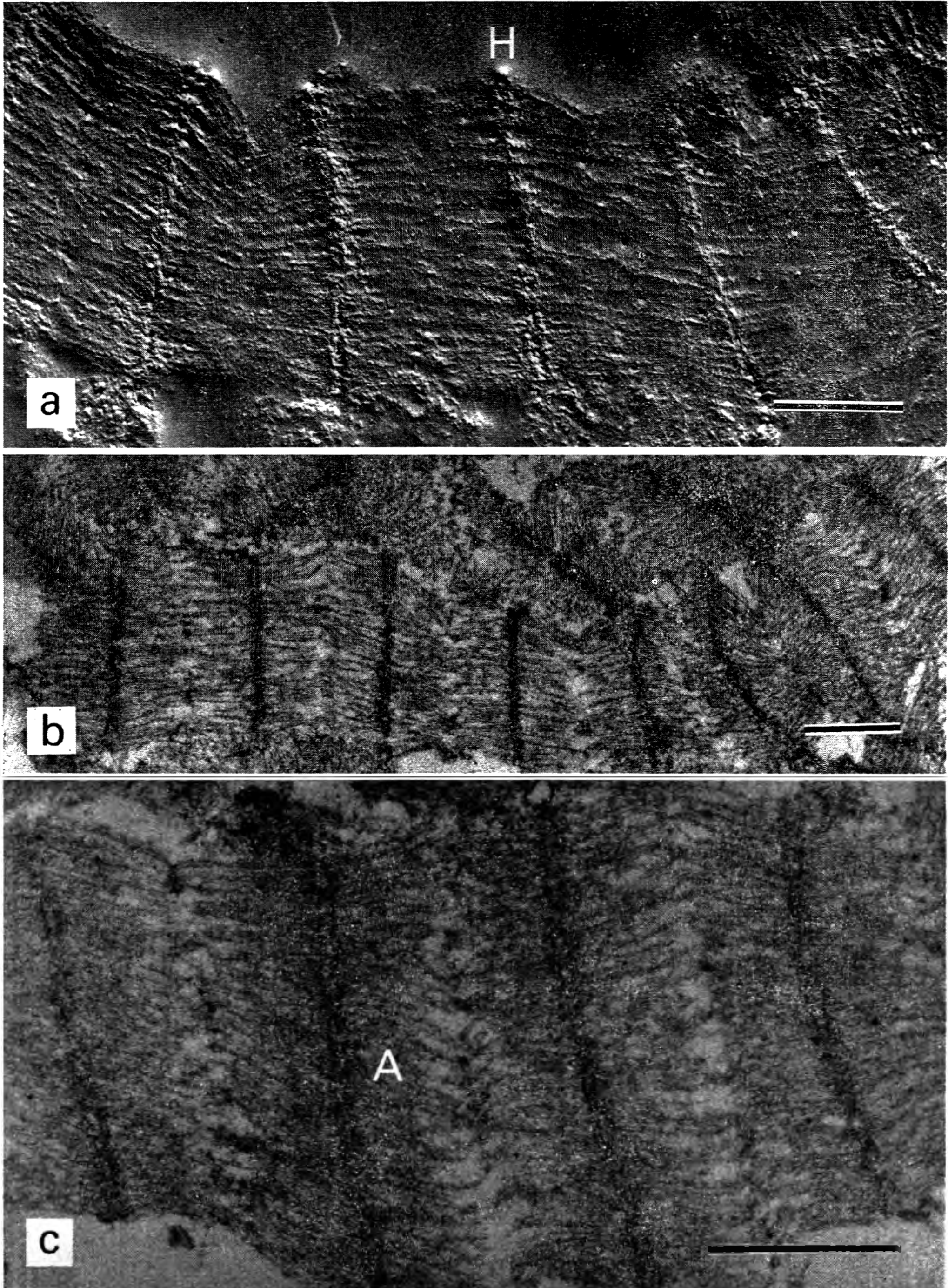

PLATE 2. (a) Longitudinal fracture (freeze-etch replica) of a residue from KCl-extracted myofibrils incubated with 13 μmol linoleic acid/g muscle. Note similar structural features as in Plate 1c. × 18 000. (b) Longitudinal section of a residue from KCl-extracted myofibrils incubated with 1·6 μmol LAHPO/g muscle. Note density of original A-bands. × 13 500. (c) Myofibril residue from the same specimen as (b) showing obvious retention of material in original A-bands. Note also absence of interruptions at the level of original H-zones. × 27 000. Scales represent 1 μm.

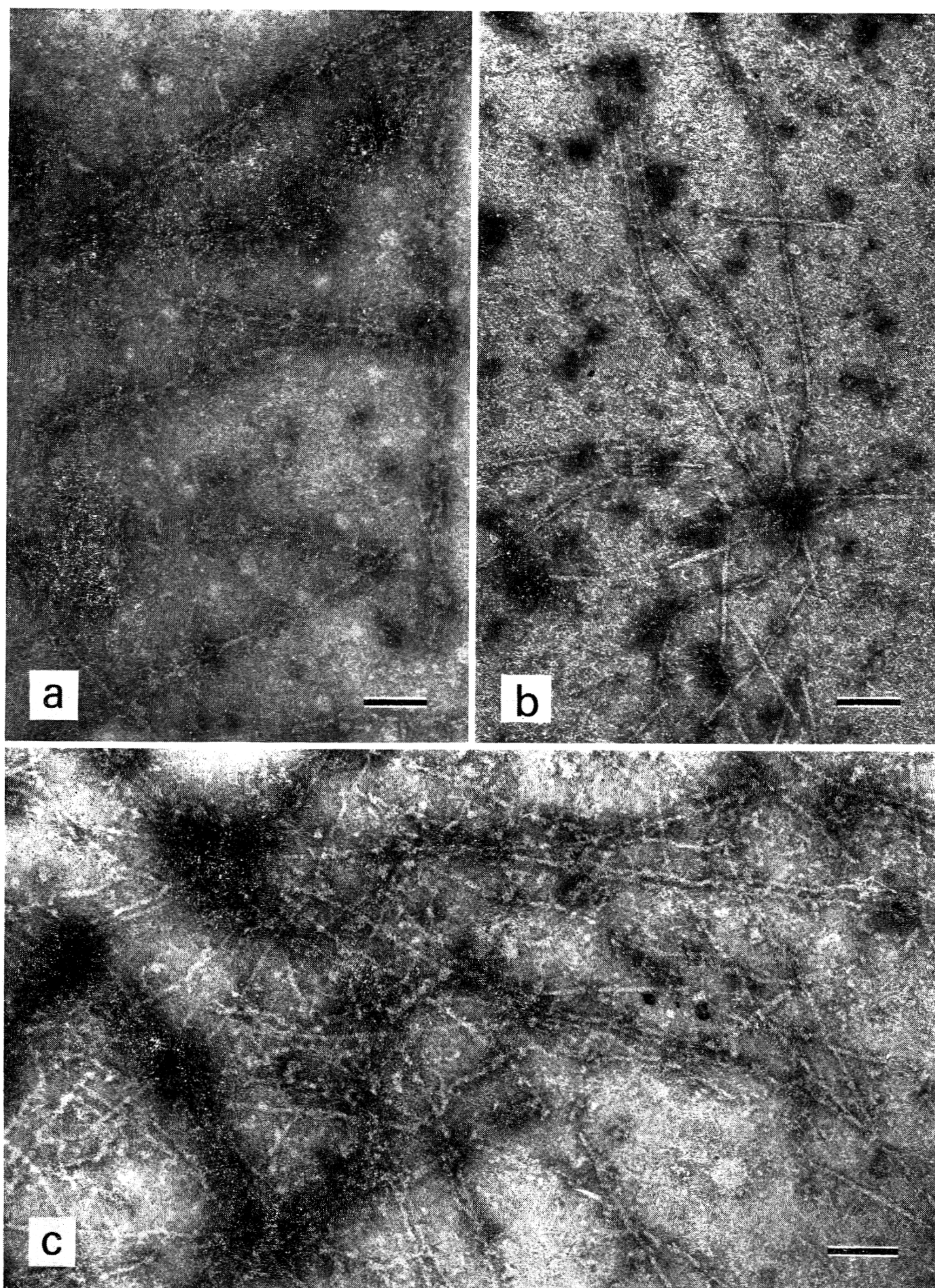

Plate 3. (a) Actomyosin filaments in the KCl-extract from myofibrils incubated with $6 \cdot 2$ μmol linoleic acid/g muscle. Note myosin decorations along the filaments. × 90000. (b) Filaments in the KCl-extract from myofibrils incubated with $1 \cdot 6$ μmol LAHPO/g muscle. Note absence of myosin decoration along the filaments. × 90 000. (c) Aggregated actomyosin filaments in the sedimented gel-phase of KCl-extracted myofibrils incubated with $0 \cdot 53$ μmol LAHPO/g muscle. Note parallel alignment of several filaments. × 99 000. Scales represent $0 \cdot 1$ μm.

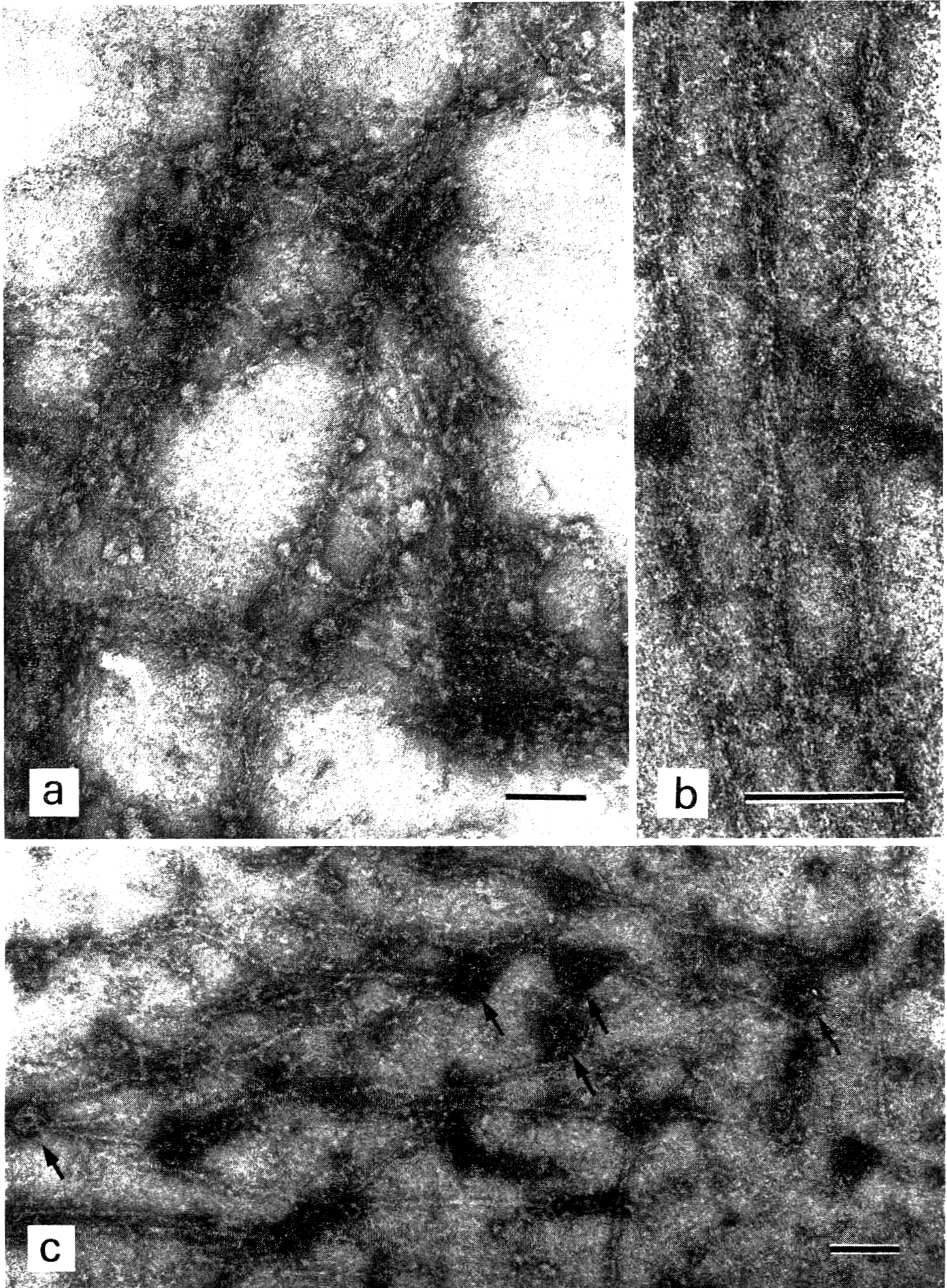

PLATE 4. (a) Aggregated actomyosin filaments in a mixture of linoleic acid and myofibrillar protein (9 μmol linoleic acid/mg protein). Note fatty acid micelles randomly adsorbed along the filaments. $\times$ 112 500. (b) Actomyosin filaments from a mixture of linoleic acid and myofibrillar protein (3 μmol linoleic acid/mg protein). Note fairly regular myosin decoration along the filaments. $\times$ 225 000. (c) Aggregated filaments in the supernatant of extracted myofibrillar proteins incubated with 15·8 μmol LAHPO/mg protein. Arrows point to dark spots possibly representing LAHPO-micelles. $\times$ 99 000. Scales represent 0·1 μm.

The transparent gel sedimenting upon centrifuging KCl-extracts of LAHPO-incubated myofibrils was found to consist of aggregated actomyosin filaments. Negative staining of dilutions of this gel-phase revealed filaments in a parallel lateral arrangement (Plate 3c). This filament arrangement shows similarities to the one obtained with a reduction of the ionic strength, in which case the aggregation takes place by interactions between myosin molecules attached to different actomyosin filaments (Jarenbäck & Liljemark, 1975b). The gel-phase may also contain other components, e.g. actomyosin filaments connected to Z-disc material.

Structure of myofibrillar proteins incubated with linoleic acid and LAHPO

In negatively stained mixtures of myofibrillar protein and emulsified linoleic acid large aggregates were found. The presence of fatty acid in the aggregates could be demonstrated only at amounts of linoleic acid equal to or exceeding that of myofibrillar protein. Plate 4a shows micelles of fatty acid adhering to the surface of actomyosin filaments. The adhering fatty acid may fuse several actomyosin filaments, thus giving rise to larger aggregates. Outside the aggregates, actomyosin filaments appeared not to be affected by the presence of linoleic acid, as far as the myosin decoration pattern is concerned (Plate 4b).

In myofibrillar protein solutions incubated with increasing amounts of LAHPO a gradual precipitation of protein occurred (Fig. 4). Negative staining was only applied to centrifuged solutions of myofibrillar protein to which more than 15 nmol LAHPO/mg protein had been added. The material not sedimenting after centrifugation at 10 000 *g* for 30 min was found to contain a few aggregates of actomyosin filaments (Plate 4c). These appeared similar to the aggregates of filaments found in the gel-phase of KCl-extracts from LAHPO incubated myofibrils (cf. Plate 3c).

Discussion

In this work, linoleic acid was chosen as test substance owing to its general use in studies of this type and particularly because its primary oxidation products can be easily prepared. Although linoleic acid is present only in small quantities *in situ* (Olley & Duncan, 1965; Addison *et al.*, 1968), it belongs to one of the major systems of polyunsaturated fatty acids found in cod muscle (Ackman, 1967). The amounts of linoleic acid added to myofibrils were chosen as falling within the concentration range of FFA maximally produced in cod during storage at $-10°C$ to $-15°C$ (Dyer & Fraser, 1959; Olley *et al.*, 1962; Bligh & Scott, 1966).

The marked reduction in the protein content of KCl-extracts from linoleic acid-incubated myofibrils (Fig. 1) is ascribed to the fatty acid itself, rather than to oxidation products, although a slight oxidation during the 20-hr extraction period cannot be entirely excluded. However, the use of propylgallate as an antioxidant did not change the effect of linoleic acid significantly.

In this case, the myofibrils were incubated with linoleic acid at pH 7·6. Anderson *et al.* (1965) found a maximal effect of sodium linolenate in reducing the solubility of isolated cod myofibrillar protein at pH 7·2 and at an ionic strength of 0·5. This effect was ascribed mainly to electrostatic interactions between fatty acid anions and charged groups on the proteins. In the pH range 6·4–6·8, closer to the pH values of frozen and stored cod (Kelly, 1969), no immediate precipitation of cod myofibrillar protein by sodium linolenate (Anderson *et al.*, 1965) or linoleic acid (Hanson & Olley, 1965) took place when added in concentrations sufficient to cause total precipitation at pH 7·2. The effect of pH may possibly be explained in terms of changes in the physical state of fatty acid, with a lower degree of dissociation and solubility prevailing at lower pH (Mabrouk & Dugan, 1961). Compared to the *in situ* behaviour this pH-dependence may, however, make the possibilities for interaction between FFA and protein questionable.

In addition to electrostatic interactions, proteins and detergents as fatty acids are known to interact by non-specific hydrophobic forces (Chapman, 1969; Tanford, 1972). Hydrophobic interaction between the hydrocarbon chains of FFA and nonpolar amino acid groups in the myofibrillar proteins was also suggested as a mechanism for the *in situ* effect of FFA (Anderson & Ravesi, 1970).

The high salt content (0·45 M KCl) necessary to extract and keep myofibrillar proteins in solution in *in vitro* experiments, may affect the FFA-protein interaction. A high salt concentration increases the size of fatty acid micelles in solution as well as the micellar weight of fatty acids already bound to protein by a general salting-out effect (Allen, 1974). The salt effect together with a non-specific co-operative association of FFA to protein (Tanford, 1972) could in turn increase the interaction between proteins, and explain the rapid change in protein solubility over a very narrow range of increasing fatty acid concentrations, obtained by most investigators.

The electron microscopic examination gave further information on the gradual reduction in protein content of KCl-extracts from linoleic acid-incubated myofibrils. Low concentrations of linoleic acid induced an aggregation of extracted proteins. Higher concentrations of linoleic acid caused an increasing retention of the original myofibril shape but no obvious impairment of the release of myosin from thick myofilaments (Plates 1c and 2a). Whether linoleic acid was initially associated with myofilaments or just adsorbed on the surface of myofibrils and later redistributed to actomyosin filaments in solution could not be determined by the structural observations. Based on the physical state of linoleic acid in the system used, a micellar solution, a surface effect on myofibrils may be the most probable mode of interaction.

In connection with frozen storage of cod, FFA may be implied in changes of membrane integrity, seen as a collapse of the sarcoplasmic reticulum (Jarenbäck & Liljemark, 1975a). Membrane changes may further contribute to the increased cohesion between myofibrils also observed upon frozen storage (Anderson & Ravesi, 1970; Jarenbäck & Liljemark, 1975b). Anderson & Ravesi (1968) found that in-

creasing the FFA-content of cod muscle, by storing for up to 39 days in ice, caused an increased cohesion between myofibrils but only a slight reduction in the amount of extractable protein. Childs (1973) observed a decreased extractability of whole myofibrils following frozen storage of Pacific cod and noticed later a reduced suspensibility of myofibrils by added FFA (Childs, 1974).

These observations, together with the surface effect of linoleic acid on cod myofibrils indicated here, suggest that FFA may exert an *in situ* effect on the textural qualities of frozen and stored cod by promoting an increased interaction between myofibrils. In living systems, e.g. hen erythrocytes, fatty acids are known to be powerful agents in fusing cells by increasing the membrane fluidity (Ahkong *et al.*, 1973). In membrane changes the damaging effects of lipid peroxides must also be considered (Tappel, 1973).

LAHPO were found to reduce the protein content of KCl-extracts from incubated myofibrils at a ten times lower concentration range (Fig. 3) compared with linoleic acid (Fig. 1). Whether the effect of LAHPO was caused by the peroxides as such or they reacted further in the presence of myofibrils and exerted their effect by virtue of secondary reaction products was not established in the present work. However, no recovery of LAHPO from myofibril incubation media was found. Judged from Fig. 1 the 19% unreacted linoleic acid in LAHPO solutions has no effect in the concentration range of LAHPO used. Olley & Duncan (1965) calculated that 0·1–0·15 mg of fatty acid peroxide would insolubilize all the myosin of 1 g cod muscle. In this case 1·5 μmol LAHPO, equivalent to 0·47 mg LAHPO/g muscle, caused a 90% reduction in the protein content of KCl-extracts where also an interaction with other myofibrillar proteins must be taken into account.

The structural examination revealed that LAHPO may exert a direct effect on myosin in the thick myofilaments of the myofibrils. This was seen as a retention of A-bands following KCl-extraction of LAHPO-incubated myofibrils (Plate 2b and c) and indirectly by a decreased myosin decoration of solitary filaments in corresponding KCl-extracts (Plate 3b). In this respect the structural changes induced by LAHPO show similarities with those found after frozen storage of cod in which case a progressive fixation of myosin in thick myofilaments takes place (Jarenbäck & Liljemark, 1975b).

Extracted myofibrillar proteins were precipitated by LAHPO. A possible explanation of the leveling out of the curve in Fig. 4 at LAHPO concentrations exceeding 15 nmol/mg protein is that when the majority of the actomyosin in solution has been aggregated and sedimented, myosin even though in an aggregated form remains suspended. About 30% protein remaining in solution (Fig. 4) is slightly less than the proportion of free myosin in cod myofibrillar extracts given by King (1966). Negative staining of centrifuged myofibrillar protein solutions given high amounts of LAHPO in fact revealed very little actomyosin although some aggregated filaments were still found in solution (Plate 4c).

A destabilizing effect of LAHPO on proteins and enzymes has been observed in many studies. Matsushita, Kobayashi & Nitta (1970) compared the effect of linoleic acid and LAHPO on the inactivation of RNase, trypsin and pepsin. Linoleic acid and LAHPO affected the enzymes to different degrees depending on pH. The effect of linoleic acid was ascribed to non-specific hydrophobic binding while LAHPO in addition could react with certain amino acids. With the same enzymes Gamage & Matsushita (1973) showed that LAHPO influenced the enzyme activities mainly by interaction with methoinine, tyrosine, lysine, cystine and histidine. In the oxidizing system of methyl linoleate and myosin used by Braddock & Dugan (1973), methionine, lysine and histidine were the amino acids most readily lost.

A higher efficiency of LAHPO compared to linoleic acid in reducing the protein content of KCl-extracts from incubated myofibrils was found. This may be explained not only by a stronger influence of LAHPO on the myofibrils but also by a higher capacity to aggregate protein once in solution.

Linoleic acid apparently aggregates actomyosin filaments by a non-specific adsorption of fatty acid micelles where at high linoleic acid to protein ratios the fatty acid proper could be visualized (Plate 4a). Chemical reaction with myosin in the actomyosin complex need not take place. As reported by Buttkus (1967), malonaldehyde reacted with ϵ-amino groups in trout myosin but with linoleic acid methyl ester or FFA from trout muscle no reaction involving the ϵ-amino groups of myosin was observed. LAHPO, by virtue of their reaction with amino groups, may be more extensively bound over the whole actomyosin filament surface. This may increase the hydrophobicity of the filaments causing a stronger interaction between individual filaments and give the gel-like precipitate with parallel alignment of filaments (Plate 3c). An additional non-specific effect, as described for linoleic acid, may participate in the LAHPO aggration of filaments since the physical state of LAHPO in the systems used is probably also a micellar solution (Plate 4c).

The significance for the involvement of lipid peroxides in changes of cod myofibrillar protein extractability must await an answer whether lipid oxidation takes place or not during frozen storage. According to Castell *et al.* (1968) the apparent absence of lipid oxidation in frozen cod muscle is solely because an increase in thiobarbituric acid reactive substances or in peroxide value has not been observed. When isolated, however, the phospholipids and FFA of cod muscle have been proven to be susceptible to oxidation (Roubal, 1967). The spontaneous interaction of LAHPO with proteins of cod muscle as demonstrated here may illustrate why the accumulation of lipid oxidation products in stored cod muscle has not been detected.

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References

ACKMAN, R.G. (1967) *J. Fd Technol.* **2,** 169.

ADDISON, R.F., ACKMAN, R.G. & HINGLEY, J. (1968) *J. Fish. Res. Bd Can.* **25,** 2083.

AHKONG, Q.F., FISHER, D., TAMPION, W. & LUCY, J.A. (1973) *Biochem. J.* **136,** 147.

ALLEN, G. (1974) *Biochem. J.* **137,** 575.

ANDERSON, M.L. & RAVESI, E.M. (1968) *J. Fish. Res. Bd Can.*, **25,** 2059.

ANDERSON, M.L. & RAVESI, E.M. (1970) *J. Fd Sci.* **35,** 551.

ANDERSON, M.L. & STEINBERG, M.A. (1964) *J. Fd Sci.* **29,** 327.

ANDERSON, M.L., KING, F.J. & STEINBERG, M.A. (1963) *J. Fd Sci.* **28,** 286.

ANDERSON, M.L., STEINBERG, M.A. & KING, F.J. (1965) In: *The Technology of Fish Utilization* (Ed. by R. Kreuzer), p. 105. Fishing News (Books) Ltd, London.

BABBIT, J.K., CRAWFORD, D.L., LAW, D.K (1972) *Agric. Fd Chem.* **20,** 1052.

BLIGH, E.G. & SCOTT, M.A. (1966) *J. Fish. Res. Bd Can.* **23,** 1025.

BRADDOCK, R.J. & DUGAN, L.R., JR (1973) *J. Am. Oil Chem. Soc.* **50,** 343.

BUTTKUS, H. (1967) *J. Fd Sci.* **32,** 432.

CASTELL, C.H. (1971) *J. Am. Oil Chem. Soc.* **48,** 645.

CASTELL, C.H., BISHOP, D.M. & NEAL, W.E. (1968) *J. Fish. Res. Bd Can.* **25,** 921.

CASTELL, C.H., SMITH, B. & DYER, W.J. (1973) *J. Fish. Res. Bd Can.* **30,** 1205.

CHAPMAN, D. (1969) *Lipids,* **4,** 251.

CHILDS, E.A. (1973) *J. Fd Sci.* **38,** 718.

CHILDS, E.A. (1974) *J. Fish. Res. Bd Can.* **31,** 111.

CONNELL, J.J. (1958) *Biochem. J.* **70,** 81.

CONNELL, J.J. (1968) In: *Low Temperature Biology of Foodstuffs* (Ed. by J. Hawthorn & E.J. Rolfe), p. 333. Pergamon Press, Oxford.

DESAI, I.D. & TAPPEL, A.L. (1963) *J. Lipid Res.* **4,** 204.

DOLE, V.P. (1956) *J. Clin. Invest.* **35,** 150.

DYER, W.J. & FRAZER, D.I. (1959) *J. Fish. Res. Bd Can.* **16,** 43.

DYER, W.J. & MORTON, M.L. (1956) *J. Fish. Res. Bd Can.* **13,** 129.

ERIKSSON, C.E. & LEU, K. (1971) *Lipids,* **6,** 144.

GAMAGE, P.T. & MATSUSHITA, S. (1973) *Agric. Biol. Chem.* **37,** 1.

HANSON, S.W.F. & OLLEY, J. (1965) In: *The Technology of Fish Utilization* (Ed. by R. Kreuzer), p. 111. Fishing News (Books) Ltd, London.

IKEDA, S. & TAGUCHI, T. (1967) *Bull. Jap. Soc. Sci. Fish.* **33,** 567.

JARENBÄCK, L. & LILJEMARK, A. (1975a) *J. Fd Technol.* **10,** 229.

JARENBÄCK, L. & LILJEMARK, A. (1975b) *J. Fd Technol.* **10,** 309.

KELLY, T.R. (1969) *J. Fd Technol.* **4,** 59.

KING, F.J. (1966) *J. Fd Sci.* **31,** 649.

KING, F.J., ANDERSON, M.L. & STEINBERG, M.A. (1962) *J. Fd Sci.* **27,** 363.

KWON, T.W., MENZEL, D.B. & OLCOTT, H.S. (1965) *J. Fd Sci.* **30,** 808.

LOWRY, O.H., ROSEBOROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951) *J. Biol. Chem.* **193,** 265.

MABROUK, A.F. & DUGAN, L.R., JR (1961) *J. Am. Oil Chem. Soc.* **38,** 9.

MATSUSHITA, S., KOBAYASHI, M. & NITTA, Y. (1970) *Agric. Biol. Chem.* **34,** 817.

MATSUSHITA, S., NITTA, Y., MAKINO, H. & KOBAYASHI, M. (1969) *Agric. Biol. Chem.* **33,** 1205.

MENZEL, D.B. & OLCOTT, H.S. (1964) *Fed. Proc.* **23,** (No. 2, part 1), 529.

OLLEY, J. & DUNCAN, W.R.H. (1965) *J. Sci. Fd Agric.* **16,** 99.

OLLEY, J. & LOVERN, J.A. (1960) *J. Sci. Fd Agric.* **11,** 644.

OLLEY, J., PIRIE, R. & WATSON, H. (1962) *J. Sci. Fd Agric.* **13,** 501.

OTA, F. & NISHIMOTO, J. (1963) In: *Progress in Refrigeration Science and Technology* (Proceedings of the XIth International Congress on Refrigeration, Munich, 1963), Vol. 2, p. 911. Pergamon Press, Oxford.

POWRIE, W.D. (1973) In: *Low Temperature Preservation of Foods and Living Matter* (Ed. by O.R. Fennema, W.D. Powrie & E.H. Marth), p. 282. Marcel Dekker, Inc., New York.

ROUBAL, W.T. (1967) *J. Am. Oil Chem. Soc.* **44,** 325.

ROUBAL, W.T. (1971) *Lipids*, **6,** 62.

ROUBAL, W.T. & TAPPEL, A.L. (1966a) *Arch. Biochem. Biophys.* **113,** 5.

ROUBAL, W.T. & TAPPEL, A.L. (1966b) *Arch. Biochem. Biophys.* **113,** 150.

SIDDIQI, A.M. & TAPPEL, A.L. (1956) *Arch. Biochem. Biophys.* **60,** 91.

SVENSSON, S.G. & ERIKSSON, C.E. (1972) *Lebensm.-Wiss. u. Technol.* **5,** 124.

TANFORD, C. (1972) *J. Mol. Biol.* **67,** 59.

TAPPEL, A.L. (1973) *Fed. Proc.* **32,** 1870.

TROUT, D.L., ESTES, E.H. & FREIDBERG, S.J. (1960) *J. Lipid Res.* **1,** 199.

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J. Fd Technol. (1975) **10,** 453–464.

Utilization of edible protein from meat industry by-products and waste

III. The isolation and spinning of proteins from lung and stomach

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Summary

Proteins isolated in quantity from porcine lung and stomach have been modified into fibrous forms. Stable dispersions could be prepared from the isolates on alkali treatment, the final viscosity depending upon protein and alkali concentration and the pH of isolation. Fibrous products spun from these protein sources appear to have lower elasticity than those spun from concentrated blood plasma. The spinning of admixtures of lung and stomach proteins with plasma gave rise to poor mechanical properties in the resultant fibres. However, amino acid analysis has indicated that such procedures effectively supplement isoleucine and methionine, deficient in spun plasma products.

Introduction

It has previously been shown that blood plasma can be fabricated by fairly straightforward procedures into spun products which could prove to be acceptable meat analogues (Young & Lawrie, 1974a). Additional work has indicated that proteins may be isolated in quantity from lung and stomach and that the electrophoretical character of the protein isolates depends upon the raw material (Young & Lawrie, 1974b). By virtue of the high yields of protein isolates obtainable from lung and stomach, it appeared useful to assess the possibility of spinning the recovered proteins and to compare the resultant products with those spun from plasma. The present study also sought to establish the feasibility of spinning admixtures of the protein isolates with concentrated plasma. It was thought that such treatment might allow variation of the textural and nutritional properties of products spun from meat waste proteins.

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Materials and methods

Isolation of protein from stomach and lung

Porcine stomachs and lungs were obtained fresh from the slaughterhouse. After thorough washing, about 1000 g of each tissue was minced. Portions of the minced tissues were homogenized for 1 min with distilled water, the solvent : tissue ratio being 10 : 1. The pH was adjusted to 10·0 with 1N NaOH and the suspension left overnight at 0 °C with stirring. The supernatant was recovered by centrifugation at 1000 **g** for 15 min and further clarified by filtration through glass wool. Protein isolates were prepared at two pH values. The supernatant was divided into two samples, the pH of one being adjusted to 4·5 and the pH of the other to 5·4. After storing overnight at 0 °C, each of the samples was again divided. One half was centrifuged at 12 000 **g** for 20 min and the other half at 15 000 **g** for 20 min. Thus, isolates of differing protein contents could be obtained. The concentration of protein in each isolate was determined on 1 g of the sample by the microkjeldahl method.

Treatment of protein isolates with alkali

The effect of alkali on the viscosity of protein dispersions from porcine lung and stomach was investigated at three levels of alkali : protein ratios for each sample. Previous trials had shown that an alkali : protein ratio of 0·05 resulted in redissolution of protein and an increase in viscosity. Consequently, the NaOH : protein ratios used in the study were 0·04, 0·05 and 0·06.

Ten grams of each isolate of protein contents varying from 98 to 150 mg/g was accurately weighed out in triplicate. The correct amount of alkali was provided from a stock solution of 0·1 g/ml NaOH. Aliquots of this stock solution were taken and diluted to 25 ml to give the concentrations required for addition to the isolates. One millilitre of the final dilution was added to the protein isolate and thoroughly mixed on a magnetic stirrer until a homogeneous slurry or solution was formed. Small volumes were taken at intervals for viscosity determination.

Viscosity measurement

Viscosity was determined using the Ferranti-Shirley cone and plate viscometer as described by Wilkinson (1960). For the determination of the rheological flow curves for the dispersions, about 0·3 ml of the alkali-treated sample was placed in the centre of the viscometer plate using a Pasteur pipette. The samples were sheared at varying rates by setting the rotational velocity of the cone at values between 1 and 100 rev/min. Readings of torque were taken at 6–8 different shear rates.

For studying the changes in viscosity with time, a cone velocity of 30 rev/min (equivalent to a shear rate of 517 sec^{-1}) was chosen for all measurements. Readings of torque at this shear rate were taken at varying intervals of time up to 4 hr from the addition of alkali.

Preparation of proteins for spinning

Three-hundred-gram portions of protein isolates recovered from porcine stomach and lung were used for spinning. The protein contents were adjusted to 100 mg/g in each case and an amount of NaOH added to give an alkali : protein ratio of 0·05. It was found necessary to emulsify the alkali-treated proteins for 1 min to obtain homogeneous dispersions. These dispersions were left to age for about 30 min prior to spinning.

Spinning dopes were prepared from bovine plasma using procedures previously described (Young & Lawrie, 1974a). For the preparation of protein mixtures, concentrated plasma protein was initially modified into a spinning dope. Lung or stomach protein isolate in the required proportion was then mixed with the plasma and the mixture stirred using a mechanical impeller for 10 min. The mixture was filtered through muslin cloth and again left to age for about 30 min before spinning. Lung and stomach proteins were each included at levels of 50% and 20% (w/w) of the total protein in the mix, protein contents being maintained at 100 mg/g.

Spinning procedure

The technique employed for spinning fibres was similar to that described elsewhere (Young & Lawrie, 1974a). The coagulating solution comprised 20% (w/v) NaCl in 1 N acetic acid. Protein, moisture and ash contents of the spun samples were analysed.

Amino acid analysis

Amino acid patterns for the proteins before and after spinning were determined by ion-exchange chromatography (Moore & Stein, 1963) using an EEL 194 autoloading analyser.

Tryptophan was measured colourimetrically using the procedure reported by Miller (1967) incorporating p-dimethylamino-benzaldehyde. The method of Carpenter (1960) was used for the determination of available lysine.

Results

Viscosity behaviour of the alkali-treated proteins

The plots of shear rate against shear stress for the alkali-treated proteins show a similar pattern indicating that they possess non-Newtonian fluid properties (Fig. 1). The curves are of a time-independent nature and their shape suggests that the fluids approximate to a Bingham plastic at high shear rates whilst exhibiting pseudoplastic behaviour at lower shear rates. Due to their non-Newtonian properties, values of viscosity for the protein dispersions will depend upon the particular shear rate at which the viscosity is measured and should be quoted as apparent values.

In general, the viscosities of lung protein isolates sharply increased during the first 10 min after the addition of alkali. Following the initial rise, viscosities stabilized to

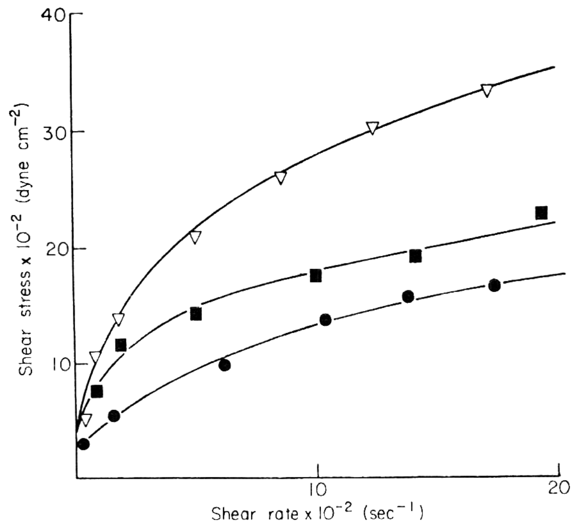


FIG. 1. Rheological flow curves for alkali-treated proteins. ▽, Concentrated bovine plasma; ■, isolated porcine stomach protein; ●, isolated porcine lung protein.

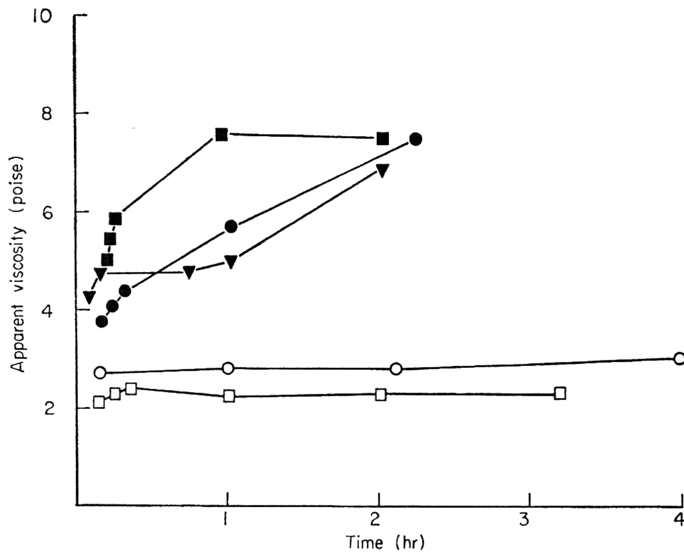


FIG. 2. The alteration in viscosity of isolated lung protein (porcine) with time as affected by protein concentration at an alkali : protein ratio of 0·04. □, 98 mg/g protein (pH 5·4 isolate); ○, 113 mg/g protein (pH 5·4 isolate); ▼, 120 mg/g protein (pH 4·5 isolate); ●, 128 mg/g protein (pH 4·5 isolate); ■, 150 mg/g protein (pH 4·5 isolate).

values which depended on protein and alkali concentrations (Figs 2–4). At a protein concentration of 150 mg/g isolate, gelation occurred under all conditions of alkali treatment indicating that concentrations of such magnitude may not be desirable for spinning. Variation of the ratio of alkali: protein between 0·4 and 0·6 had a negligible effect on the final values of viscosity using protein concentrations of 98 and 113 mg/g isolate.

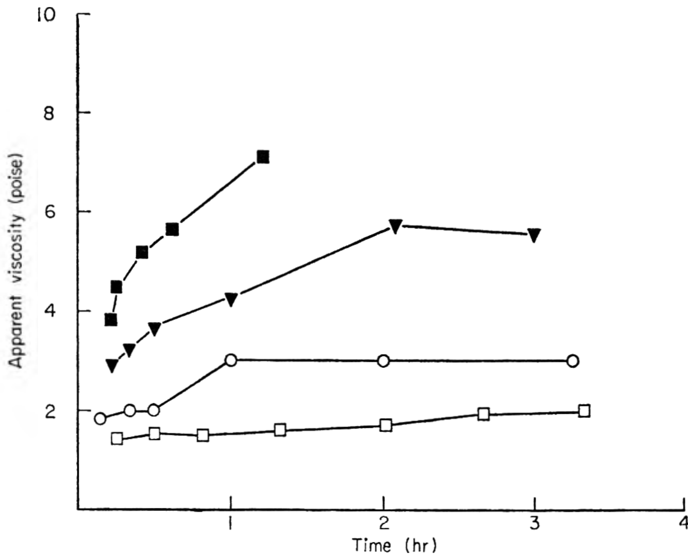


Fig. 3. The alteration in viscosity of isolated lung protein (porcine) with time as affected by protein concentration at an alkali : protein ratio of 0·05. □, 98 mg/g protein (pH 5·4 isolate); ○, 113 mg/g protein (pH 5·4 isolate); ▼, 120 mg/g protein (pH 4·5 isolate); ■, 150 mg/g protein (pH 4·5 isolate).

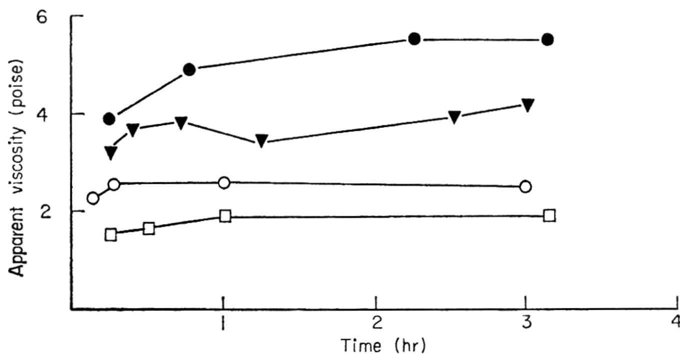


Fig. 4. The alteration in viscosity of isolated lung protein (porcine) with time as affected by protein concentration at an alkali : protein ratio of 0·06. □, 98 mg/g protein (pH 5·4 isolate); ○, 113 mg/g protein (pH 5·4 isolate); ▼, 120 mg/g protein (pH 4·5 isolate); ●, 128 mg/g protein (pH 4·5 isolate).

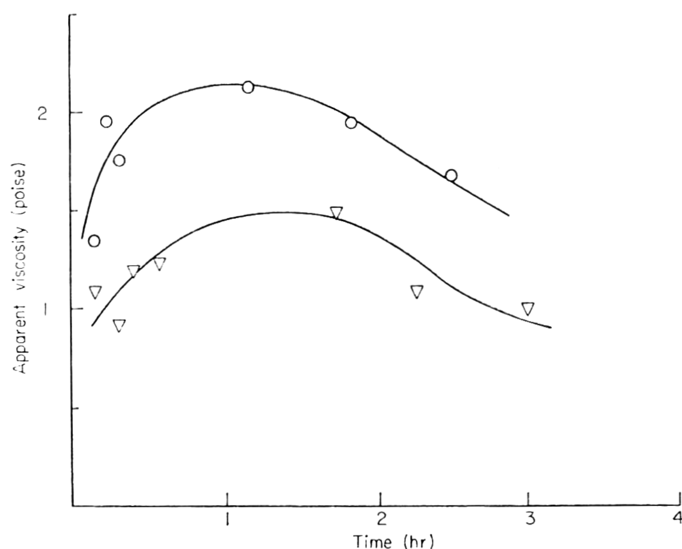


Fig. 5. The alteration in viscosity of isolated stomach protein (porcine) with time as affected by alkali : protein ratio. ○, alkali : protein = 0·055; ▽, alkali : protein = 0·05.

Apparent viscosities of about 2 and 3 poise respectively were recorded in each case, the modified dispersions being of suitable consistency and stability for spinning. However, for the isolates of higher protein content (120–150 mg/g), the rise in viscosity appeared to depend upon the concentration of alkali present.

It was interesting to observe that higher viscosity increases were exhibited in the samples isolated at pH 4·5 than in those isolated at pH 5·4. This is particularly obvious from Fig. 2 where the differences in viscosity behaviour appear too great to be accounted for by protein content alone.

The overall changes in viscosity with time for alkali-treated stomach protein were similar to those for the lung protein isolates. However, a notable feature common to the stomach protein was that a peak viscosity occurred 1–2 hr after the addition of alkali. This is illustrated in Fig. 5. Nevertheless, the viscosity appeared to be sufficiently stable to allow the dope to be spun into fibres. The values of apparent viscosity recorded for alkali-treated stomach protein were of the same order as those for alkali-treated lung protein at corresponding protein and alkali concentrations.

Chemical analysis and general appearance of spun fibres

General analyses of samples taken throughout the course of the study are given in Table 1. All the determinations were carried out 24 hr after spinning, the samples having rested in sealed bottles. Spun plasma protein was found to have a greater protein content, and concomitantly less moisture, than spun lung and stomach proteins. In most cases, spun stomach protein had the lowest protein content, a value as low as

TABLE 1. General analyses of spun protein fibres

	Source of protein		
	Plasma	Lung	Stomach
Protein (%)	16·5–19·7	14·0–15·3	11·5–14·8
Moisture (%)	72·8–75·3	75·1–78·9	79·1–82·2
Ash (%)	7·5–8·6	8·1–8·5	7·7–8·4

11·5% being recorded in one instance. An ash content of about 8% was common to all spun samples and reflected the extent to which salt was carried over from the coagulating bath.

An impression of the differences in appearance of spun products obtained from serum, lung and stomach proteins may be gained from Plates 1 and 2. Although in each case the fibres could be extruded and drawn without difficulty, those fabricated from the isolated lung and stomach proteins had a more coarse appearance than the spun plasma product. An obvious characteristic of spun lung protein was the presence of brown

TABLE 2. Amino acid compositions (g/16 g N) of bovine plasma protein and soluble protein extracts from porcine stomach and lung

Amino acid	Plasma	Lung	Stomach
Isoleucine*	3·5	4·1	5·5
Leucine*	10·9	10·3	9·9
Lysine*	10·1	8·3	8·1
Methionine*	1·9	3·3	4·2
Cystine*	Trace	Trace	Trace
Phenylalanine*	5·3	—	4·0
Threonine*	5·9	3·7	4·4
Valine*	6·9	5·8	6·0
Tryptophan*	1·4	1·3	1·1
Arginine†	6·3	6·2	6·5
Histidine†	3·8	4·1	—
Alanine	3·7	5·5	5·0
Aspartic acid	7·9	7·9	7·0
Glutamic acid	12·7	11·3	12·0
Glycine	2·9	3·9	4·0
Proline	8·1	—	—
Serine	6·0	4·5	4·5
Tyrosine	5·4	3·6	3·6

* Essential; † essential for infants.

metmyoglobin. Moreover, in the absence of neutralization of the fibres, the intensity of the colour tended to increase on storage due to further acid denaturation. Incorporation of lung protein at a level of 20% in admixtures with serum protein resulted in a slightly darker spun product than that from serum protein alone. The latter product together with spun stomach proteins was creamy white in colour.

Regarding the spinning of admixtures of proteins, extreme difficulty was encountered in collecting fibres spun from 50 : 50 mixtures of lung or stomach protein and plasma protein due to their fragile nature. Inclusion of either of the former proteins at a level of 20% in the mix, however, appeared to have little effect on the mechanical strength of the spun fibres.

Assessment of protein quality

The amino acid profiles of normal bovine plasma and soluble alkaline protein extracts from porcine stomach and lung are presented in Table 2. The essential amino acids were generally well represented although lower amounts of isoleucine and methionine (3·5 and 1·9 g/16 g N respectively) were recorded in plasma protein. Isoleucine and methionine were present in porcine lung protein at concentrations of

TABLE 3. Amino acid compositions (g/16 g N) of spun plasma, lung and proteins stomach

Amino acid	Spun plasma protein	Spun lung protein	Spun stomach protein
Isoleucine*	3·3	4·0	4·9
Leucine*	9·1	9·4	8·7
Lysine*	9·3	7·3	7·7
Methionine*	2·0	3·3	3·4
Cystine*	Trace	Trace	Trace
Phenylalanine*	5·6	4·1	4·2
Threonine*	7·3	4·8	4·9
Valine*	7·7	5·5	4·8
Tryptophan*	1·5	1·4	1·1
Arginine†	6·6	6·0	5·7
Histidine†	3·1	3·1	2·7
Alanine	5·2	5·7	5·2
Aspartic acid	10·0	8·9	7·5
Glutamic acid	12·5	12·9	13·0
Glycine	3·5	4·2	4·2
Proline	5·4	5·0	—
Serine	6·5	5·0	5·0
Tyrosine	5·0	3·7	4·0

* Essential; † essential for infants

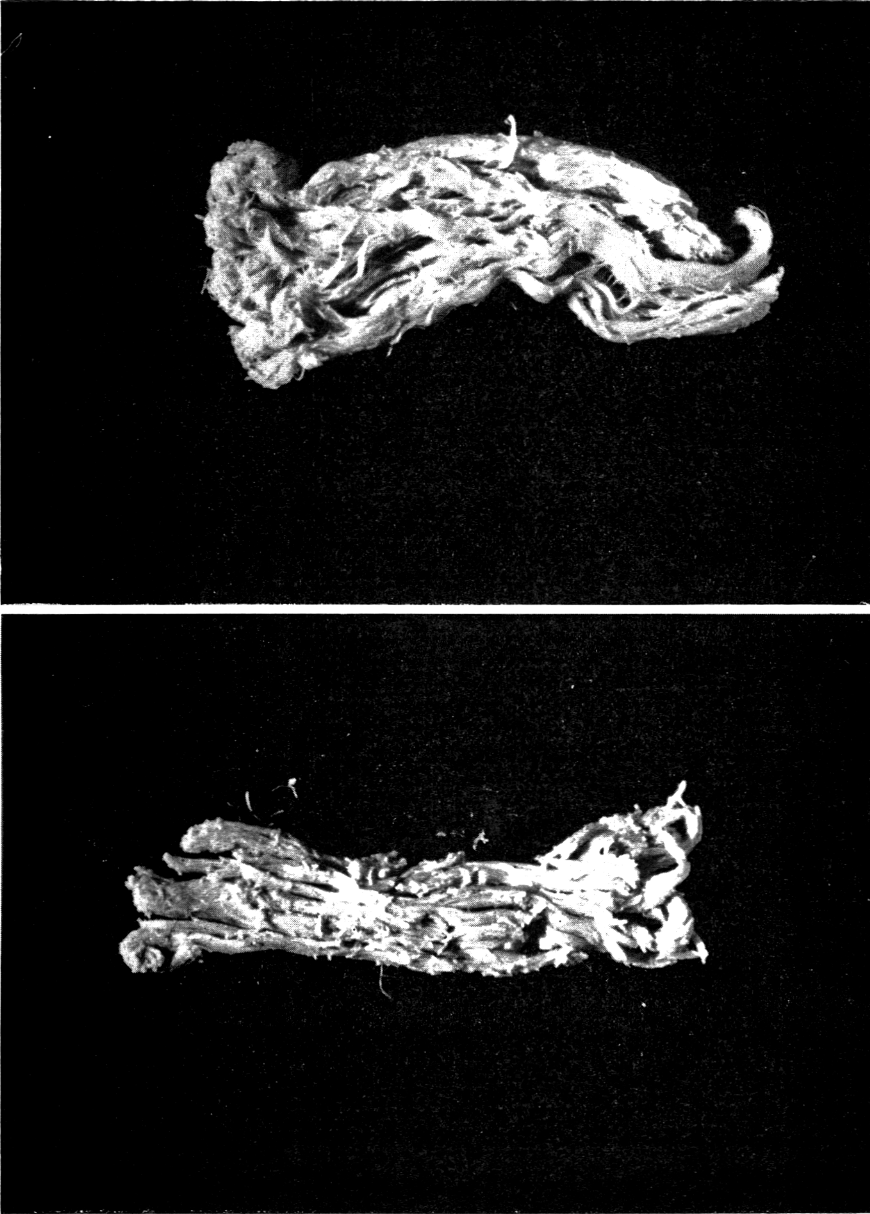
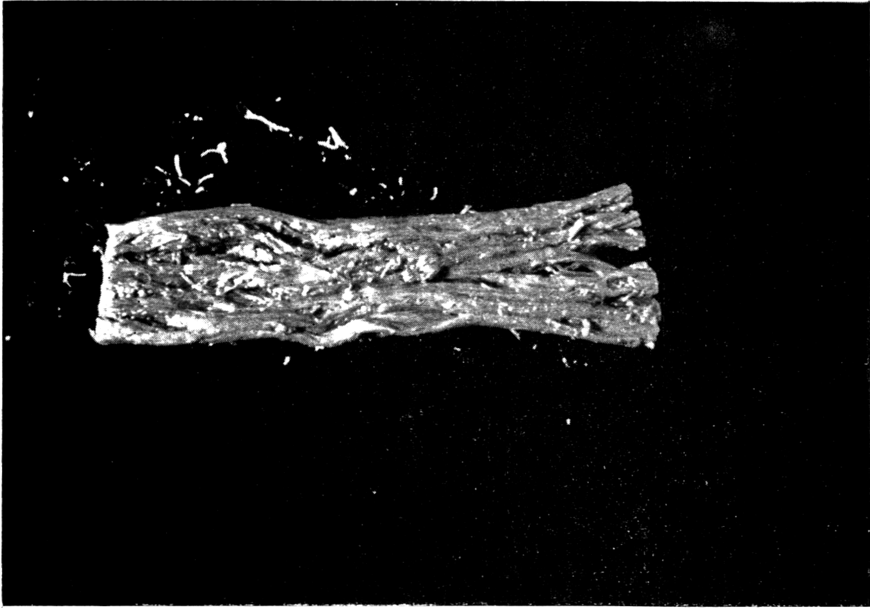


PLATE 1. Spun products fabricated from (a) plasma protein; (b) 80% plasma/20% lung protein.

(*Facing p.* 460)



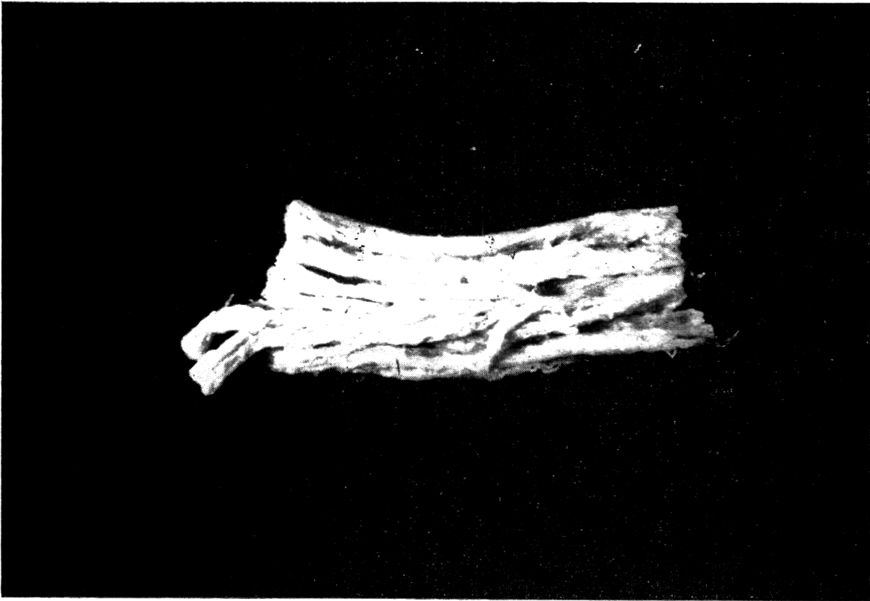


PLATE 2. Spun products fabricated from (a) lung protein; (b) stomach protein.

4·1 and 3·3 g/16 g N respectively, and at concentrations of 5·5 and 4·2 g/16 g N respectively in porcine stomach protein. Serum protein comprised greater amounts of the other essential amino acids.

The amino acid profiles of spun products from these proteins are shown in Table 3. No significant changes in the patterns occurred on spinning but slight losses of isoleucine, leucine and lysine were consistently observed. For example, the concentrations of isoleucine, leucine and lysine in plasma protein were altered from 3·5, 10·9 and 10·1 g/16 g N respectively prior to spinning to 3·3, 9·1 and 9·3 g/16 g N after spinning in an acetic acid/salt solution. Similar losses were recorded on spinning lung and stomach proteins.

Table 4 shows the amino acid patterns of spun products obtained from 50 : 50 and 80 : 20 mixtures of plasma protein with lung and stomach protein. In the products from all these protein mixtures the concentrations of isoleucine and methionine were higher than those of the plasma product. The degree of supplementation of isoleucine and methionine was greater in products from spun mixtures of plasma and stomach proteins. In this case, the incorporation of stomach protein at a level of only 20% promoted a marked increase in the isoleucine and methionine concentration in the spun

TABLE 4. Amino acid compositions (g/16 g N) of spun products from admixtures of plasma with lung and stomach proteins

Amino acid	80 : 20 plasma : lung protein fibre	50 : 50 plasma : lung protein fibre	80 : 20 plasma : stomach protein fibre	50 : 50 plasma : stomach protein fibre
Isoleucine*	3·4	3·8	4·0	4·4
Leucine*	9·1	9·4	10·0	9·0
Lysine*	8·9	7·2	9·6	8·0
Methionine*	2·4	3·1	2·6	3·3
Cystine*	Trace	Trace	Trace	Trace
Phenylalanine*	5·3	4·4	5·3	4·4
Threonine*	6·6	5·6	6·6	5·7
Valine*	6·7	6·0	7·4	7·1
Tryptophan*	1·5	1·5	1·3	1·3
Arginine†	6·0	5·0	6·1	5·6
Histidine†	3·1	2·7	3·0	2·8
Alanine	5·5	5·3	5·6	5·3
Aspartic acid	10·0	9·6	8·9	8·9
Glutamic acid	12·7	13·3	14·3	13·9
Glycine	4·3	3·8	4·0	4·4
Proline	4·8	5·0	—	—
Serine	6·8	5·8	7·0	6·5
Tyrosine	5·0	3·9	5·1	4·4

* Essential; † essential for infants.

TABLE 5. Available lysine (g/16 g N) in spun protein fibres

Spun protein	Available lysine (g/16 g N)
Bovine plasma	9·3
Porcine lung	7·0
Porcine stomach	6·9

product to 4·0 and 2·6 g/16 g N respectively. At the 50% level, these concentrations were further increased to 4·4 and 3·3 g/16 g N respectively. Higher amounts of the other essential amino acids were maintained in the mixed products, by virtue of the presence of plasma protein, than in the products spun from lung or stomach proteins alone.

Mean values of available lysine for spun proteins are given in Table 5. It was particularly interesting to observe that the available lysine content of spun protein was remarkably high (9·3 g/16 g N) and equalled the total lysine value. It was apparent from the results that little, if any, reduction in the availability of lysine had occurred during the spinning procedure.

Discussion

The fabrication of palatable food products is a natural progression from the isolation of recoverable protein in a concentrated form. From the results of this study, it appears that lung and stomach protein isolates may be modified into fibrous meat-like forms. However, the behaviour of these isolates on alkaline treatment differed substantially from that previously observed for concentrated plasma protein (Young & Lawrie, 1974a). Moreover, spun products engineered from isolated lung and stomach proteins exhibited lower elasticity than spun plasma protein.

The exponential viscosity rise terminating in gelation which was characteristic of alkali-treated plasma and necessitated stabilization with acid was not observed for the isolated lung and stomach proteins. The latter exhibited an initial sharp increase in viscosity stabilizing to a plateau after a few minutes in a similar manner to that reported for acid-precipitated soy protein (Kelley & Pressey, 1966). It is likely that this type of viscosity behaviour on alkaline treatment is characteristic of isolated proteins in general. Indeed, studies have indicated that the viscosity of plasma protein isolated by acid and salt treatment also stabilizes under alkali treatment alone (R. H. Young, unpublished data). The effect may be related to the incidence of preliminary aggregation of protein at the isolation stage which could occur to some extent due to its amorphous nature. Furthermore, the higher viscosities observed in the lung protein samples isolated at pH 4·5 may also be due to excessive protein aggregation with the exclusion of water at

this pH. The similarity of the viscosity curves observed for isolated proteins suggests that the state of the protein is of greater significance in determining the viscosity behaviour in alkali than the particular protein type. This is an important factor, not only because a high degree of stability of viscosity is required in spinning dopes, but also because the state of the protein in the dope may influence the mechanical properties of the resultant spun fibres.

It was interesting to note that products spun from lung and stomach protein isolates appeared to be less elastic than those fabricated from concentrated plasma. Electrophoretical differences between the proteins of lung, stomach and plasma have been established (Young & Lawrie, 1974b) and these differences in protein character may in part be responsible for variations in the physical properties of the spun products. However, the manner in which the protein has been treated prior to spinning may have a more appreciable effect on the fibre properties. On the basis of these preliminary trials, it would appear that fibrous products from concentrated plasma possess more desirable properties than those formed from lung or stomach protein isolates. This may be related to the fact that the isolation stage is omitted during the preparation of plasma protein for spinning.

The differing states of concentrated plasma protein and lung or stomach protein isolates caused problems in the preparation of protein admixtures for spinning. Fibres spun from 50 : 50 mixtures of these proteins were excessively weak probably due to insufficient denaturation of a portion of the protein in the mix by alkali or to the inability of the proteins to suitably interact. It would appear therefore that this approach requires more detailed experimentation.

There were no appreciable differences between the amino acid profiles of spun products and original protein extracts which correlated well with human requirements (WHO/FAO, 1973). Workers concerned with the nutritional value of soy protein preparations have reached similar conclusions (Bressani *et al.*, 1967). Moreover, the high values obtained for available lysine in the products suggest that little nutritional damage had occurred on spinning. A possible drawback regarding spun plasma protein was the slight deficiency in isoleucine and methionine. It was demonstrated, however, that these amino acids were supplemented in the products spun from admixtures of plasma protein with lung or, more especially, stomach proteins. Incorporation of the latter proteins at a level of only 20% in the mixture effected a high degree of supplementation. In view of the probable nutritional significance of products spun from admixtures of these proteins, it would seem worthwhile to direct further attention to the improvement of their inferior mechanical properties.

Acknowledgments

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References

BRESSANI, R., VITERI, F., ELIAS, L.G., ZAGHI, S., ALVARADO, J. & ODELL, A.D. (1967) *J. Nutr.* **93,** 349.
CARPENTER, K.J. (1960) *Biochem. J.* **77,** 604.
KELLEY, J.J. & PRESSEY, R. (1966) *Cereal Chem.* **43,** 195.
MILLER, E.L. (1967) *J. Sci. Fd Agric.* **18,** 381.
MOORE, S. & STEIN, W.H. (1963) *Meth. Enzym.* **6,** 819.
WHO/FAO (1973) *Energy and Protein Requirements*. WHO Techn. Rep. Ser. No. 522.
WILKINSON, W.L. (1960) *Non-Newtonian Fluids*, p. 124. Pergamon Press, Oxford.
YOUNG, R.H. & LAWRIE, R.A. (1974a) *J. Fd Technol.* **9,** 171.
YOUNG, R.H. & LAWRIE, R.A. (1974b) *J. Fd Technol.* **9,** 69.

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Utilization of edible protein from meat industry by-products and waste

IV. Studies on the physical and mechanical properties of spun fibrous products

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Summary

The effect of various parameters on the mechanical properties of fibrous products fabricated from meat waste protein has been investigated. The shear resistance of spun plasma protein was significantly affected by the extrusion rate of protein through the spinneret and by the drawing velocity of the fibres. Fibrous products spun from isolated lung and stomach proteins consistently exhibited a low resistance to shear. These findings are related to the fine structure of the protein constituting the fibre and their significance with regard to the manipulation of texture in the spun products is discussed.

Introduction

The previous paper in this series described procedures for fabricating spun products from proteins isolated from lung and stomach. Moreover, preliminary investigations revealed differences in the mechanical properties of products spun from lung, stomach and plasma proteins. It would appear that a number of factors may influence the mechanical properties of the spun fibres. These include the protein type, the mode of preparation of protein prior to spinning and the conditions selected for spinning. Information regarding the effect of these parameters on fibre properties is minimal. Indeed, only recently have the spinning dynamics of any protein been systematically studied (Balmaceda & Rha, 1974). The structural alterations in the proteins which are responsible for the mechanical properties of the spun fibres have also not been fully elucidated. Lundgren (1949) related molecular orientation and crystallization to fibre properties. These theories of unfolding and alignment of peptide chains were used to explain the nature of alkali-treated protein dopes and the changes in fibre strength on

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stretching (Boyer, 1954). More recently, however, Tombs (1970) has shown that in general the artificial protein fibres are cylindrical gels and suggests that the mechanical properties of groundnut, casein and soy based fibres depend more upon the gel mesh dimensions than on the possibilities of interaction between extended peptide chains.

Since a variety of protein fibres have been fabricated from meat waste sources, it was thought useful to establish more clearly the effect of spinning parameters and protein type on the mechanical properties of the fibres. The spinning parameters considered were the viscosity of the spinning dope, the gear pump velocity (i.e. extrusion rate) and the velocity of the take-away reel (i.e. drawing velocity). In addition an attempt was made to relate the mechanical properties to the structural arrangement of the proteins constituting the fibre using the technique of electron miscroscopy.

Materials and methods

Preparation of proteins for spinning

Five-hundred-millilitre volumes of bovine plasma concentrated by partial freeze drying were used for spinning, alkaline modification being effected on the basis of data previously obtained (Young & Lawrie, 1974a). The viscosity behaviour of the plasma was followed on a 100-ml aliquot which was poured into a falling-ball viscometer. The time of addition of acetic acid could then be calibrated against a particular fall-time from the viscometer so that desired values of final viscosity could be acquired.

Protein isolates from porcine lung and stomach were recovered at pH 5·0 and treated with 40% (w/v) NaOH to resolubilize and increase the viscosity of the protein dispersion. Three-hundred-gram portions of protein isolate were used for spinning, the amount of NaOH added calculated to give an alkali : protein ratio of 0·05. The dispersions were emulsified for 1 min and left to age for about 30 min prior to spinning.

Spinning procedure

The apparatus used to spin the protein fibres has been described in an earlier publication (Young & Lawrie, 1974a). In each case, the denatured protein dispersion was poured into the reservoir of the spinning apparatus and a pressure of 20 psi maintained with the manual pump to force the dispersion to the gear pump. The rotational velocity of the gear pump could be varied between 1 and 10 rev/min. Above the latter limit, difficulty in obtaining and collecting fibres was experienced. The coagulating solution for these experiments consisted of 1N acetic acid containing 20% (w/v) NaCl (pH 2·3). Fibres were fed from the solution to the rotating take-away reel which could then draw the fibres continuously from the spinneret. Differing degrees of stretch were applied by varying the speed of the take-away reel between 200 cm/min and 600 cm/min. Bundles of fibres were removed from the reel, drained of excess liquid and stored at 0°C in screw-capped bottles.

Experimental design 1: effect of spinning parameters on the shear resistance of spun fibres

In this experiment, the effect of dope viscosity, gear pump velocity and rotational speed of the take-away reel on the shear resistance of spun samples was investigated using concentrated plasma as the raw material.

A randomized block experiment in two blocks was designed incorporating high and low levels of the parameters under investigation. Thus, eight different treatments were carried out in duplicate to give sixteen runs. The protein concentration of the plasma was maintained at 97·5 mg/ml for each treatment. The high and low levels of apparent viscosity were about 7 and 2 poise respectively (as measured at a shear rate of 517 sec^{-1} using a Ferranti–Shirley cone and plate viscometer). The desired level of viscosity was acquired by varying the time of addition of acetic acid to the alkali-treated plasma. Gear pump velocity was 10 rev/min at the high level and 2 rev/min at the low level, the corresponding values for the speed of the take-away reel being 580 cm/min and 245 cm/min respectively. The shear resistance of spun samples from each different treatment was measured in triplicate using the Volodkevitch texturometer as described by Sale (1960). Results were subjected to an analysis of variance to determine the significance of differences.

Experimental design 2: effect of protein character on the shear resistance of spun fibres

In order to investigate this aspect, a randomized block experiment in two blocks was again designed. Concentrated bovine plasma (97·5 mg/ml protein) was denatured and spun as before at high and low levels of gear pump and take-away reel speeds. The values of these parameters were equal to those selected for the previous experiment. Thus, spun samples were obtained from four different sets of conditions. Similarly, denatured porcine lung and stomach protein isolates of the same protein content (97·5 mg/ml) were spun under corresponding conditions. Consequently, twelve different treatments were carried out in duplicate to give twenty-four runs. The shear resistance of spun samples from each treatment was measured in triplicate as before using the Volodkevitch texturometer. Again, the results were subjected to an analysis of variance to determine the significance of differences.

Electron microscopy

Protein fibres were separated and fixed in 6% (w/v) glutaraldehyde in acetic acid buffer for 12 hr. The buffer had the same composition as the coagulating bath in the spinning process, i.e. 20% (w/v) NaCl in 1N acetic acid (pH 2·3). After primary fixation, the fibres were washed in buffer for about 24 hr (with six changes of buffer) to remove all traces of glutaraldehyde. The fibres were then post-fixed for 1 hr in 2% aqueous osmium tetroxide. After dehydration with ethanol, the specimens were impregnated with epoxy propane and embedded the following day. The embedding medium was a mixture of araldite epoxy resin, dodecenyl succinic anhydride and

dibutyl phthalate. A 1–2% (w/v) solution of benzyldimethylamine was added as accelerator to polymerize the plastic. Polymerization took place at 60 °C.

Sections of transverse protein fibres were cut on a Reichert OM U2 ultramicrotome and picked up on copper grids. The sections were stained for 25 min in 1% aqueous uranyl acetate and for 5 min in Reynolds lead citrate. After washing in running distilled water and drying, the sections were examined in a J.E.M. 6C electron microscope.

Results

The effect of spinning parameters on the shear resistance of spun plasma protein

Values of shear resistance for the spun samples prepared in experiment 1 are tabulated in Table 1. The results of the analysis of variance are presented in Table 2.

TABLE 1. Variation in shear resistance of spun plasma products from experiment 1 as determined by Volodkevitch shear jaw system

	Viscosity (poise)	Pump speed (rev/min)	Take-away reel speed (cm/min)	Mean shear resistance (relative values)
Block 1	2	2	245	0·77
	2	2	580	0·49
	2	10	245	0·18
	2	10	580	0·35
	7	2	245	0·81
	7	2	580	0·45
	7	10	245	0·21
	7	10	580	0·18
Block 2	2	2	245	0·70
	2	2	580	0·43
	2	10	245	0·21
	2	10	580	0·21
	7	2	245	0·69
	7	2	580	0·49
	7	10	245	0·17
	7	10	580	0·25

Both pump velocity (V) and reel speed (G) had a significant effect on the shear resistance of the resultant fibre bundles. The V effect was very highly significant ($P<0{\cdot}001$) with much reduced shear resistance readings being obtained at the high level of V than at the low level. Similarly, the G effect was highly significant ($P<0{\cdot}01$). At high levels of G, the shear resistance of spun products was low. It appeared, therefore, that the pressure at which the protein dope was forced through the spinneret and the

TABLE 2. Analysis of variance table for experiment 1

Source	Degrees of freedom	Sum of squares	Mean square	Variance ratio	Significance
Blocks	1	0·0053	0·00526	4·129	—
Viscosity (μ)	1	0·0005	0·00051	0·398	—
Pump velocity (V)	1	0·5891	0·58906	462·761	***
$\mu \times V$	1	0·0023	0·00226	1·773	—
Error (A)	3	0·0038	0·00127		
Main	7	0·6009			
Reel speed (G)	1	0·0495	0·04951	11·876	**
$\mu \times G$	1	0·0011	0·00106	0·253	—
$V \times G$	1	0·1106	0·11056	26·520	**
$\mu \times V \times G$	1	0·0008	0·00076	0·181	—
Error (B)	4	0·0167	0·00417		
Total	15	0·7794			

General mean = 0·4119.
Coefficient of variation = 8·66% for main plots; 15·68% for sub plots.
** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$.

speed at which the fibres were drawn from the bath were critical factors in determining the texture of the spun product. Moreover, there was a highly significant $V \times G$ interaction ($P < 0{\cdot}01$) which reflected the fact that shear resistances were not affected to such a degree at the high level of V by a change in G as at the low level of V. The viscosity of the protein dope had no significant effect on the shear resistance values determined during the experiment.

Consistently higher readings of shear resistance were obtained for spun products produced at a combination of low V and G values. It was noticed during the spinning procedure that under these conditions the fibres were maintained under tension as they emerged from the spinneret. At high levels of V, however, the fibres were forced rapidly to the surface of the coagulating solution and pulled along the surface prior to collection on the take-away reel. Thus, the fibres were less orientated along the direction of the bundle than when the former conditions were applied in the process.

The effect of protein character on the shear resistance of spun products

Values of shear resistance for the spun samples prepared in experiment 2 are tabulated in Table 3. The results of the analysis of variance are presented in Table 4.

It appeared that there was a significant interaction between the three factors under investigation, i.e. protein type (M), pump velocity (V) and take-away reel speed (G). This being so, all individual factors and interactions considered in the analysis also show significantly high variance ratios and little information can be gained from the table. A diagrammatic representation of the results is given in Fig. 1 which shows the extent

TABLE 3. Variation in shear resistance of spun products from experiment 2 as determined by Volodkevitch shear jaw system

	Protein source	Pump speed (rev/min)	Take-away reel speed (cm/min)	Mean shear resistance (relative values)
Block 1	L	2	245	0·22
	L	2	580	0·20
	L	10	245	0·12
	L	10	580	0·13
	P	2	245	0·69
	P	2	580	0·44
	P	10	245	0·20
	P	10	580	0·28
	S	2	245	0·30
	S	2	580	0·27
	S	10	245	0·13
	S	10	580	0·15
Block 2	L	2	245	0·18
	L	2	580	0·18
	L	10	245	0·11
	L	10	580	0·13
	P	2	245	0·75
	P	2	580	0·50
	P	10	245	0·25
	P	10	580	0·28
	S	2	245	0·29
	S	2	580	0·26
	S	10	245	0·13
	S	10	580	0·17

L, lung; P, plasma; S, stomach.

and direction of texture changes occurring in the spun product for each protein type on altering the level of G. Thus, two plots are given for each of the lung, stomach and plasma protein types investigated, one plot showing the effect on texture at the low V level and the other at the high V level.

At corresponding values of V and G, the spun plasma product gave a significantly greater value of mean shear resistance than both the lung and stomach products. This is evident from Fig. 1 and is borne out by the fact that the overall factor M means from the analysis of variance were 0·159 for lung, 0·424 for plasma and 0·213 for stomach. Since the standard error of the difference was 0·0157, it is apparent that the difference in mean shear resistance between the plasma product and the lung and stomach products is highly significant. The mean values of 0·159 and 0·213 for lung

TABLE 4. Analysis of variance table for experiment 2

Source	Degrees of freedom	Sum of squares	Mean square	Variance ratio
Blocks	1	0·0004	0·00042	0·422
Protein type (M)	2	0·3140	0·15699	159·109
Pump velocity (V)	1	0·2017	0·20167	204·392
$M \times V$	2	0·0799	0·03995	40·494
Error (A)	5	0·0049	0·00099	
Main	11	0·6009		
Reel speed (G)	1	0·0060	0·00602	42·471
$M \times G$	2	0·0130	0·00650	45·912
$V \times G$	1	0·0253	0·02535	178·941
$M \times V \times G$	2	0·0233	0·01164	82·147
Error (B)	6	0·0009	0·00014	
Total	23	0·6694		

General mean = 0·2650.
Coefficient of variation = 11·85% for main plots; 4·49% for sub plots.

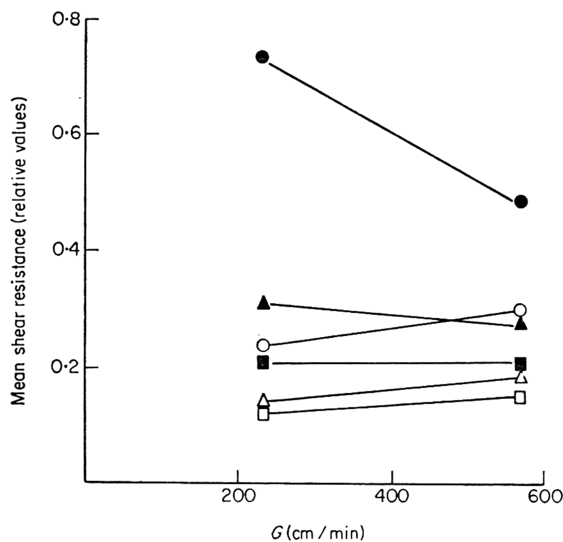

FIG. 1. Plot to show the effect of process variations on the mean shear resistance values for spun plasma, lung and stomach proteins. V, gear pump velocity; G, drawing velocity. ●, Spun plasma protein (low V); ○, spun plasma protein (high V); ▲, spun stomach protein (low V); △, spun stomach protein (high V); ■, spun lung protein (low V); □, spun lung protein (high V).

and stomach respectively also differ by an amount in excess of the standard error. It would appear, therefore, that spun stomach protein generally exhibits a significantly greater shear resistance than spun lung protein.

At the low level of V, the mean shear resistance of all the spun products was reduced by changing from the low to the high value of G. This is shown clearly in Fig. 1. However, comparison of the texture differences with the standard error for the $M \times V \times G$ interaction of 0·0238 indicates that the effect on the plasma product was highly significant whereas the effect on the lung product was not significant and the effect on the stomach product was only of dubious significance.

At the high level of V, there was also consistency in the direction of the texture change on altering the value of G. In this case, however, the mean shear resistance readings were increased by changing from the low to the high value of G. Again, the texture difference for the spun plasma product was significant whereas the effect on the texture of the spun lung and stomach products was not significant.

Observations on the fine structure of protein fibres as determined by electron microscopy

It was immediately apparent from the studies on plasma protein fibre that there was no orientation of protein molecules along the axis of the fibre. Observation of an electron micrograph of a cross-section of normal serum protein fibre (Plate 1a) indicates that the fibre consists of spherical protein particles linked together in chains or strands to form a three-dimensional mesh incorporating pores of varying sizes. There is also evidence of more random aggregation is some regions. At increased magnification (Plate 1b) the strands of protein particles may be seen more clearly.

Plate 2 shows electron micrographs of cross-sections of plasma fibres spun under different conditions and consequently of differing mechanical strength. In the fibre of high shear resistance (Plate 2a) the gel structure of the fibre is more clearly defined and the protein strands are easily discernible. A similar structure is apparent in the fibre of medium shear resistance (Plate 2b), but in this case the three-dimensionally linked chains are not as obvious and some fracturing of the system appears to have taken place. The fibre of low shear resistance (Plate 2c) is not structurally well-defined and in this case a higher degree of random aggregation of protein has occurred.

Fibres spun from lung protein isolate exhibited a markedly different cross-sectional appearance (Plate 3a) to those spun from plasma protein. There was minimal evidence of specifically oriented aggregation of protein to form a gel structure. Randomly aggregated protein appeared to predominate and the pores between the protein aggregates were of much larger dimensions than those occurring in the plasma protein fibres. The stomach protein fibre had a structure more closely related to a gel and strands of protein particles were detectable in the micrographs Plate (3b). However, the dimensions of the pores between the strands were again relatively large.

Discussion

A knowledge of the parameters which significantly affect the textural properties of the spun products is obviously desirable so that optimum conditions may be selected in any

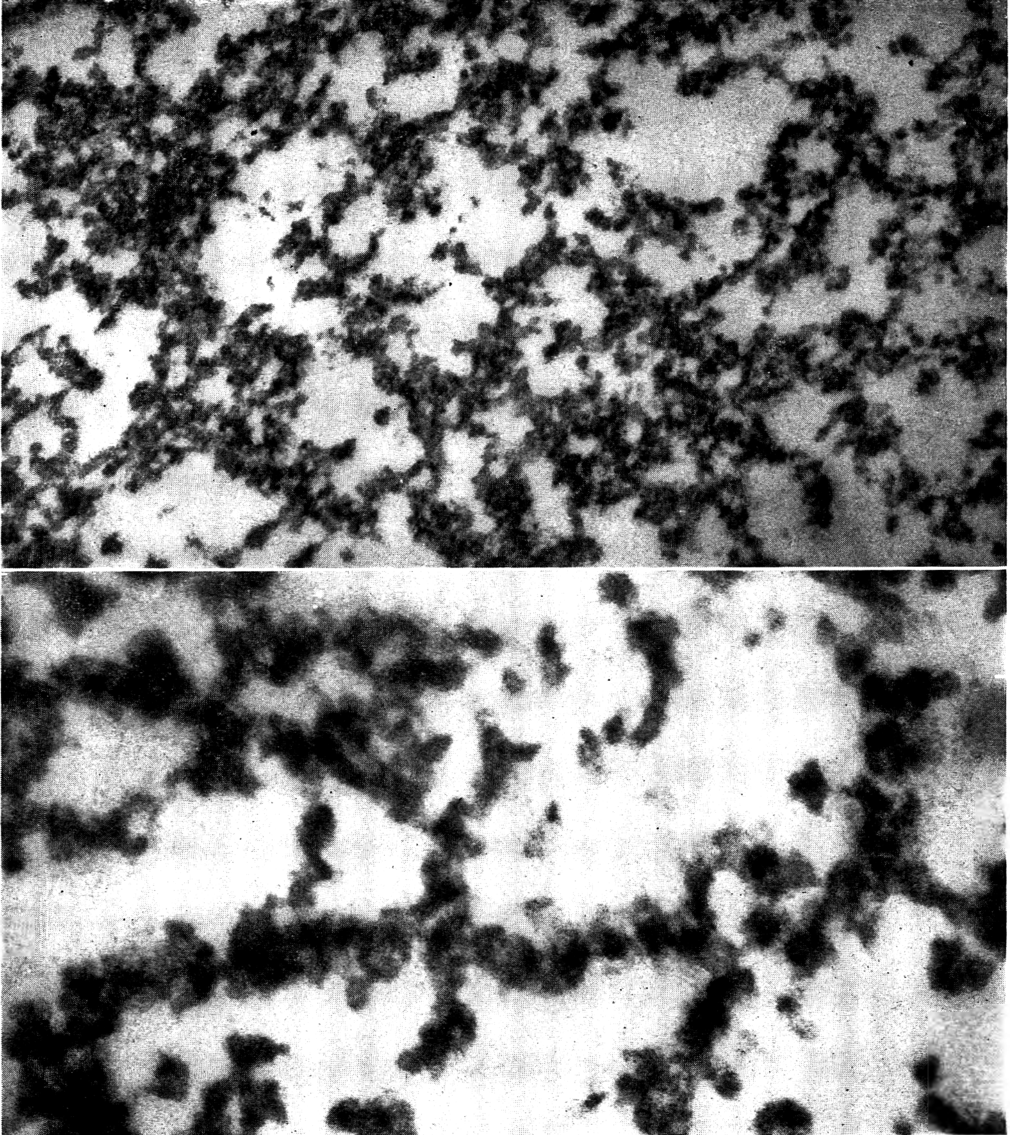

PLATE 1. Electron micrographs showing cross-sections of plasma protein fibre spun at $V = 2$ rev/min, $G = 245$ cm/min. (a) × 28 000 (b) × 89 250.

(*Facing p.* 472)

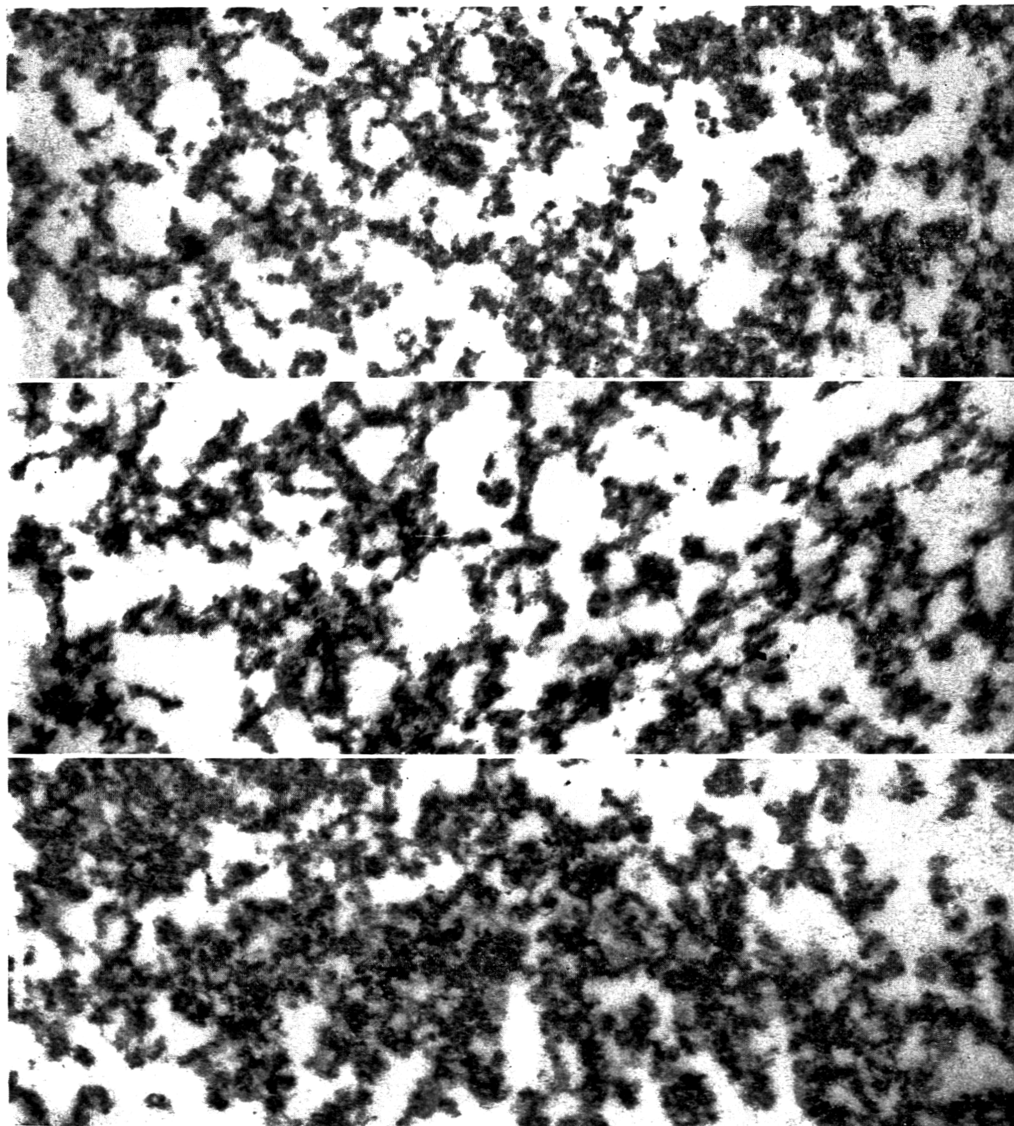

PLATE 2. Electron micrographs (× 28 000) showing cross-sections of plasma protein fibres spun at (a) $V = 2$ rev/min, $G = 245$ cm/min; (b) $V = 2$ rev/min, $G = 580$ cm/min; (c) $V = 10$ rev/min, $G = 580$ cm/min.

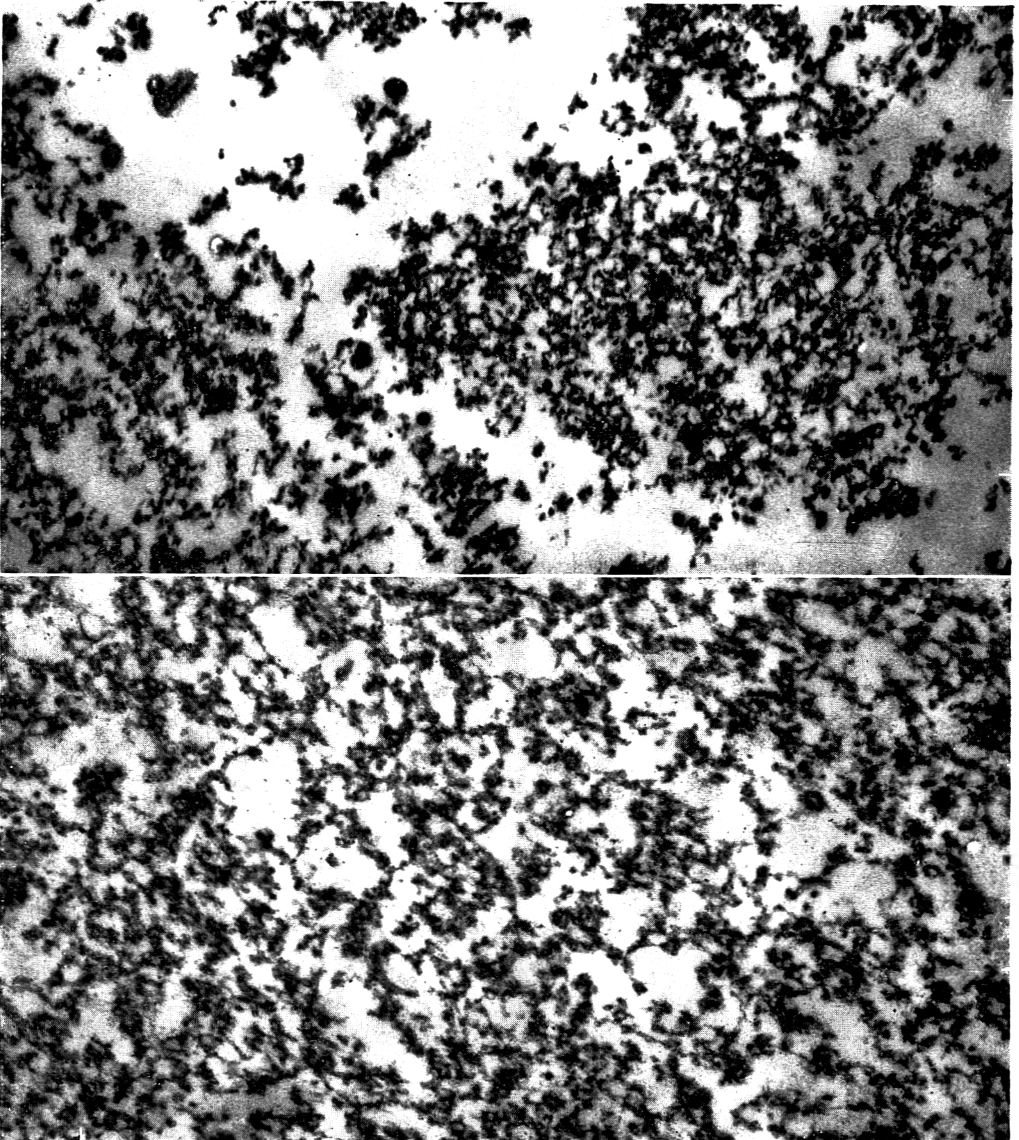

Plate 3. Electron micrographs showing cross-sections of (a) lung protein fibre (× 12 950); (b) stomach protein fibre (× 16 800).

future processing. The criticality of gear pump velocity, and hence extrusion rate, in determining the shear resistance of spun plasma protein was particularly obvious from the results of experiment 1, necessitating accurate control of this parameter in any large-scale production. A parameter of secondary importance appeared to be the speed of removal of fibres from the coagulating bath. The finding that higher values of take-up speed reduced the shear resistance of spun plasma products conflicts with the claims of the original patents concerning spun soy protein (Boyer, 1954). However, these early reports, which suggest that increasing the degree of stretch of the fibres orientates the protein molecules conferring greater toughness on the product, appear to oversimplify the situation. The results of experiment 1 also indicated that the shear resistance is dependent on the interaction between extrusion rate and drawing velocity. This is in agreement with the findings of Balmaceda & Rha (1974) who concluded, in defining the spinnability curves for zein, that the maximum drawing velocity of the fibres was determined by the extrusion velocity. It became clear throughout the course of the investigation that, by maintaining the fibres just under tension as they were drawn from the bath, the maximum shear resistance values were obtained in the spun product. It was convenient, although perhaps surprising, that dope viscosity did not significantly affect the shear resistance of spun serum products. This being so, the inevitable variations in viscosity which occur in the dope prior to spinning would appear to be unimportant in this respect.

The information on fibre structure gained from the electron microscopy studies could be correlated to some extent with the texture analysis. Low values of shear resistance in the spun product were attendant with a less oriented gel structure in the fibre. Higher drawing velocities tended to fracture the protein strands whilst higher extrusion velocities promoted a greater degree of random aggregation of proteins. The occurence of large pores in the fibres, especially in those spun from lung and stomach isolates, appeared to be an important factor in reducing the resistance to shear of the spun product. Even when the optimum conditions were applied to the lung and stomach proteins, poorly defined structures and large pores were found in the fibre giving rise to low mechanical strengths. The inferior structure of these fibres may reflect the degree of protein aggregation occurring during treatment prior to spinning which differs from that applied to plasma. Alternatively, the nature of protein constituting the lung and stomach extracts may be such that the opportunity for oriented interactions is minimized due to the absence of the groups required for such linkages. Electrophoretically lung protein has been shown to comprise, in the main, low molecular weight components, the major portion of stomach protein being of higher molecular weight (Young & Lawrie, 1974b). The fact that the stomach protein fibre was found to have a higher resistance to shear and a more oriented gel structure than the lung protein fibre may be due solely to protein composition since both fibres were prepared under identical conditions.

Clearly, a complex variety of parameters involved prior to and during the spinning

process may interact to determine the mechanical properties of spun fibres. As Tombs (1970) pointed out, these properties reflect the dimensions of the protein gel forming the fibre. It would appear that, regarding the meat waste proteins investigated in this study, a stronger fibre may be derived from blood plasma by virtue of the fact that the protein is not isolated during its preparation.

Acknowledgments

This work was supported by a grant from the Meat and Livestock Commission which is gratefully acknowledged. The authors are also indebted to Mrs A. Tomlinson for her technical assistance with the electron microscopy studies.

References

Balmaceda, E. & Rha, C. (1974) *J. Fd Sci.* **39**, 226.

Boyer, R.A. (1954) U.S. patent 2682466.

Lundgren, H.P. (1949) *Adv. Prot. Chem.* **5**, 305.

Sale, A.J.H. (1960) *Measurement of Meat Tenderness.* Monograph no. 7, Society of Chemical Industry, London.

Tombs, M.P. (1970) Alterations to proteins during processing and the formation of structures. In: *Proteins as Human Food* (Ed. by R.A. Lawrie), pp. 126–38. Butterworth, London.

Young, R.H. & Lawrie, R.A. (1974a) *J. Fd Technol.* **9**, 171.

Young, R.H. & Lawrie, R.A. (1974b) *J. Fd Technol.* **9**, 69.

(*Received* 20 *December* 1974)

J. Fd Technol. (1975) **10**, 475–482

Book Reviews

Vitamin C. Ed. by G. G. Birch and K. Parker.
London: Applied Science Publishers Ltd, 1974. Pp. xx+259. £10.

The proceedings of a symposium in April 1974 on vitamin C, which was organized under the auspices of the National College of Food Technology, have been published with the sub-title 'Recent aspects of its physiological and technological importance'. This qualification of the programme title might at first sight be considered too limiting in that, after the opening remarks by E. J. Rolfe, historical aspects of work up to the mid 1930s are given in an interesting contribution by I. M. Sharman. However, relatively few references to the subsequent period up to 1960, when much of the basic work on vitamin C was carried out, are to be found in the majority of chapters which follow; research of the last decade is well reviewed.

After introductory papers on the technical uses and chemical estimation of the vitamin by H. Kläui and J. R. Cooke respectively, technological aspects of vitamin C are considered. Individual chapters deal with fruit juice and vegetable processing (G. G. Birch *et al.*), canning and freezing (J. D. Henshall), meat products (M. D. Ranken), soft drinks and fruit juices (D. M. Gresswell) and breadmaking (B. H. Thewlis). Treatment by the contributors varies from detailed descriptions of original work to review papers with both natural and added synthetic ascorbic acid being considered. The standards achieved are variable. As might be expected from the chapter headings, there is occasional overlapping of information. The possibility that biologically inactive substances in processed foods may interfere in the chemical and physical methods applied for estimation of the vitamin does not always seem to have been fully appreciated; use of the biological method as the standard of reference for vitamin C in this context is not reported.

Interposed in this section on technological matters are three chapters on nutritional aspects of vitamin C. R. E. Hughes deals with the interaction with heavy metals, C. L. Walters with nitrosamine formation and D. Hornig with recent advances in vitamin C metabolism in humans and animals.

The remaining section of the book relates to physiological aspects of the vitamin. E. Degkwitz and Hj. Staudinger are concerned with microsomal cytochromes, E. Ginter with lipid metabolism and atherosclerosis, C. W. M. Wilson with tissue metabolism, oversaturation, desaturation and compensation and S. Lewin with recent advances in the molecular biology of vitamin C. These contributions form a useful review of modern thought on vitamin C in animal physiology. A paper on aspects of the vitamin in plant physiology would have given added value to this section of the symposium.

Well recorded discussions in question and answer form which follow each chapter are valuable in exposing weaknesses in addition to introducing new lines of thought.

This is a somewhat uneven book, some sections of which need to be read critically. The experienced food scientist and technologist will, however, find much to inform and to stimulate ideas. If only for the purpose of demonstrating different methods of approach which have been adopted for increasing our knowledge of vitamin C, copies should be available in both academic and industrial establishments.

The publishers and editors are to be congratulated on bringing out these proceedings in the same year as the symposium took place.

MAMIE OLLIVER

Vegetable Protein Processing: Food Technology Review No. 16. By L. P. HANSON. New Jersey: Noyes Data Corporation, 1974. Pp. xii + 308. US$36.

This book is one of a series of food technology reviews published by the Noyes Data Corporation and gives information on more than 200 US patents relating to the processing of vegetable proteins, from agricultural sources, to make them usable as food products. The patents cover the period from June 1954 to May 1974.

Those of us who are familiar with patent literature and who have an interest in vegetable proteins will be grateful to the author for having presented the patent information in a form which is readable and not complicated by legalistic jargon. Assessment of the value and importance of any patent has been left to the reader, as the author, no doubt using some discretion, has refrained from making comments in this respect.

The subject matter has been organized under various headings which, together with indexes on companies, inventors and patent numbers, enables one to locate patents of specific interest without too much difficulty. As the review consists mainly of one patent following another there is no requirement to read in a continuous manner from beginning to end, and most benefit is likely to be obtained by using the book as a reference for locating patents of specific interest for closer study.

About one third of the review is concerned with, not unexpectedly, the soybean and its derivatives, a smaller section dealing with other vegetable proteins such as cottonseed, grain, sunflower, safflower, groundnut, alfalfa, coconut and mistletoe. The remainder of the review is concerned with patents on lipid removal, detoxification, hydrolysis, fibre production, textured protein gels and consumer products such as simulated milk and other dairy-like products, flavouring materials and Tofu, Miso and Tempeh, the latter three products being soy-based and more suited to the oriental palate. Some of the patents covered have received attention in other Noyes publications but this could be considered as unavoidable and even necessary for the sake of completion.

In a world where it is becoming increasingly difficult to keep abreast of new technical information this type of review is of value and can be recommended to those with an interest in this important area of processing technology.

T. F. McMichael

Micro-organisms in Foods, Vol. 2. Sampling for microbiological analysis: principles and specific applications. International Commission on Microbiological Specifications for Foods.
Toronto: University of Toronto Press, 1974. Pp. xii+213. US$25.

This book achieves what it sets out to do, namely, to dispel much of the controversy, confusion and indecision surrounding microbiological methods for appraising food quality and is the first really comprehensive publication dealing with statistically based sampling plans broadly applicable to the field of food microbiology. An authoritative text such as this has been sorely needed for many years. It is an international book, being based on the knowledge of fifty-three experienced food microbiologists and statisticians from twenty-four countries; these workers have contributed five years of study and discussion under the auspices of the International Commission on Microbiological Specifications for Foods (ICMSF), a standing commission of the International Association of Microbiological Societies.

The volume is divided into two sections. Part I defines the statistical concepts and terms used in the text, describes the kind of sampling plans available and their purposes, outlines the procedures for selecting the best plan for the food and organism in question, and explains the principles of randomization. Part II describes in detail the practical application of these principles for various foods including fish, shellfish, vegetables, dried foods, frozen foods, milk and milk products, raw meats, processed meats and shelf-stable canned foods. The text ends with general conclusions, a glossary of special terms, a list of references and a comprehensive index.

The sampling plans proposed provide a rational basis for the recommended microbial limits for the majority of foods found in international trade. The specific limits given express the principle that stringency of examination of a food should be related to the severity of the hazard to be anticipated under normal conditions of use. The adoption of three-class sampling plans is an important innovation which, by accommodating a tolerable number of microbiological values that may appear to exceed an 'ideal' limit, recognizes normal distribution ranges and thereby removes an important prejudice against the use of microbiological limits and standards.

There is a growing interest in microbiological specifications for foods and many international organizations other than the ICMSF such as Codex Alimentarius, the World Health Organization and the European Economic Community are considering sampling plans, methodology and specifications. With so many agencies involved it is

imperative that cooperation and agreement be the order of the day, if there is not then the food microbiologists in industrial and government laboratories, the food inspectorate and public health administrators will be faced with a confusing complexity of data.

This authoritative book from the ICMSF will be of great benefit to food technologists and is an absolute 'must' for food microbiologists and those responsible for quality control and public health. Not a book the reader can leaf through in an hour or two, it requires careful reading but all who do will benefit.

R. J. GILBERT

Affinity Chromatography. By C. R. LOWE and P. D. G. DEAN. London: J. Wiley & Sons, 1974. Pp. xi+272. £6.25.

Affinity chromatography represents the logical development of the adsorption phenomenon in chromatography by conferring on an adsorbent an affinity for an adsorbate through the provision of adsorption sites particularly suited to that adsorbate. So far, descrimination is provided by known specific relationships in biochemistry, and the technique finds application almost exclusively in this field.

The technique is introduced by the demonstration, in turn, of the limitations in specificity of classical techniques of protein separation, the degree of discrimination obtained by linking the specific binding of ligands to classical methods and, finally, the exploitation of the specificity of the ligand-protein interaction in the form of affinity chromatography. The principles of operation are displayed by discussing the influences of the various components of the chromatographic system and of the operational variables on separations. The discussion involves a minimum of theory and of experimental observation, providing a useful piece of guidance for the would-be explorer of the technique but, at the same time, leaving the impression that empiricism has not yet been superseded in chromatography.

The resolving power of the method is illustrated by reference to the group of selectively interacting molecules comprising enzyme, cofactor, substrate and inhibitor. Examples are given of the use of group specificity, difference in degree of affinity and specificity of order of association, to achieve separations within the group. The further range of scope of the method is then displayed by examples of separations depending on other types of affinity such as those between antigen and antibody, hormone and receptor, substrate and transport agent. Uses are also indicated in which separation is not the primary objective. These include the investigation of mechanisms of enzyme action and of hydrophobic bonding between molecules.

The last chapter describes in detail the methods used for the preparation of adsorbents by the attachment, firstly, of the spacer arm to the inert matrix and, subsequently, of the ligand. The significance of the properties of the spacer arm and the mode of coupling of the ligand is made clear, thereby implying the difficulties encountered in securing

for an adsorbent the maximum, or indeed any, activity. Thus the book provides very useful practical information for the novice, not least among which is the item which deals with the avoidance of explosions with cyanogen bromide.

J. E. McKay

Bakery Products—Yeast Leavened: Food Technology Review No. 20. By D. J. de Renzo.
New Jersey: Noyes Data Corporation, 1975. Pp. xii + 456. US$36.

This book is a compilation of many of the patents granted in the US since 1960 in the field covered by the title. The contents are classified under subject headings, which serve as a crude index and include continuous breadmaking, fermentation, emulsifiers, improvers, preservatives, flavour and speciality breads. Additional useful indexes are by company, inventor and patent number. The boast of the publishers that they have closed the time gap between manuscript and completed book seems to be borne out by the appearance of at least two patents dated June 1974, which have presumably already suffered the usual delay before publication by the patent office.

The author's method is to introduce sections or individual patents with brief presentations of general principle or historical background and then leave the patents to speak for themselves. However, they speak with more than usual clarity, thanks to the omission of legal jargon, and the author is to be congratulated on his skill in presenting the abbreviated essence, including examples, in easily readable form.

It is not difficult to criticize aspects of the book. Some selection has admittedly been exercised and it is claimed as a virtue that this is without 'bias'; it is difficult to avoid the suspicion that this is a euphemism for 'without discrimination'. Only the informed reader can know which of the patents heralded significant innovations and which describe stillborn inventions. What about the commercial successes never patented and thus unrecorded in these pages? However, this is to carp. The author would reply, with justice, that here is a compendium of ideas which were thought likely to lead to commercially successful processes or products; many of them did.

The book can be recommended to anyone working in research and development in this field as a source of reference to information often not well covered by the more conventional journal or text book. It is well produced and readable and a thoughtful browse through its pages could well stimulate the next breakthrough – or reveal that it had already been made.

Norman Chamberlain

Sugars in Nutrition. Ed. by H. L. Sipple and K. W. McNutt.
New York: Academic Press, 1974. Pp. xxi + 768. £21.60.

Covering over 700 pages, this book is, in fact a collection of papers based on an International Conference on Sugars in Nutrition held in 1972. Almost every chapter offers a section on 'Research needs' and I find this approach an extremely valuable one which

could well be adopted in other books of similar character. For any young scientist entering the carbohydrate research field, the problem will be what to choose, for there is certainly no lack, and it is clear that science, as yet, has only touched the fringe of the problems of carbohydrate in nutrition.

The first section describes nutrition as an art, but surely knowledge of the subject has sufficiently advanced (as, indeed, is witnessed by this book) for it to be called a science. This I found put right later in the section. Both the chapters are historical; the second is, also, in some senses, a review of some of the ensuing chapters and, as such, is very welcome at the commencement of the book. This author's emphasis on the difference between 'normal individuals' and the smaller number of others prone to metabolic aberrations is, I think, well taken and serves as a warning not to treat data solely as a mathematical exercise. The second section covers the psychology of sweetness —always a difficult area. The underlying mechanism of sweet taste sensation and the biochemistry of chemoreceptors is discussed. The great difficulties in measuring sweetness become apparent, but nothing is said about such synergistic effects as can be obtained by mixtures of saccharin and sodium cyclamate; and I should have liked more to have been said about the effects of such fashions as slimming on preference assessments.

In the third section, dealing with the occurrence of sugars in food, only glucose, fructose, sucrose and maltose are listed, but I suppose it is the rarer sugars in fruits and vegetables, such as mannoheptulose in avocardo pears and alpha-alpha-trehalose in mushrooms, which give them particular properties, and these could have been discussed with advantage. The section leads on to two papers dealing with sucrose supplies and consumption and, the effect of the consumption of a much higher proportion of sucrose in prepacked foods is assessed.

The four chapters covering recent technological developments emphasize the growth of the so-called fructose bearing syrups, suggesting that these have achieved a more rapid prominence in America than in Great Britain. Their present growing importance in the latter make any discussion on them valuable. The emphasis that the statistics of consumption are based on the disappearance of foods from commercial stores, *not* on the sugars as present in food when eaten, I found helpful. A useful Patent list is given.

Four chapters are devoted to digestion and absorption of sugars and serve to indicate what a wide area of research is still open. For instance, the influence of carbohydrate on gastric emptying is a largely unexplored field, and the exact mechanism of glucose and sodium transport is unknown. There is a chapter on enzyme deficiencies which suggests an even greater area of ignorance. Important comparisons between cow's milk and human milk in favour of the latter, tend to support the modern emphasis on breast feeding, though this competes with the economic necessity of the mother earning her living and the convenience of bottle feeding.

The importance of the metabolism of sugars is emphasized in the six chapters that follow in the next section, but I was surprised to find three of them devoted to the

polyols. These *are* important and bear an important relationship to carbohydrate, but they can scarcely be described as sugars. The metabolism of polyols is for the specialist. The chapter by Macdonald, dealing with orally ingested carbohydrates, which, after all, is the way most of us take our carbohydrate, makes useful distinctions between maltose and the higher polysaccharides.

Four sections, a matter of 180 pages, are devoted to disorders and another section of 104 pages is given over to therapeutic uses of sugars, making a somewhat unbalanced allocation of 284 pages to subjects which are likely to have, mainly, only specialized interest.

The last section of the book deals with dental problems. These are of much more general interest and could have received more emphasis, if necessary to the exclusion of some of the more specialized matter. Thus, although the papers have been assembled to attempt a balanced review, I think these emphases have rather spoiled the effort.

The book is, inevitably, out of date. For instance, in my own field there have been important advances in the study of the influence of carbohydrate ingestion on athletic performance and brain function. It is, too, unfortunately necessary now to speak of joules rather than calories.

It is said to be a United Kingdom edition, yet it bristles with American spellings. I found these, and one or two uses of nouns as verbs, particularly irritating. Out of fifty authors, only two are British, but quite a lot of work on carbohydrate has been done in Britain since this conference. I should be very surprised if British research were found to be lagging behind, though this might be the impression reached from the list of authors.

There are one or two misprints and errors, and the cross references to the authors of chapters is not consistent. For instance, on p. 12 it refers to Wurhmann *et al.* as authors of chapter 20, whereas it should read Cristofaro *et al.*; yet the authors of chapter 29 are correctly referred to as Cohen *et al.*

Important items in a technical work are the indices. This work has both an author and subject index. There is a serious criticism of the former, which can best be illustrated by the first name, Abei, T., which is said to appear on p. 607 and in the complete reference for chapter 34, on p. 612. In fact, it cannot be found on p. 607 and is tucked away as the *second* author following Kameda, H., on p. 612. It would have been better to list Abei, T. as 'see Kameda, H. *et al.*'. The subject index is excellent; in a random selection of ten different subjects, no errors were detected.

Generally speaking, the book is very readable, although one or two chapters are more like long lists of references. I can recommend it to be purchased, although its price may cause private individuals to think more than once.

H. L. Green

Books Received

Toxicological Evaluation of Some Food Colours, Enzymes Flavour Enhancers, Thickening Agents and Certain Other Food Additives. World Health Organization Food Additives Series, No. 6.
WHO: Geneva, 1975. Pp. 204. Sw. fr. 13.
A series of monographs on the toxicological evaluation of a number of food additives, prepared by the Joint FAO/WHO Expert Committee on Food Additives in 1974.

Nutrition for the Growing Years. By MARGARET MCWILLIAMS.
London: John Wiley & Sons, 1975. Pp. 452. £5.95.
A comprehensive elementary textbook for students and those interested in child nutrition.

Improvement of Food Quality by Irradiation. Proceedings of a panel meeting.
Vienna: International Atomic Energy Agency, 1974. Pp. 188. £3.80.
Proceedings of panel organized jointly by the Food and Agriculture Organization and the International Atomic Energy Agency in 1974. The papers discuss a variety of changes brought about in foods by irradiation.

Getting the Most out of Food.
London: Charles Knight & Co., 1975. Pp. 136. £1.00 (soft cover), £2.50 (hard cover).
A selection of essays by various authors, sponsored by van den Berg & Jurgens, on topical aspects of nutrition, which affect particularly consumers in this country.

Cereals. By J. SCADE.
London: Oxford University Press, 1975. Pp. 70. £1.00.

Sugar and Chocolate Confectionery. By S. CAKEBREAD.
London: Oxford University Press, 1975. Pp. 60. £1.00.

Milk and Dairy Foods. By D. W. G. PORTER.
London: Oxford University Press, 1975. Pp. 64. £1.00.
Three short introductory texts, in a general series, 'The Value of Food', for students of home economics and others entering the food industry.

Freezer Facts. Ed. by MARGARET LEACH.
London: Forbes Publications Ltd, 1975. Pp. vi+190. £4.75.
A collection of articles, written by a number of experts, on various aspects of frozen foods including changes during storage and economic considerations for the use of freezers in the home.

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The Kinetics of Mycelial Growth. By A. L. Koch
Generation Times of Bacteria. By P. R. Painter
The Synthesis of β-Galactosidase by Constitutive and Other Regulatory Mutants of *E. coli* in Chemostat Culture. By C. J. L. Macleod, P. Dunhill and M. D. Lilly
Attraction of Plasmodia of the Myxomycete, *Badhamia utricularis*, by Extracts of the Basidomycete, *Stereum hirsutum*. By M. F. Madelin, F. Audus and D. Knowles

BIOCHEMISTRY
The Lipids of *Agaricus bisporus*. By P. F. S. Byrne and P. J. Brennan
Loss of D-Alanine during Sublethal Heating of *Staphylococcus aureus* s6 and Magnesium Binding during Repair. By A. Hurst, A. Hughes, M. Duckworth and J. Baddiley
Enzymic Activity of Purified Plasma Membranes from the Yeast and Mycelial forms of *Candida albicans*. By M. S. Marriott

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Temporary Expression of Flagellar Phase-1 in Phase-2 Clones of Diphasic *Salmonella*. By T. Iino, T. Oguchi and T. Hirano
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CAMBRIDGE UNIVERSITY PRESS

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32 East 57th Street, New York, N.Y. 10022

REFINED CARBOHYDRATE FOODS AND DISEASE

Some Implications of Dietary Fibre

edited by D. P. Burkitt
Medical Research Council External Scientific Staff, England

and H. C. Trowell
Former Consultant Physician, Uganda

Both formerly of Makerere University and Mulago Hospital, Kampala, Uganda

August/September 1975, xiv + 352 pp., £7.80/$20.75
0.12.144750.2

"Once every ten years or so a new idea emerges about the cause of disease that captures the imagination and, for a time, seems to provide a key to the understanding of many of those diseases whose aetiology was previously unknown. Such ideas have included the concepts of hormone deficiency and excess, inborn errors of metabolism, vitamin deficiency, septic foci, psychosomatic disease, stress, and auto-immunity. To these we may now add a deficiency of dietary fibre."
Sir Richard Doll, *Regius Professor of Medicine, University of Oxford.*

Recent years have seen few significant breakthroughs in the understanding of many of the major health problems characteristic of modern western civilization. This important and extensively documented book provides considerable evidence that many of these diseases may be related to a reduction in consumption of dietary fibre. With the modern tendency to process foods for convenience, cereal foods in particular suffer from the removal of this fibre and research shows its deficiency can be one of the causes of such critical diseases as ischaemic heart disease, tumours of the large bowel, diverticular disease, gallstones, appendicitis, varicose veins, haemorrhoids, obesity and diabetes.
In this book the authors suggest simple methods of avoiding a great deal of suffering and health expenditure in the western world, and of checking the expected rapid increase of these diseases in developing countries.
This book should be of extremely wide interest, not only to general practitioners and all medical specialists, but also to members of the general public who are genuinely concerned with the relationship between diet and health. In addition it will be invaluable to public health authorities, nutritionists, dieticians, food technicians and the food processing Industry.

Contents

Disease and environment. Refined carbohydrate foods. Refined carbohydrate foods in the gastro-intestinal tract. Diseases of the large intestine. Diseases associated with constipation and straining at stool. Other disorders related to refined carbohydrate foods. Index.

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The Second Edition

Handbook of Flavour Ingredients

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

The new data incorporated into this well-known reference is so extensive that the work has been expanded into two volumes. The general format and contents of the first edition have been retained and the information provided is essential to anyone concerned in the use or development of flavour ingredients.

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Volume 26, Number 4, June 1975

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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'.

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Journal of Food Technology

Volume 10 Number 4 August 1975

Contents

375 The heating of foodstuffs in a microwave oven
D. Kirk and A. W. Holmes

385 Comparison of water vapour sorption by sugar beet root components
Héctor A. Iglesias, Jorge Chirife and José L. Lombardi

393 Further studies on ginger storage in salt brine
B. I. Brown

407 Yeasts in ginger storage brines
Annice C. Lloyd

415 Effects of γ-irradiation on quality and enzyme activities of prepacked cut chicory
Yoshikazu Tanaka and Dick Is. Langerak

427 The relationship between respiration, atmosphere and quality in intact and perforated mushroom pre-packs
R. Nichols and J. B. W. Hammond

437 Ultrastructural changes during frozen storage of cod (*Gadus morhua* L.). III. Effects of linoleic acid and linoleic acid hydroperoxides on myofibrillar proteins
L. Jarenbäck and A. Liljemark

453 Utilization of edible protein from meat industry by-products and waste. III. The isolation and spinning of proteins from lung and stomach
R. H. Young and R. A. Lawrie

465 Utilization of edible protein from meat industry by-products and waste. IV. Studies on the physical and mechanical properties of spun fibrous products
R. H. Young and R. A. Lawrie

475 Book Reviews

482 Books Received

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