Volume 14 Number 1 February 1979

Journal of Food Technology

Published for the Institute of Food Science and Technology (U.K.) by Blackwell Scientific Publications
Oxford London Edinburgh Melbourne

JOURNAL OF FOOD TECHNOLOGY

Institute of Food Science and Technology (U.K.)

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The Journal of Food Technology is published bimonthly, six issues form one volume. The annual subscription is £45.00 (U.K.), £54.00 (Overseas), $120.00 (N. America including cost of airfreight). Back volumes are still available.

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J. Fd Technol. (1979) **14**, 1–8

Protein-rich flours from palm kernels

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Summary

A method is proposed for recovering protein-rich flour suitable for human consumption from palm kernels. The method combines grinding, extraction, and screening. A mathematical model of the process, which is based on surface cleaning, is presented.

Introduction

Protein-rich flours suitable for human consumption have been successfully prepared from a variety of oil seeds, but apparently not from palm kernel cake on a commercial scale. Over one million tons of palm kernels are harvested annually in the world. Assuming that palm kernels have protein content of 9% and that 40–50% of this protein would be retained in a refined product, close to 40 000 tons of protein could be produced from palm kernels if suitable processing facilities were available. It is the object of this report to describe a method of preparation that might form the basis of a commercial extraction procedure for producing a protein concentrate from palm kernels. It is believed that this concentrate would be suitable for human consumption.

Material and methods

The oil palm

The fruit of the oil palm consists of a soft outer skin (epicarp), a pulpy fibrous layer containing the palm oil (mesocarp), and an inner cell (endocarp) that encloses the kernel (endosperm). The endosperm, which makes up one-

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0022-1162/79/0200-0001 $02.00

third to one-half the volume of the fruit, is a white cellular mass covered by a tough black membrane, the testa. A typical endosperm consists of 50% lipid, 9% crude protein, 5.5% cellulosic fibre and tissue, and digestible starches and sugars (Crombie, 1956). This cellulosic material is not fit for human consumption and must somehow be separated from any possible food product. The discussion here will focus on the recovery of a protein concentrate from palm kernels of a quality suitable for a human food source.

Palm kernel microstructure

A microsection from a palm kernel shows many cells, each of which can be viewed as a closed sack of cellulosic films, more or less oval in shape and typically $150 \times 80\,\mu m$. Examination of these sections was facilitated by selective staining; Sudan III was used for lipids; ninhydrin for proteins, polypeptides, and amino acids; and iodine-potassium iodide for starch (amylose). As reported by Stahl & Kaltenbach (1961), the purple-black colour of the starch-iodine complex could not be observed at 25°C. In the present work, however, a blue-coloured complex was observed when the section was heated to 60°C (Aghazu, 1972).

As is typical of oil seeds, the palm kernel cells are filled with a crude lipid that melts above ambient temperature. This fat breaks down upon heating into an oil similar to the palm oil derived from the pericarp. We observed granules of protein and starch embedded in this fat. Only a few protein granules per cell are found in the first layers of cells lying nearest the testa. For the next seven or so cell layers, the number of protein granules per cell increases, then diminishes again. The size of the protein granules is greatest in the interior cells, averaging about $5\,\mu m$. The starch granules in the palm kernel cells are about the same size as the protein granules but are distributed more uniformly throughout the endocarp cells.

Close examination indicated that, in the cells, the protein granules float within a sea of oil and appear to be not very firmly attached to the cell walls. This finding suggested that a mechanical method might be found for extracting the protein granules.

Mechanical separation

This study was conducted to develop a simple, inexpensive method of extracting the protein from palm kernels along the following lines:

Stage 1. The kernels would be subjected to a mechanical treatment to break open the cells of the endosperm, thus producing a mixture of protein granules, fat, fibres, cell wall fragments, polysaccharides, etc., in which the protein granules would be free and unattached. Success in freeing the protein particles would be confirmed by microscopic examination of the product.

Stage 2. The mixture from *Stage 1* would then be subjected to a separation procedure to isolate a fraction high in protein and starch but free from oil, indigestible fibre, and cellulosic membranes. To be acceptable as a diet supplement, the concentrate should contain at least 40% crude protein. Until now, such an objective has been considered unattainable because of the low average protein content of palm kernels.

Study of possible mechanical methods for use in *Stage 1* was undertaken using Nigerian palm kernels. These were air-freighted from a plantation in the vicinity of Lagos, Nigeria, and were kept refrigerated during this work to prevent spoilage. These kernels were air-dried before use in these studies.

Stage 1 studies of crushing kernels or sections of kernels were generally carried out in a lipid solvent, which served to reduce the pastiness of the mass to a manageable level. It was found that, even in solvents, hammering did not adequately free protein granules from the associated fibres and indigestible polysaccharides. Ball milling was equally unsuccessful, as was cutting and chopping with knives. Satisfactory results were obtained, however, by milling the kernels together with solvent in a Waring Blender. Figure 1 presents typical

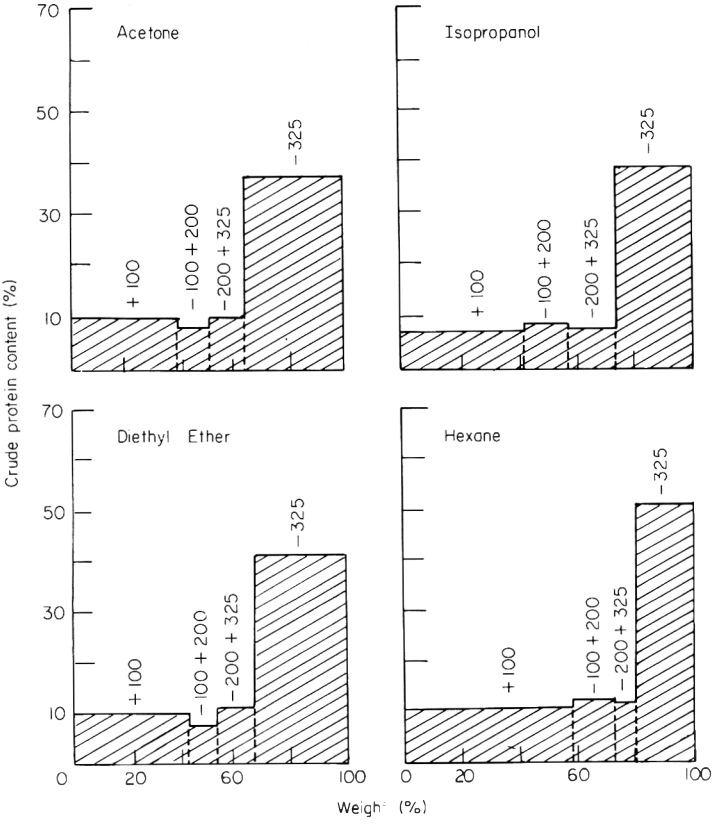

Figure 1. Protein content of sieve fractions. Processing history: 50 g palm kernels and 100 ml solvent at 14 700 rev/min in a Waring Blender for 4 min, followed by sieving. Abscissa gives weight percentage of recovered protein (75% of original).

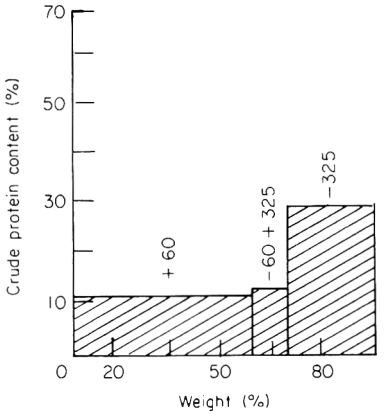

Figure 2. Protein content of sieve fractions. Processing history: dry grinding and trichlorethylene slurrying, then sieving.

results with various solvents when the blender was operating for 4 min at 14 700 rev/min with a loading of 0.5 g of palm kernel per ml of solvent (Aghazu, 1972).

Similar protein recoveries were obtained by passing palm kernels, or palm kernel cake from which oil had been extracted by pressing, through a grinding mill such as a coffee mill. Results on palm kernel cake, as treated, were as shown in Fig. 2. Recovery of free protein granules during such grinding depends somewhat upon the size of the serrations on the grinder. It was concluded that a shearing and tearing action was needed for successful freeing of the protein granules. Results were similar for all solvents tested: hexane, acetone, carbon tetrachloride, trichlorethylene, isopropanol, and diethyl ether.

For *Stage 2*, a sieving procedure was adopted to separate protein granules from other solids in the crude slurry from *Stage 1*. The slurry tested was typically formed in *Stage 1* either by the grinding of 100 g of palm kernels in 200 ml (or more) of fat solvent, or, in the case of dry grinding, by the addition of solvent to the previously ground kernels. The slurry was passed through five screens, each with a surface area of about 0.15 m^2, arranged in order of increasing fineness (32, 60, 100, 200 and 325 mesh). Most of the coarse fibres and cell fragments were retained as a loose mat on the first screen, and lesser amounts of finer undesirable solids were caught by the subsequent screens. Batch size for filtration must be chosen so that the mat of retained solids on the first screen does not exceed 12.7 mm in thickness, since a thicker layer would impede the free passage of protein and starch granules suspended in the solvent. After a slurry batch was filtered, about 100 ml of solvent was passed through the screens to dislodge remaining protein particles from the mats. The mats were then removed from the screens, and a new batch of crude slurry was processed. The protein and starch granules suspended in the filtrate and wash liquors were recovered by a method that involved settling, filtering, and then washing them with solvent that had been separated from the oil by distillation and recycled. The granular solid was dried to form the product flour.

Recovery of crude protein from the palm kernels increased with the intensity of grinding, reaching a maximum of about 75% of the protein originally present in the kernel. The distribution of protein in the various sieve fractions of the dried flour is shown in Fig. 1 for Waring Blender operations, and in Fig. 2 for large coffee-grinder operations. In addition to protein, this flour contained about 2% crude fibre, 10% ash, and carbohydrates.

Crude analysis

The palm kernels were ground in a blender in the presence of a solvent and finally in a mortar with a pestle. Undissolved solids were recovered by filtration, and fat content was determined as weight loss after refluxing with diethyl ether in a Soxhlet extractor. Crude protein was in all cases determined by the Kjeldahl method, which involved digestion of the protein in concentrated sulfuric acid, addition of excess base, steam distillation to evolve ammonia, and titration of the condensate with standard acid. The protein content was assumed to be related to nitrogen content by a factor of 6.25 (Bradstreet, 1965). Both ash and crude fibre contents were determined by the procedures of the Association of Official Agricultural Chemists (AOAC, 1965).

Results

Model

The overall recovery of protein in the −325 fraction was 40–50% of that originally present in the kernel. In all experiments, the fraction of protein in the original kernels that was not recovered in the −325 fraction was found to vary with the fraction of oil that was not recovered (Fig. 3) (high protein recoveries were always associated with high oil recoveries). The following model presents the experimental findings:

(1) Each palm kernel contains many small cells formed by a three-dimensional network of cellulosic membranes. These cells are all filled with fat, within which granules of protein and carbohydrates are suspended.

(2) The cell walls are sturdy and do not rupture easily during hammering. Again, since the cells are small, a slicing procedure severs only a few cells per cut. A more effective action is therefore needed to make the contents of each cell accessible to the solvent. Blender action appears to be successful because the blades continuously damage and peel off the external layers of membrane from the kernel as it diminishes in size. The fat in the newly exposed external cells is dissolved away as these external cell walls are bruised by the blades. This defatted external layer of membranes is then peeled away, and the process continues as the individual kernels diminish in size. In other words, when a blender is used, relatively little breakage or slicing occurs. Instead, cell walls on the

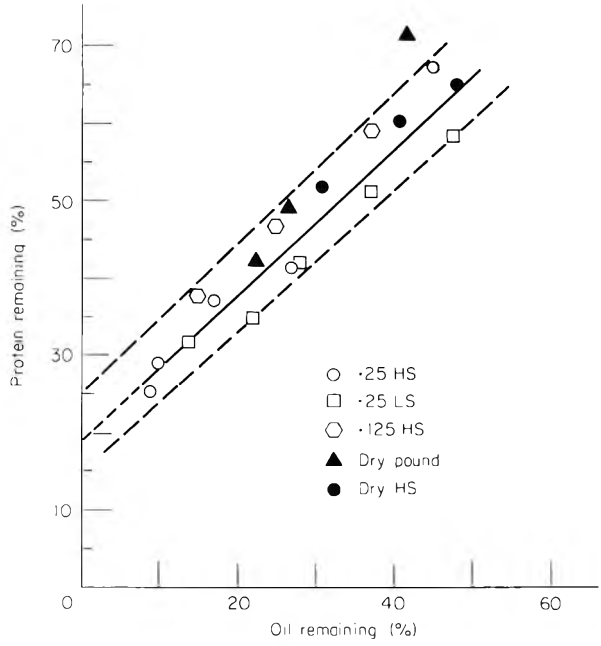

Figure 3. Protein residual function. HS indicates high speed (14 700 rev/min, LS indicates low speed (10 900 rev/min). Grams of palm kernel per ml solvent indicated by 0.25, 0.125.

surfaces of the kernels are successively bruised, defatted, and peeled away while each kernel decreases in size. This model is called the surface-cleaning model.

(3) The dissolution rate of the fat is proportional to the exposed surface of a kernel. If the weight of fat in a kernel at any time θ is W, the surface area of the kernel at any time is proportional to $W^{2/3}$. The rate of fat weight loss per kernel, i.e. $\mathrm{d}W/\mathrm{d}\theta$, is then proportional to A, and thus proportional to $W^{2/3}$.

(4) The vigour of the cleaning action of the blades, when travelling at a constant rotational speed, is also proportional to the kernel momentum, i.e. to its mass. Since total mass is proportional to W, $\mathrm{d}W/\mathrm{d}\theta$ is proportional to both surface area and to the mass, or

$$\frac{\mathrm{d}W}{\mathrm{d}\theta} = -kW^{5/2} \tag{1}$$

Integrating, combining constants, and defining W/W_0 as U, where W_0 is the weight of oil at the outset, gives:

$$\left(\frac{1}{U^{2/3}}\right)_{\text{final}} - \left(\frac{1}{U^{2/3}}\right)_{\text{initial}} = 1 - k\theta \tag{2}$$

The factor k increases with increasing blade speed and also with a decreasing kernel/solvent ratio at a given blade speed, since at this time the mass is less likely to rotate as a unit with the rotating shafts.

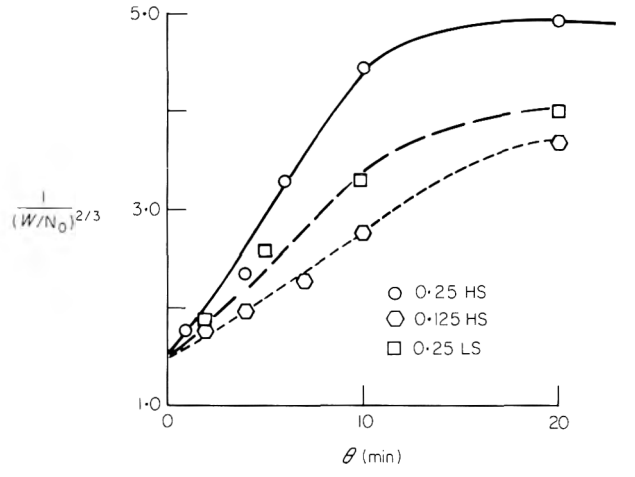

Figure 4. Variations of $1/(W/W_0)^{2/3}$ *v.* time, supporting equation 2.

Inspection of Fig. 4 shows that, at least for the first 10 min the points of $(W/W_0)^{2/3}$ *v.* θ fall on straight lines and are thus consistent with eqn (2). The fact that these lines all start from a W/W_0 value of about 1.5 rather than zero suggests that some initial breakage of the kernels occurs before 'surface peeling' becomes the predominant action. The departure of these curves from linearity after 10 min of blender action presumably reflects the fact that the mass of the individual particles has fallen below the level where impact with the moving blades is great enough for surface peeling to occur.

Discussion and conclusions

Commercial processing

The simple two-stage process outlined here promises to add a valuable by-product to the palm nut industry. Operations might start with palm kernel cake from which the oil has already been extracted, as is generally produced in existing installations. For *Stage 1* processing, machines analogous to blenders are available, such as the Welex High Intensity Mixer. Alternatively, large-scale coffee grinders or the equivalent could be used. Wet grinding in a solvent gives a slightly better protein yield than dry grinding, and the selection of one or the other method will depend upon local economics. For *Stage 2*, an initial filtration to separate the bulk of the fibre and cell fragments could be carried out using as a filter medium a very coarse cloth, possibly supported on a metal net of some sort. This initial screen must be cleaned after each slurry batch to avoid excessive mat build-up, which would prevent free passage of suspended protein granules. The final screening to remove the fine fibres would be done, as in the laboratory, on fine screens, again preventing excessive build-up of the mat. These granules would then be recovered from the filtrate and washed with

a fat solvent. Great care must, of course, be used in handling and recovering the solvent to minimize solvent losses. Such a two-stage process for making an edible protein concentrate promises to be simple enough for plantation use. The possibility of further improving the yield by recycling the coarse fibre mats to the grinders deserves consideration.

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(Received 11 January 1978)

J. Fd Technol. (1979) **14**, 9–19

A tentative evaluation of the potentiokinetic polarization technique in studies of localized corrosion of lacquered tinplate

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Summary

Preliminary results are presented on the evaluation of the potentiokinetic polarization technique for studies of localized corrosion behaviour of lacquered tinplate samples. Evaluation was performed with a 5 μm thick layer of an epoxy-phenolic lacquer on tinplate and in 1% model solutions of citric, oxalic, lactic and acetic acids (2.3–2.8 pH range) and in 1% model solutions of the sodium salts of the same acids (6.4–8.0 pH range) by means of scannings of the potential first anodically and then, in the reverse direction, at a rate of 50 mV/min.

The shape of the current-potential curves obtained, dependent on the anion type and pH of the solution, suggests the development of passive regions with localized penetration of the organic coating by the conducting phase. We interpret these results as a lacquer breakdown process that involves transport of anions at sites of low resistance electrolytic paths in the organic coating with their specific effect on the dissolution of tin from below the organic coating. These results correlate with the visible localized corrosion behaviour of the lacquered samples from direct corrosion experiments.

Introduction

In the past decade much work has been expended by many laboratories on devising rapid corrosion tests employing electrometric methods in an effort to obtain more information about the mechanism of protection of metals by organic coatings, to select materials best suited to particular cases as well as to avoid the very long exposure periods needed in direct tests. General techniques used have included the measurement of potential–current and potential–time

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0022-1163/79/0200-0009 $02.00

relationship (Wormwell & Brasher, 1949; Pourbaix, 1965), polarization resistance measurements (Bird, Jones & Warner, 1971; Kleniewski, 1975), cathodic polarization of scratched lacquered specimens (Gonzalez, Josephic & Oriani, 1974; Albu-Yaron, Semel & Berzin, 1977) related to passivation film characteristics (Sherlock, 1976) or measurements of the coating impedance of a polymer coated steel as a function of time and coating thickness (Kending & Leidheiser Jr. 1976). Wolstenholme (1973) reviewed and summarized most of the work done between 1936–70 with comments on some special problems associated with electrochemical measurements on painted steel. Nevertheless, these methods do not elucidate completely the phenomena that are observed in some systems and do not explain the mechanism by which a polymer coating protects a metal substrate against corrosion.

The applicability of potentiokinetic polarization measurements to corrosion phenomena, inhibition and corrosion protection of passive metals and alloys has been discussed in detail in the literature (Greene, 1962; Pourbaix, 1965; Pourbaix *et al.*, 1963; Bohni & Uhlig, 1969) and the measurements have been used extensively in the study of passivation breakdown. However, the validity of using this technique as an index in evaluating corrosion behaviour of lacquered samples in the above manner has been questioned.

Assumptions have been made by other authors that, (1) the electrical responses of the electrolyte/coating/metallic substrate system can be correlated with corrosion behaviour of the metal substrate (Mayne, 1952; Craig & Olson, 1976); its potential being determined by the relative importance of the separate cathodic and anodic rates, and that (2), the corrosion of polymer-coated metal is associated with a porous penetration of the aqueous phase randomly distributed within the organic coating (Kending & Leidheiser, 1976). In view of this, we undertook the present work in an attempt to demonstrate the applicability of this method to define localized corrosion resistance of lacquered tinplate samples.

The current study presents preliminary results of the potentiokinetic polarization behaviour of an epoxy-phenolic lacquer on tinplate exposed to 1% model corrosive solutions of varying pH's and organic acids found in foods.

Materials and methods

Materials

All polarization tests were performed on unlacquered and lacquered tinplate samples supplied (by a French tinplate manufacturer) as 10 × 10 cm coupons. The tinplate was electrolytic, No. 1.00 lb/bb, with the conventional cathodic passivation treatment in sodium dichromate. The 5 μm thick layer non-pigmented epoxy-phenolic lacquer (curing temperature 200°C) used in these experiments was applied by centrifugation by the manufacturer.

The electrolytes in all of the experiments were 1% aqueous solutions of citric, oxalic, lactic and acetic acid (pH range of 2.1–2.8) as well as 1% aqueous

Table 1. Experimental details (tinplate, E No. 1.00 lb/bb; lacquer system, non-pigmented epoxyphenolic; lacquer thickness, 5 μm; adhesion, 100%)

Acid system	pH 1% acid	pH 1% sodium salt
Citric	2.5	8.0
Oxalic	2.1	6.8
Lactic	2.3	6.4
Acetic	2.8	7.5

solutions of the sodium salts of these acids (pH range of 6.4–8.0) prepared from analytical grade chemicals and doubly distilled water. Information on the experimental details of this work is given in Table 1.

Cell and instrumentation

Experiments were performed in a simple three-electrode cell consisting of 1.8 cm thick and 6.5 cm long perspex tubing with four 1.5 cm diameter necks for connecting the salt bridge from the reference electrode to the solution, and for adding and emptying out the electrolyte solutions. The two open ends (each of 6.5 cm inner diameter) were closed on each side by two circular perspex end blocks that also served as holders for the auxiliary electrode on the one side and for the sample (working) electrode on the other side (Fig. 1).

The auxiliary electrode consisted of a platinum sheet with an exposed circular area of 6.5 cm in diameter concentric with the working electrode opening. The working electrode consisted of test specimen coupons 10 × 10 cm held vertically in the perspex holder. The experimental areas were delimited by a circular aperture of 1.6 cm diameter bordered by a rubber 'O' ring. The exposed area of each test specimen was 2 cm^2. It can be seen that with this opening and a 10 × 10 cm coupon, edge effects were not introduced. The two blocks were held in place by an insulated clamp. The reference electrode was a saturated calomel electrode (SCE), Tacussel-type, connected to the cell solution through a Luggin capillary.

The basic instrument used for corrosion experiments consisted of a Tacussel-type PRT 20-2 potentiostat with a millivoltmeter rack model S-70 AS/R coupled to a drive-unit Servovit-10A pilot scanner for automatic programming of the operating potentials. Current potential curves were obtained on a Tacussel potentiometric recorder model EPL-28, equipped with the current-recorder plug-in T-I-20G.

Methods

Electrochemical measurements. Polarization curves were obtained by the following procedure which was adopted for the sake of uniformity: after an

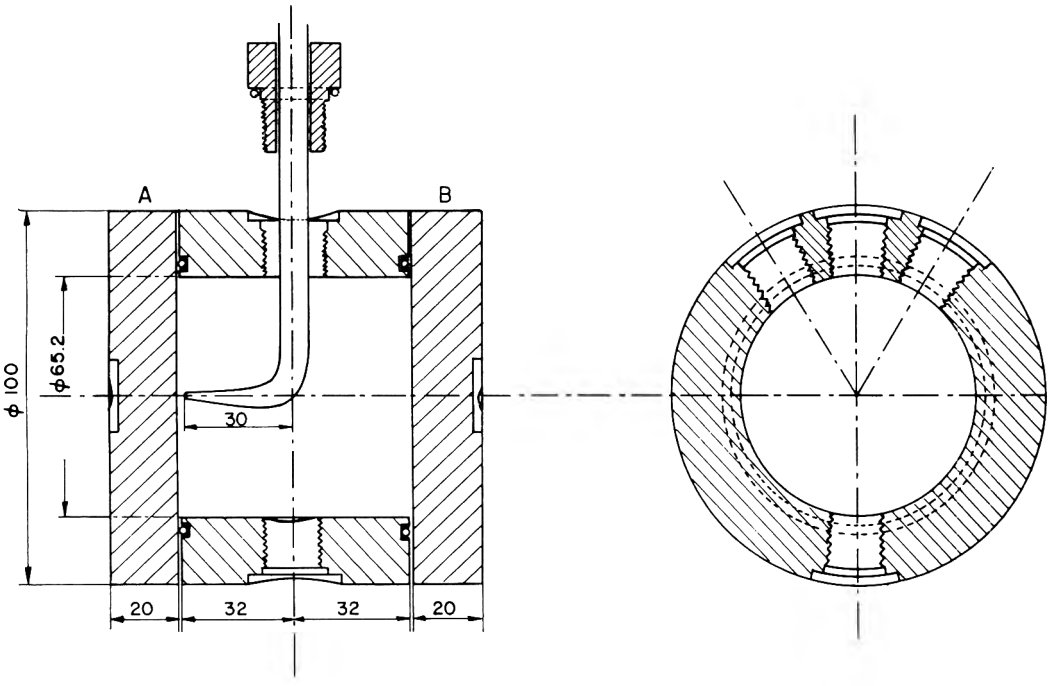

Figure 1. Cross-section through perspex corrosion cell and end blocks. A, sample holder end-block; B, auxiliary (Pt-sheet) electrode holder end-block.

initial exposure period of 5 min in the respective solution, the free corrosion potential (Eo) was measured. The potential of the specimen was then activated at Eo – 500 mV(SCE) and the potential scanning in the noble direction started at a rate of 50 mV/min, within the Eo – 500 to Eo + 500 mV range; the potential scanning was then reversed. Currents were recorded simultaneously. All measurements were carried out in duplicate.

Tests were performed in electrolyte solutions in contact with air at the ambient laboratory temperature (20°C), without stirring. A fresh solution was used for each test. The occurrence of localized corrosion during polarization was indicated by an increase in the polarization current, as well as by the presence of hysteresis in the polarization curve during the backscanning of the potential.

Direct corrosion experiments. Lacquered specimens of 2 cm^2 area, of which the metallic back surface as well as the cut edges were masked with paraffin, were immersed in sufficient volumes of the same model solutions and stored at 35–38°C in a thermostated cupboard for 500 h. After this period all surfaces were compared for performance. The extent of undermining on these samples was assessed by visual inspection. All tests were carried out in duplicate.

Results and discussion

Potentiokinetic polarization curves of tinplate samples lacquered with a 5 μm thick epoxy-phenol layer in several organic acid solutions found in foods and

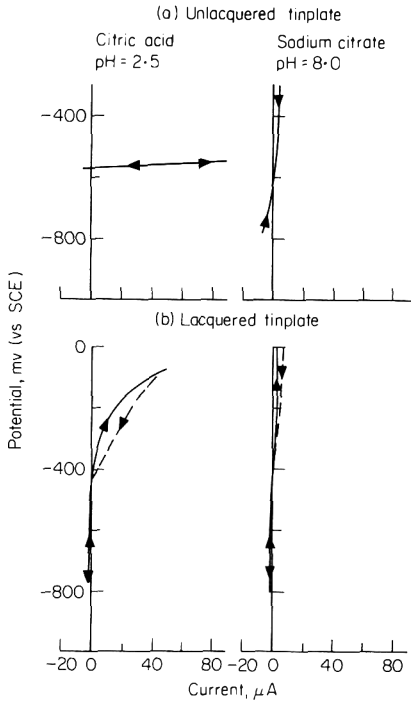

Figure 2. Potentiokinetic polarization curves for (a), unlacquered tinplate, and (b), lacquered tinplate samples exposed to 1% citric acid solution (pH = 2.5) and to 1% sodium citrate solution (pH = 8.0).

the effect of the organic acid anion type and pH on the shape of these curves are shown in Fig. 2b–5b. We were concerned in this work only with the aspects of localized corrosion at sites of breakdown in the lacquer structure. Samples of perfect coatings, without scratches or cuts through the organic coating were chosen for these experiments.* The polarization curves for unlacquered specimens of identical tinplates are given in Fig. 2a–5a.

Organic acid solutions (citric, lactic and acetic acids)

Different anodic polarization characteristics of the two surfaces can be observed in all of these acid solutions in the lower pH range: 2.2–2.8. Polarization curves for the coated tinplate samples (Fig. 2b, 4b, 5b) exhibit a region of very small currents both in the anodic and cathodic sides of the corrosion potential when scanning the potential from Eo – 500 mV in the anodic direction to Eo + 500 V, indicating a negligible corrosion rate. The anodic branch of the polarization curve exhibits a passive region between the corrosion potential and a certain potential (Ebr), over which the anodic current begins to increase.

*A special device tester, based on variation in conductivity between the dielectric (lacquer) and air with acoustic signals, was used for non-destructive examination of the coating continuity.

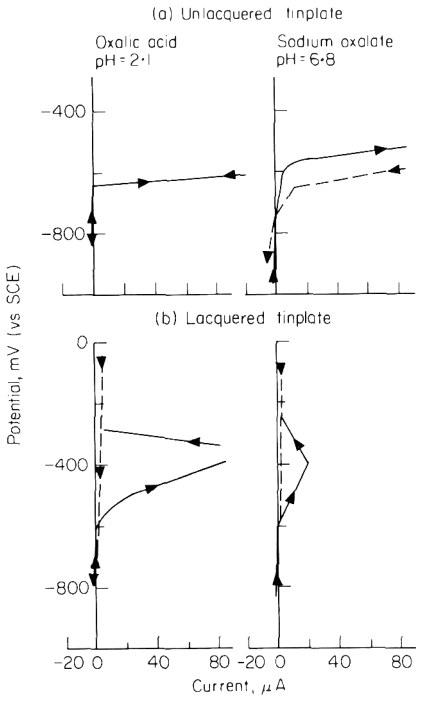

Figure 3. Potentiokinetic polarization curves for (a), unlacquered tinplate and (b), lacquered tinplate samples exposed to 1% oxalic acid solution (pH = 2.1) and to 1% sodium oxalate solution (pH = 6.8).

On backscanning the potential, the polarization current continued to increase with decreasing potentials for some time, then decreased at a potential less noble. This indicates the occurrence of 'pitting corrosion' presumably due to breakdown processes taking place through the organic coating followed by underfilm anodic corrosion of tin and/or iron substrate.

Curves in Figs 2a–5a show polarization behaviour of the unlacquered tinplate samples in the same organic acid solutions. Polarization curves exhibit a continuous increase of anodic currents at the corrosion potential indicating that the unlacquered surface is active in all of these solutions and rapid corrosion occurs. Curves do not show any range of passive currents; during backscanning the potential in the reverse direction, identical curves were obtained.

Results of these experiments show clearly that in these low pH organic acid solutions in which the tinplate surface is anodically active, the lacquered tinplate surface initially exhibits protection (like a porous film) which breaks down first to give localized corrosion and then general corrosion. We associate the initial breakdown with conductive sites within the organic coating, through which ions from the surrounding solution are carried by means of corrosion currents. The specific effect of each anion on the mechanism of underfilm corrosion of the tinplate substrate is presumably due to different diffusion rates through the unbroken organic layer as well as different degrees of chemical interactions with the metal substrate.

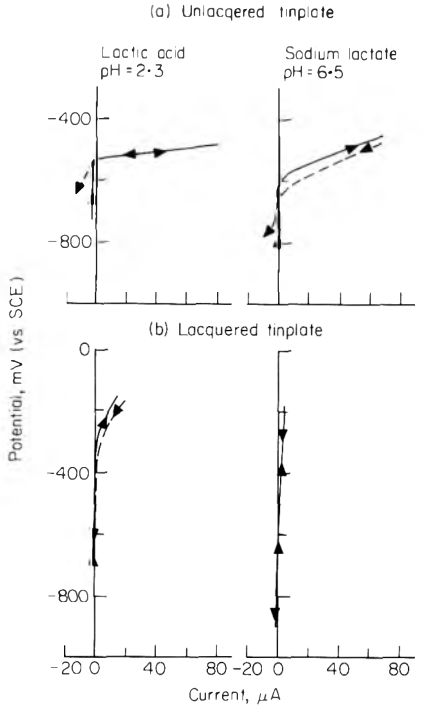

Figure 4. Potentiokinetic polarization curves for (a) unlacquered tinplate and (b) lacquered tinplate samples exposed to 1% lactic acid solution (pH = 2.3) and to 1% sodium lactate solution (pH = 6.5).

The results of these experiments are in good agreement with conclusions previously noted by Mayne (1972), Leidheiser & Kending (1976), Kending & Leidheiser (1976) and Koehler (1977) who studied this phenomenon in detail. As Koehler (1974) has shown, corrosion of organic-coated metal is enhanced by the flow of corrosion currents directly through the organic coating to the extent that such corrosion currents are possible. Whenever the organic coating can conduct corrosion currents, breakdown processes take place through the organic coating.

Kending & Leidheiser (1976) showed that in some localized areas the coating does not behave as a dielectric (highly crosslinked polymer with inert electric behaviour) but exhibits electrolytic conductivity as a consequence of polymer breakdown such that electrolyte is dissolved within a hydrolysed region or because of penetration by the electrolyte through electrolytic paths. These authors considered the entire system, coating/metal oxide/metal, to consist of electrically activated conducting regions penetrating the coating randomly over the surface and depth. In agreement with Kending & Leidheiser's (1976) and Koehler's (1977) work, our results also show that the local conducting paths at which corrosion begins are points at which later organic coating breakdown occurs.

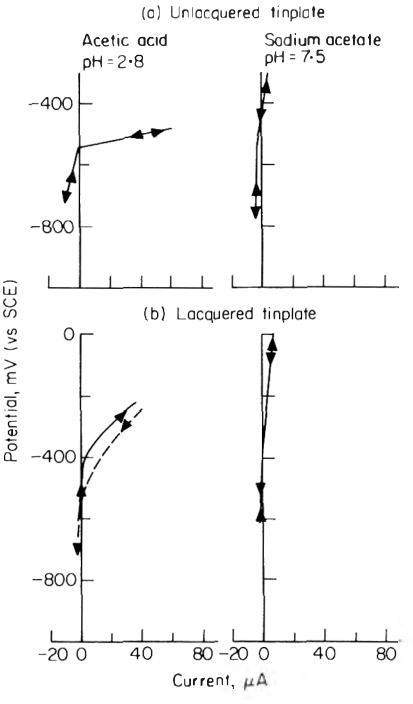

Figure 5. Potentiokinetic polarization curves for (a), unlacquered tinplate and (b), lacquered tinplate samples exposed to 1% acetic acid solution (pH = 2.8) and to 1% sodium acetate solution (pH = 7.5).

Near neutral 1% salts of the organic acid solution (citrate, lactate and acetate)

Potentiokinetic polarization scanning on the two surfaces yield similar results in the sodium citrate solution (pH = 8.0) (Fig. 2a, b) and sodium acetate solution (pH = 7.5) (Fig. 5a, b); curves exhibit a passive region with polarization currents of the order of few μA over the entire range of the potential scannings indicating negligible corrosion rates. Curves obtained under these conditions are characteristic of a non-corrosive surface. Identical curves were obtained when polarizing in the reverse direction.

Scannings in pH = 6.5 sodium lactate solutions with the two types of surfaces resulted in different polarization curves (Fig. 4a, b). The lacquered surface was passive and exhibited low passive corrosion currents over the entire range of potential tried, whereas the unlacquered surface exhibited a region of passivity with a sudden increase of the anodic current at which passivity broke down. When the potential scan was reversed the polarization current continued to increase with decreasing potentials for some time then decreased at a potential less noble, indicating the possibility of localized corrosion.

Results of these experiments in neutral or near neutral solutions of the organic acid salts show that the tinplate itself is resistant to corrosion at these pH. No active passive transitions were observed on either surface (except the

unlacquered tinplate surface in sodium lactate solution) and the lacquer cathodically protects the tinplate.

By comparison with results in acid model solutions, the data obtained indicate that low acid environments more rapidly degrade the quality of the coating than do near neutral environments. These results agree with those of Leidheiser & Kending (1976).

Oxalic acid solutions

The potentiokinetic polarization curves of the lacquered tinplate surface in oxalic acid solutions at pH 2.1 as well as in the neutral sodium oxalate solution (pH 6.8) were of a different shape than curves obtained in the three other organic acid solutions (Fig. 3b). An initial active-passive transition was observed at increasing polarization in the positive direction, the critical anodic current for passivity being nearly five times as great in the pH 2.1 solution, as in the pH 6.8 solution. Hence, the ease of passivation increases with increasing pH.

The decrease in the current, when scanning potentials in the positive direction presumably corresponds to the formation of a tight corrosion product layer of tin oxalate at points where breakdown of lacquer allows the flow of oxalate ions to arrive at the tin oxide/tin metal interface. During polarization in the reverse direction, a curve of very low currents was obtained over the entire range of potentials thus indicating that the oxidation reaction which occurs at the higher potentials does not modify the surface. These results are in agreement with findings in actual practice, as for example, in lacquered cans containing lemon juice (which contains a high percentage of oxalic acid) – a solid tin oxalate layer can be observed under the undamaged lacquer.

The unlacquered surface displayed potentiokinetic curves which indicates rapid corrosion in the pH 2.1 oxalic acid solution and a region of passivation with possibility of localized corrosion in the pH 6.8 oxalate solution (Fig. 3a).

Table 2. Visual assessment of corrosion of tinplate coated with a 5 μm thick layer of an epoxyphenolic lacquer, after immersion for 500 hr*

Model acid solutions (1%)	pH	Appearance
Citric acid	2.5	Few isolated corrosion spots
Oxalic acid	2.1	Corrosion spots with delamination of the lacquer
Sodium oxalate	6.8	Small corrosion spots
Lactic acid	2.3	Few isolated corrosion spots
Acetic acid	2.8	Small corrosion spots
Sodium acetate	7.5	No visible corrosion spots

* In the presence of atmospheric oxygen.

Correlation with storage test

Results obtained from the duplicate of the electrochemical measurements were correlated with 500 h storage test. The reproducibility of the polarization measurements was within ± 20 mV (location of potentials) and agreement between polarization measurements and 500 h storage tests was good for all model solutions used in the electrochemical measurements.

The appearance of very small corrosion spots on the surface of the lacquered specimen which underwent accelerated immersion tests (Table 2) in the same model solutions (500 h, 35–38°C), provides visual evidence that further reinforces the results from potentiokinetic polarization measurements.

The results presented are preliminary. Further investigations already in progress providing more detailed information will be published in a subsequent paper.

Acknowledgments

The experimental part of this study was carried out during the senior author's (A.A.Y.) leave at the Laboratoire de Corrosion, Ecole Normale Supérieure de Chimie, Paris, and at the Centre National de l'Emballage et du Conditionnement, Trappes.

She wishes to express her thanks to Professor J. Talbot and Mr Jobard for their kind permission to work in their respective Institutes.

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(Received 18 March 1978)

J. Fd Technol. (1979) **14**, 21–31

Texture studies on edible protein fibres produced by a wet spinning technique

I. Fibres produced from casein and carrageenan

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Summary

Solutions of casein and carrageenan, prepared at neutral pH, were used to produce edible protein fibres by a wet spinning technique. Variation in fibre breaking strength, shear force, stress relaxation and elasticity as a result of changes in such process variables as fibre wind-up rate, immersed length, precipitation bath salt concentration and precipitation bath temperature were followed. The effect of spinning solution carrageenan level on fibre texture was also investigated. It was possible to alter certain textural properties by changes in the conditions of fibre production.

Introduction

In recent years, there has been a great deal of interest in the production of edible protein fibres for use as meat extenders. Wet spinning is an important method for the production of such fibres. This importance is reflected in the volume of patent literature referring to the technique (Gutcho, 1973). While most work has tended to concentrate on the use of vegetable protein from a number of sources e.g. soya bean, cottonseed, safflower, zein etc. (see review by Burke, 1971), the formation of edible fibres from animal protein has been reported in some cases. Meat industry by-products and waste such as lung and stomach protein (Young & Lawrie, 1975) and blood plasma (Young & Lawrie, 1975; Swingler & Lawrie, 1977) have been the chief sources of such animal protein. There has also been some interest in the use of casein for edible fibre production (Schwartz, 1940; Schmandke *et al.*, 1976b).

While edible casein fibres have advantages in terms of colour, flavour and nutritional value over similar fibres produced from vegetable protein, a major functional disadvantage has been their relatively high solubility in water (Ashton, Burke & Holmes, 1970). Attempts to decrease fibre solubility have been made by the incorporation of other, mainly vegetable, proteins in the

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0022-1163/79/0200-0021 $02.00

spinning solution (Szczesniak & Engel, 1960; Schmandke & Schmidt, 1977), the similar incorporation of polysaccharide (Giddey, 1962; Ishler, Macallister & Engel, 1963; Atkinson, 1969) or chemical modification of formed fibres (Atkinson, 1969; Schmandke & Hartman, 1976; Schmandke & Schmidt, 1977). Edible casein fibres have been prepared in this laboratory by inclusion in the spinning solution of the sulphated polysaccharide carrageenan which reacts in a highly specific manner with milk proteins (Payens, 1972).

Consumer acceptance of edible protein fibres in food depends largely on the texture of such fibres. Food texture measurement has as its aim the development of objective mechanical test methods which correlate well with taste-panel (i.e. subjective) assessments of such parameters as tenderness, juiciness etc. (Szczesniak & Torgeson, 1965; Iles, 1971). Shear force has been the textural parameter measured in most instances because of its correlation with tenderness, especially in meat (Kramer, Burkhardt & Rogers, 1951) but Pool & Klose (1969) have suggested that reliance solely on this parameter is not to be recommended. Stanley, Pearson & Coxworth (1971) examined a number of other physical properties of muscle in order to detect those which produced the best correlation with sensory ratings of tenderness. Among those reported were fibre breaking stress, stress relaxation and elasticity.

Textural investigations of textured protein products have previously been reported (Stanley, Cumming & de Man, 1972a; Young & Lawrie, 1975; Maurice, Burgess & Stanley, 1976; Schmandke *et al.*, 1976a; Swingler & Lawrie, 1977). With the exception of Stanley *et al.* (1972a) none reported the measurement of more than one textural property of the fibres under examination (shear force) and only Swingler & Lawrie (1977) monitored changes in any such property with alterations in the conditions of fibre production. No wide-ranging analysis of the variation in textural properties of edible fibres produced from casein with changes in processing conditions has been published. The present investigation describes the variation in fibre breaking strength, shear force, elasticity and stress relaxation with certain changes in the spinning process. These changes were the degree of stretch imparted to the fibres, variation in precipitation bath composition and temperature, length of fibre immersed in the precipitation bath and carrageenan level in the spinning solution.

Materials and methods

Edible acid casein (90 mesh) was obtained from North Kerry Creameries Ltd, Listowel, Co. Kerry. Carrageenan, sodium salt, was supplied by BDH (BDH Chemicals Ltd, Poole, Dorset BH12 4NN). All other chemicals were 'Analar' grade, with the exception of sodium chloride.

Spinning solutions

Spinning solutions were prepared (to an approximate final pH value of 6.8) in the following manner:

Casein (15% w/v) and carrageenan (0.3% w/v) were added slowly in turn with vigorous stirring to a solution of sodium hydroxide (0.33% w/v) at 70°C. The heat source was then removed and no control was exercised over solution temperature until deaeration. Stirring was continued for about 30 min after additions were complete to allow adequate time for dissolution; at this point, batches were homogenized in a laboratory homogenizer (MSE Ltd, Crawley, Sussex) for approximately 30 sec at full speed. Deaeration of the resultant solution was effected by centrifugation at 30°C and 2000 rev/min for 5 min (MSE Coolspin Centrifuge). Solutions were then ready for immediate use.

Fibre production and collection

The viscous spinning solution was transferred from a stainless steel reservoir by a gear-pump (Slack & Parr Laboratory pump, Model No. SPL2). This pump was driven by an electric motor (Model SD1SS; approximate full load final speed 20 rev/min) which was in turn controlled by a thyristor D.C. controller. From the pump, the solution was forced through a nylon mesh filter (mesh size 100 μm), a noble metal spinnerette containing 250 holes, each of diameter 0.004 in (Courtalds Engineering Ltd, Model No. SD/127C) and into a stainless steel precipitation bath. Standard precipitation bath composition was 3% (v/v) lactic acid and 5% (w/v) sodium chloride. In all the experiments reported here, fibre extrusion rate was calculated from pump speed to be approximately 250 cm/min. Fibres were collected on a wind-up spool driven by another electric motor (Model No. SD1S, approximate full load final speed 55 rev/min) also under thyristor control. The length of fibre immersed was controlled by the position in the bath of a movable rod. Unless otherwise stated, this length was 62.5 cm, measured from the spinnerette face. After collection, fibres were removed from the spool and rinsed in 2% (w/v) sodium chloride solution at room temperature. Prior to texture analysis, fibres were stored overnight in this solution, also at room temperature.

Electric motors and thyristor D.C. control units were supplied by Parvalux Electric Motors Ltd, Wallisdown, Bournemouth.

Texture measurement

An Instron Universal Testing Machine and Recorder, Model No. 1112, was used for the measurement of fibre texture. Shear force measurements were performed on wet fibre bundles (fibre tows were collected for 5 min, removed from the wind-up reel, rinsed and then cut into eight pieces of equal length) using a V-shaped shear blade (Instron Ltd, High Wycombe, Bucks.) and a compression cell (Type 2511-203; FSD of 50 kg) suspended from the cross-head. Results are expressed as shear force (grams) per tow. Texture measurements on individual fibre tows necessitated the use of a tension load cell (Type 2511-101;

FSD 500 g and fibre clamps (Type 2A). A gauge length of 2 cm was used and fibre tows were cut into pieces approximately 3.5 cm in length. In all cases, both chart and cross-head speeds were set at 10 cm/min. Fibre breaking strength is defined as the breaking load (g) per gram of sample; prior to determining tow weight per centimetre, excess moisture was removed by gently blotting a length of tow (90 cm) between two pieces of tissue paper. Stress relaxation was taken as the loss in stress, as a percentage of the original stress, of a fibre tow held extended for a period of 1 min. For elasticity measurement, fibre tows were cycled five times only, as preliminary work indicated that no increase in precision was obtained by using a greater number of cycles. Elasticity is normally taken as the percent reduction in work required for the final cycle as compared to that required for the first. In this investigation, since the curves were close approximations to isoceles triangles, curve height was substituted for area in elasticity determination.

Chemical analysis

Protein determinations were performed by the macro Kjeldahl method and moisture levels by heating at 101°C for 2 hr in a hot air oven. Ash values were obtained following heating in a muffle-furnace at 550°C for 2½ hr, and pH determinations were made on a Radiometer model 28 pH meter.

Results and discussion

Chemical

Fibres were routinely analysed to determine moisture, protein and ash levels, typical values being 68–70%, 28–30% and 1.7–2.0% respectively. Determination of fibre carrageenan levels was not normally performed.

Textural

Shear Force Variation. Variation in the shear force per tow of casein-carrageenan fibres (expressed in logarithmic form in some cases) with wind-up rate (i.e. stretching), precipitation bath temperature, salt level and length of fibre immersed in the bath are shown in Figs 1, 2, 3 and 4 respectively. In Fig. 5 is shown the effect of change in the carrageenan concentration of the spinning solution on the same parameter.

It is apparent that the shear force per tow decreases with increasing fibre wind-up rate, i.e. as the fibres become progressively more stretched. This observation appears to be in conflict with the claim of Boyer (1954) while agreeing with the results of Young & Lawrie (1975) who investigated fibres

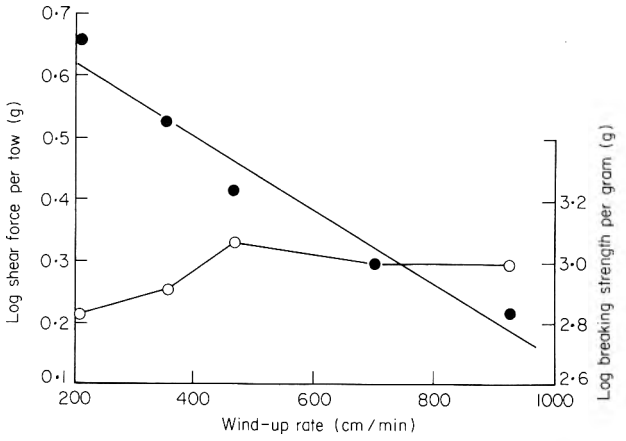

Figure 1. Variation of the logarithm of shear force per tow (●——●) and breaking strength per gram wet weight (○——○) with fibre wind-up rate.

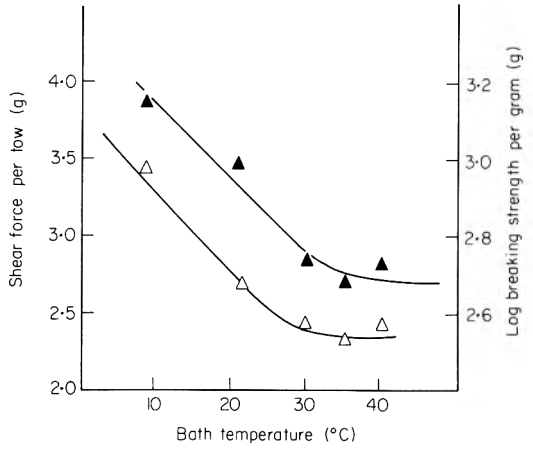

Figure 2. Variation of the shear force per tow (△——△) and logarithm of breaking strength per gram wet weight (▲——▲) with precipitation bath temperature.

produced from meat waste protein. In addition, Young & Lawrie (1975) report that subjecting the fibres to zero stretch (i.e. merely maintaining the fibres taut as they emerge from the spinnerette) produces samples of the highest shear strength, also in broad agreement with the results reported here. The reason for the apparent conflict with the claim of Boyer (1954) may lie in the observation that, while fibre shear strength per tow decreases with increasing wind-up rate, the number of fibre tows collected per minute actually increases. Thus, the shear force per bundle increases with increasing fibre stretch and this latter increase may have been assumed by Boyer to stem from an increase in individual tow shear strength.

Fibre shear force per tow increases linearly with increasing precipitation bath salt concentration, over the range investigated. Swingler & Lawrie (1977)

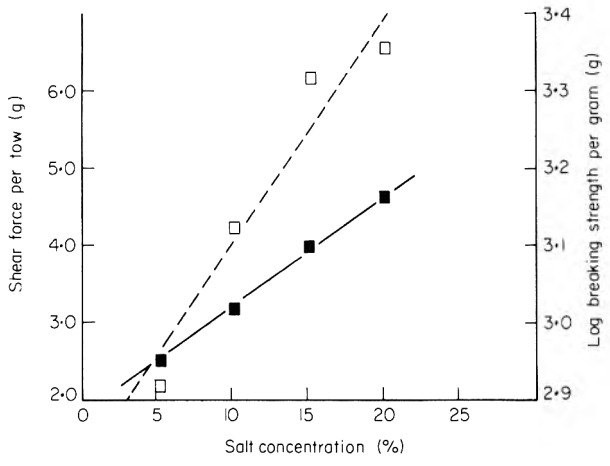

Figure 3. Variation of the shear force per tow (□——□) and the logarithm of breaking strength per gram wet weight (■——■) with precipitation bath salt concentration.

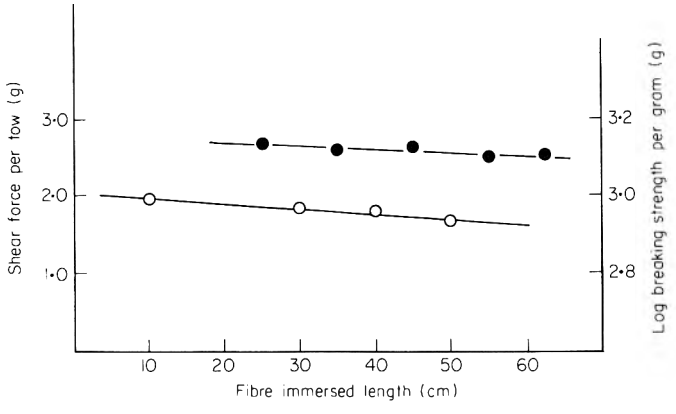

Figure 4. Variation of the shear force per tow (●——●) and the logarithm of the breaking strength per gram wet weight (○——○) with length of immersed fibre.

however, in an investigation into the influence of precipitation bath composition on the shear strength (determined on a Volodkevich texturometer) of fibres produced from blood plasma, did not obtain a simple relationship between this textural parameter and precipitation bath sodium chloride concentration at any given concentration of acid.

The effect of precipitation bath temperature is more complex, with the shear force per tow decreasing with increasing temperature to a minimum value around 30°C. Fibre texture at any temperature is the result of the combined effect of many factors among which are bath pH, and degree of saturation with respect to salt.

It may be seen in Fig. 5 that fibre shear force is independent of the carrageenan concentration of the spinning solution. The finding that fibre shear

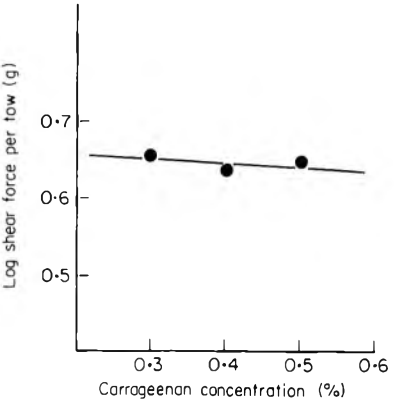

Figure 5. Variation of the logarithm of shear force per tow with the concentration of carrageenan (w/v) in the spinning solution (solutions containing less than 0.3% and more than 0.5% were not suitable for spinning).

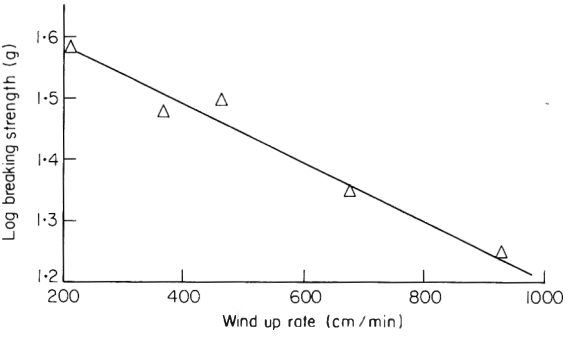

Figure 6. Variation of the logarithm of fibre breaking strength with wind-up rate.

force varies only very slightly with fibre immersed length is evidence of the rapidity and completeness of precipitation in this system. This is in contrast to the report of Swingler & Lawrie (1977) and the difference is probably due at least in part to the high pH of their protein spinning solution (pH 10.0). In addition to such process variables as extrusion rate, fibre diameter etc., intrinsic properties of the spinning solution such as protein concentration and type may be expected to have an effect on the rate of coagulation.

It is of interest at this point to refer to the report of Yamakami (1975) concerning fibre spinning at low temperatures in conjunction with stretching to improve the textural properties of fibres made from soy protein.

Breaking strength

Variations in tow breaking strength with all the process parameters mentioned in the preceding section are also shown in Figs 1, 2, 3 and 4. It may be

seen from Figs 3 and 4 that the logarithm of fibre tow breaking strength per gram wet fibre weight increases linearly with increasing precipitation bath salt concentration but decreases very slightly with increasing fibre immersed length. The relationship between fibre breaking strength per gram wet weight and bath temperature is seen to be similar to that previously described for bath temperature and fibre shear force i.e. it decreases, with increasing temperature, to a minimum value around 30°C. Increasing fibre wind-up rate has the effect of increasing the weight-normalized breaking strength only very slightly to a maximum level at a wind-up rate of approximately 500 cm/min. In contrast, the uncorrected fibre breaking-strength decreases linearly with increasing fibre stretch, as shown in Fig. 6.

Stanley *et al.* (1972a) report a value of 2.12×10^3 g/g for the breaking strength of rehydrated soy fibres, significantly higher than that previously obtained for raw (0.241×10^3 g/g) or cooked (0.427×10^3 g/g) pork tenderloin by others (Stanley *et al.*, 1972b). As mentioned above, the fibre breaking strength/gram may be tailored by judicious selection of processing conditions; changing the degree of stretch of casein–carrageenan fibres, produced variation in breaking strength from 650 to 1150 g/g while the effect of increased salt in the precipitation bath was more noticeable (830–2270 g/g). It is therefore possible to produce casein–carrageenan fibres with breaking strengths in the same range as commercial soy fibres.

It is apparent from Table 2 that fibre breaking strength per gram wet weight increases slightly with increasing carrageenan concentration. While the differences are statistically significant, organoleptically they may not be of any importance.

In Fig. 7 is shown an idealized stress–strain curve for casein–carrageenan fibres. It resembles one previously reported for rehydrated soy fibres (Stanley *et al.*, 1972a) in the sharp fall off in stress on breakage but the behaviour prior to this point is more akin to that of meat (Stanley *et al.*, 1971). It may be that spun soy fibres used without previous dehydration would produce a stress–strain curve more closely resembling that of fresh casein–carrageenan fibres.

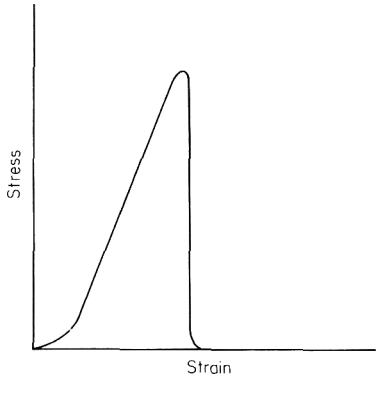

Figure 7. Typical stress–strain curve of casein–carrageenan fibres.

Stress relaxation

Values for the elasticity and stress relaxation of casein-carrageenan fibres are shown in Tables 1 and 2, each value being the mean of six replicates.

Within a given treatment batch, differences between the extreme values of stress relaxation are slight but statistically significant (at $P = 0.05$) in most cases. While change in precipitation bath temperature has essentially no effect, increasing bath salt level produces a slight increase in this parameter. Increasing the degree of fibre stretching or the length immersed in the precipitation bath decreases stress relaxation slightly. An increase in the percent elongation used in the test method, from 10% to 35% (above this value, fibre breakage becomes a problem) does not alter the magnitude of the result. Stanley *et al.* (1972a) compared the stress relaxation values obtained for rehydrated spun soy fibres and cooked meat on an equal fraction of break elongation basis, i.e. about half that required for breaking. In this case, this would correspond to an elongation of *c.* 20% but since we found no significant difference between results at 10 or 20%, our results for 10% are comparable to the extensions used by Stanley *et al.* (1972a). Those authors report 34% stress relaxation for cooked meat and 49% for soy fibres whilst the value for fibres produced in this investigation ranged from 51–58%.

Table 1. Variation of stress relaxation and elasticity with alteration of process variables

Variable	Stress relaxation (%)	Elasticity (%)
Wind-up rate (cm/min):		
210	54.2 ± 3.7	90.1 ± 3.0
360	55.8 ± 4.0	90.9 ± 4.6
460	53.5 ± 6.7	91.4 ± 2.8
670	52.6 ± 5.8	91.7 ± 2.0
925	51.7 ± 7.2	91.6 ± 4.7
Immersed length (cm):		
10	57.1 ± 6.1	91.3 ± 5.2
20	54.2 ± 4.9	91.6 ± 2.6
30	53.0 ± 3.4	91.2 ± 2.8
40	51.0 ± 4.4	91.9 ± 2.8
50	51.7 ± 4.1	91.9 ± 2.6
Bath salt concentration (% w/v):		
5	52.9 ± 4.2	90.8 ± 4.0
10	55.1 ± 4.4	93.6 ± 1.6
15	57.8 ± 3.8	92.6 ± 4.8
20	56.9 ± 3.6	94.1 ± 3.5
Bath temperature (°C):		
8.5	54.2 ± 1.5	92.1 ± 3.9
21	54.5 ± 3.3	89.4 ± 7.5
30	52.7 ± 6.1	91.9 ± 2.7
35	55.3 ± 5.4	91.6 ± 6.7
40	52.5 ± 4.5	91.9 ± 5.6

Table 2. Effect of spinning solution carrageenan level on fibre textural parameters

Carrageenan level (% w/v)	Breaking strength (g/g)	Elasticity (%)	Stress relaxation (%)
0.3	1152 ± 245	–	53.5 ± 6.7
0.4	1163 ± 178	90.5 ± 3.2	57.7 ± 3.7
0.5	1188 ± 192	91.4 ± 6.9	59.8 ± 6.0

Fibre stress relaxation shows a slight increase with increasing carrageenan level in the spinning solution, as may be seen in Table 2.

Elasticity

Elasticity is another textural property of fibres which may be of importance in the mastication process. As can be seen from Tables 1 and 2, while there may be statistically significant differences between the extreme elasticity values in any one treatment batch, these differences are very small and probably do not represent any organoleptically detectable change in fibre texture. It therefore appears that neither fibre elasticity nor stress relaxation may be altered to any organoleptically significant degree by alterations in the fibre production process. Again, extrapolation of the data of Stanley *et al.* (1972a) to the use of five cycles and a fibre elongation value of 10% as used in this work, produces a value for the elasticity of rehydrated soy fibre of approximately 69%, much lower than the results found in this study of casein–carrageenan fibres (91%). Since the soy fibres under investigation have been reported to be slightly less elastic than cooked pork, it may be that casein–carrageenan fibres are of greater elasticity.

Conclusion

It is apparent from the results presented here that it is possible to tailor certain of the textural properties of casein–carrageenan fibres within limits by simple manipulation of spinning process variables. In the case of fibre breaking strength per gram and shear force, the effects of changes in processing conditions are more marked than is the case with either elasticity or stress relaxation. Variation of these latter parameters may require chemical modification of either the spinning solution or the protein fibre. Such modification may additionally affect tow shear force (Schmandke *et al.*, 1976a) and fibre breaking strength.

Acknowledgments

The authors wish to thank Miss N. H. Hurley and Mr J. Kelly for technical assistance. Thanks are also due to the Irish Dairy Levy Fund for financial support.

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(Received 14 April 1978)

J. Fd Technol. (1979) **14**, 33–40

Texture studies on edible protein fibres produced by a wet spinning technique

II. Fibres produced from casein and alginate

G. DOWNEY* AND K. J. BURGESS†

Summary

Edible protein fibres were produced by a wet spinning technique from solutions of casein, containing sodium alginate, prepared at neutral pH. Variation of fibre breaking strength, shear force, stress relaxation and elasticity as a result of changes in such process variables as the degree of fibre stretch, length of fibre immersed in the precipitation bath, precipitation bath sodium chloride concentration and precipitation bath temperature were monitored. Changes in the conditions of spinning resulted in the production of fibres with altered textural parameters.

Introduction

Consumption of textured protein products, in their role as meat extenders, has increased in the recent past for many reasons, chiefly economic (Pearson, 1976). Such textured products have been produced from vegetable protein in the main, but some interest in their production from normally waste animal proteins (Young & Lawrie, 1975; Swingler & Lawrie, 1977) and casein (Szczesniak & Engel, 1960; Lecluse, 1975) has been reported.

Casein fibres have been previously reported to be highly soluble in water (Ashton, Burke & Holmes, 1970). In addition, solutions of casein sufficiently viscous for spinning (approximately 30% w/v) are difficult to handle when prepared at pH values close to neutrality because of the entrapment of large volumes of air. However, the addition of carbohydrate hydrocolloids to casein solutions, at pH values close to 7.0, allows the production of casein-containing fibres of low solubility (Giddey, 1962; Atkinson, 1969). Deaeration of spinning solutions containing carbohydrate hydrocolloids and casein (which have a viscosity similar to that of 30% w/v casein solutions) may be readily accomplished by centrifugation.

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0022-1163/79/0200-0033 $02.00

The preparation of spinning solution at pH values close to 7.0 is desirable in order to minimize the formation of lysinoalanine. Significant amounts of this amino-acid may be formed by exposure of proteins to heat and alkali. Lysinoalanine formation has been reported to be accompanied by reduced net protein utilization and to induce nephrotoxic reactions in rats (de Groot & Slump, 1969; Woodard & Short, 1973).

A previous publication (Downey & Burgess, 1979) described the preparation and textural characterization of fibres produced from casein and carrageenan. This study of casein-alginate fibres is an extension of that work.

Materials and methods

Sodium alginate (Alginade DC, Batch No. 314883) was supplied by Albright & Wilson (Irl. Limited) Dun Laoghaire, Co. Dublin and edible acid casein (90 mesh) was obtained from North Kerry Creameries Ltd, Listowel, Co. Kerry. All other chemicals were 'Analar' grade with the exception of sodium chloride.

Methods of fibre production, chemical analysis and texture measurement were as described in Downey & Burgess (1979) with the following exceptions. In the production of spinning solutions, carrageenan (0.3% w/v) was replaced by sodium alginate (2.5% w/v) and the spinnerette used in this investigation contained 100 holes, each of diameter 0.004 in. Fibre extrusion rate was estimated to be approximately 370 cm/min. In the measurement of stress relaxation and elasticity, extensions of 0.4 cm (20% of gauge length) were used.

Results and discussion

Chemical

Routine analyses for fibre moisture, protein and ash levels were performed. Values for these parameters were normally in the ranges 62–64%, 28.5–30.2% and 3.6–3.8% respectively. Fibre alginate levels were not determined.

Textural

Shear force variation. Variation in the shear force per tow of casein–alginate fibres with changes in fibre wind-up rate, precipitation bath sodium chloride concentration, precipitation bath temperature and the length of fibre immersed in the precipitation bath is shown in Figs 1–4.

It may be seen from Fig. 1 that the logarithm of the fibre shear force per tow decreases linearly with increasing wind-up rate. Figures 2 and 3 reveal that fibre shear force per tow increases linearly with increasing precipitation bath sodium chloride concentration over the range tested and decreases with

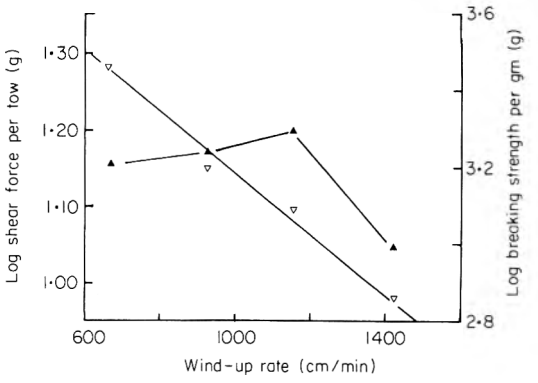

Figure 1. Variation of the logarithm of the shear force per tow (▽——▽) and the logarithm of the breaking strength per gram wet weight (▲——▲) with fibre wind-up rate.

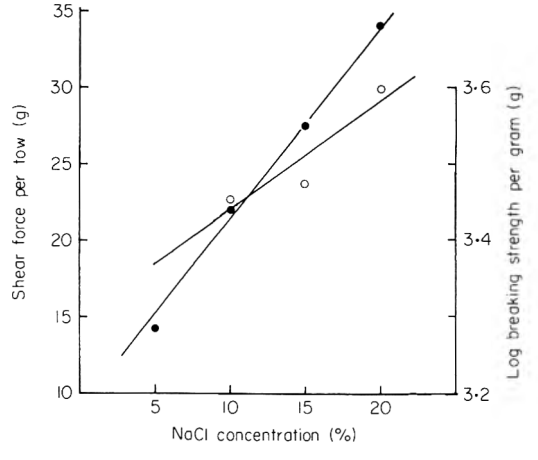

Figure 2. Variation of the shear force per tow (●——●) and the logarithm of the breaking strength per gram wet weight (○——○) with precipitation bath NaCl concentration.

increasing temperature to a minimum value at the highest temperature tested, 30°C. All these changes are qualitatively identical to those previously described for casein–carrageenan fibres (Downey & Burgess, 1979). There are, however, some important quantitative differences. In all cases, the magnitude of fibre shear force measured is much higher than the corresponding value for casein–carrageenan fibres. The ratio of these shear force values varies from approximately 2:1 (in the case of the variation in wind-up rate) to approximately 5:1 (in the case of variation in bath sodium chloride level or temperature). This is despite the fact that each tow of casein–alginate fibres contains 100 individual fibres as compared to 250 in the case of the fibres produced from casein and carrageenan.

One important difference between alginate- and carrageenan-containing fibres concerns the effect of increasing the length of fibre immersed in the

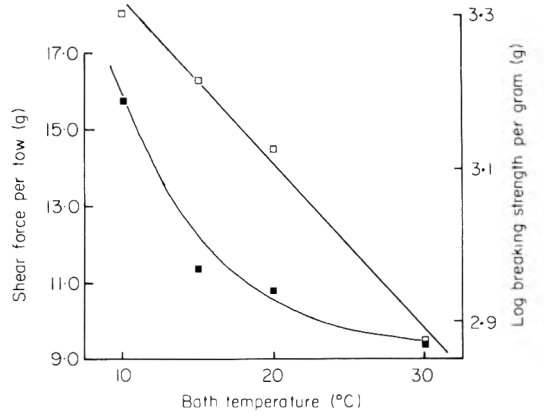

Figure 3. Variation of the shear force per tow (■——■) and the logarithm of the breaking strength per gram wet weight (□——□) with precipitation bath temperature.

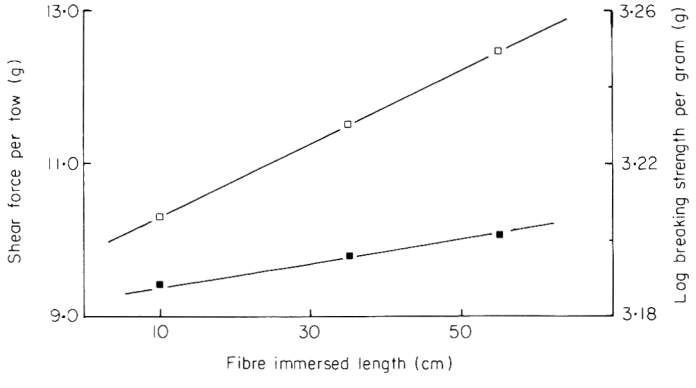

Figure 4. Variation of the shear force per tow (□——□) and the logarithm of the breaking strength per gram wet weight (■——■) with the length of fibre immersed in the precipitation bath.

precipitation bath. Whereas, the shear force per tow of carrageenan-containing fibres is independent of the length of fibre immersed, this parameter increases significantly with increased immersed fibre length in the case of casein–alginate fibres (see Fig. 4). This must stem from differences in the chemical composition of the spinning solutions. That this increase in fibre shear force per tow is not due to incomplete precipitation of casein–alginate fibres is suggested by the size of the variation in the weight-normalized fibre breaking strength (see Fig. 4). The increase (approximately 2%) in this parameter over the entire range of fibre immersed length tested is not significant. A significant variation in the weight-normalized fibre breaking strength would be expected if the degree of fibre precipitation varied significantly over the range of fibre immersed length tested.

Breaking strength

Variations in the logarithm of the weight-normalized fibre tow breaking strength with changes in the previously mentioned process variables are also shown in Figs 1–4. It may be seen that the logarithm of this textural parameter increases linearly with increasing precipitation bath sodium chloride concentration and the length of fibre immersed in the precipitation bath over the ranges tested. The extent of the latter increase is, however, slight (approximately 2%). Figure 1 shows that the weight normalized tow breaking strength increases slightly with increasing fibre stretch to a maximum at around an extrusion-to-wind-up ratio of 1:3, beyond which it decreases sharply. In contrast, the tow breaking strength (uncorrected for changes in fibre weight) decreases linearly with increasing wind-up rate as shown in Fig. 5. This may be explained as follows:

The graph of the logarithm of the weight-normalized fibre breaking strength versus fibre wind-up rate is the resultant of two separate graphs, namely that of uncorrected fibre breaking strength versus wind-up rate and fibre weight per unit length versus wind-up rate. In this case, the rate of decrease in fibre weight per unit length is slightly faster than the rate of decrease in uncorrected fibre breaking strength until a wind-up rate of approximately 1160 cm/min is reached. After this point, the rate of decrease in fibre weight per unit length is much slower than the decrease in uncorrected fibre breaking strength (see Fig. 5), producing the line of negative slope observed in Fig. 1.

The weight-normalized fibre breaking strength of casein–alginate fibres is essentially independent of the length of fibre immersed in the precipitation bath (see Fig. 4). This behaviour is identical to that previously reported by us for casein–carrageenan fibres.

It may be seen from Fig. 3, that the weight-normalized fibre breaking strength (plotted logarithmically) decreases linearly with increasing precipitation bath temperature. This behaviour is in marked contrast to that of casein–

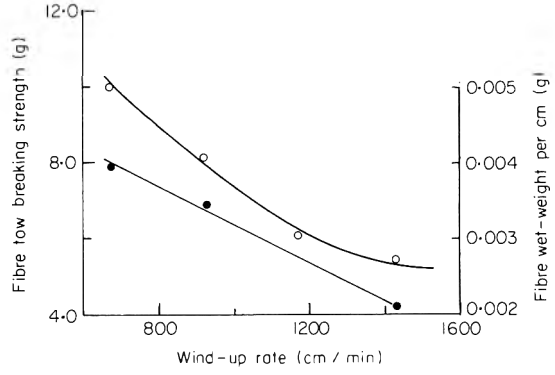

Figure 5. Variation of the fibre tow breaking strength (●——●) and fibre wet-weight per centimetre (○——○) with fibre wind-up rate.

carrageenan fibres, the breaking strength of which decreased, with increasing temperature, to a minimum value around 30°C. It was not possible to produce casein–alginate fibres at temperatures above 35°C in this investigation; however, no immediate change in the rate of decrease of fibre breaking strength above a precipitation bath temperature of 30°C appears likely. This difference in behaviour between casein–carrageenan and casein–alginate fibres must arise from differences in the chemical properties of the hydrocolloids.

Variation in the sodium chloride concentration of the precipitation bath allows the production of casein–alginate fibres with breaking strengths per gram in the range 2850–3950 g while the figures obtained from variation of wind-up rate are 1635–2000 g. These figures compare with a value of 2120 g/g in the case of rehydrated soy fibres reported by Stanley, Cumming & de Man (1972) and illustrate that casein–alginate fibres can be produced with a wide range of breaking strengths, both above and below those found for commercial soy fibres.

Elasticity and stress relaxation

Values for the stress relaxation and elasticity of casein–alginate fibres produced under various conditions are shown in Table 1. Each value is the mean of at least five replicates.

Table 1. Variation of stress relaxation and elasticity of casein–alginate fibres with alterations in process variables

Variable	Stress relaxation (%)	Elasticity (%)
Fibre wind-up rate (cm/min)		
670	62.4 ± 3.9	87.9 ± 9.4
925	59.8 ± 3.3	88.0 ± 10.0
1155	61.4 ± 4.9	91.8 ± 7.9
1430	60.9 ± 10.0	–
Length of immersed fibre (cm)		
10	57.8 ± 4.6	91.8 ± 2.9
15	58.5 ± 6.9	92.8 ± 5.7
35	61.2 ± 14.7	89.3 ± 4.9
55	63.6 ± 13.4	94.5 ± 6.2
Precipitation bath sodium chloride concentration (% w/v)		
10	66.9 ± 8.9	91.8 ± 8.8
15	65.2 ± 2.7	89.4 ± 4.4
20	65.4 ± 2.4	93.3 ± 3.2
Precipitation bath temperature (°C)		
10	55.4 ± 5.6	87.3 ± 4.6
15	54.9 ± 6.8	92.6 ± 15.5
20	57.5 ± 2.4	93.7 ± 16.0
30	56.2 ± 1.8	90.7 ± 15.3

Table 2. Variations in the textural characteristics of casein–alginate fibres with changes in the alginate concentration of the spinning solution

Alginate concentration (% w/v)	Shear force per tow (g)	Breaking strength (g/g)	Stress relaxation (%)	Elasticity (%)
2.5	10.8 ± 3.4	1345 ± 212	57.5 ± 2.4	93.7 ± 16.0
3.5	9.2 ± 1.2	950 ± 108	59.2 ± 8.5	91.3 ± 1.8

As was the case with casein–carrageenan fibres, it can be seen that there are significant statistical differences (at $P = 0.05$) between the extreme values obtained for fibre stress relaxation and elasticity within any given treatment batch. However, given the magnitude of these differences it is not possible to say whether they represent organoleptically detectable changes.

Values obtained previously for stress relaxation of casein–carrageenan fibres lay in the range 51–58%; under comparable test conditions, Stanley *et al.* (1972) reported values of 34 and 49% stress relaxation for cooked meat and rehydrated soy fibres respectively. In this investigation, the range of values obtained for casein–alginate fibres is 58–67%.

Elasticity values displayed in Table 1 range from 88–95%, close to the range previously found for casein–carrageenan fibres (89–94%). While different degrees of fibre extension were used in each case (20 and 10% of gauge length for fibres produced from casein–alginate and casein–carrageenan respectively), previously reported experience with casein–carrageenan fibres indicated that such a difference has no effect on the results obtained. This observation was confirmed for casein–alginate fibres during the present investigation. It therefore appears that there is no significant difference between the elasticity of fibres obtained either from spinning solutions containing casein and carrageenan or casein and alginate. Both types are more elastic than rehydrated soy protein fibre (Stanley *et al.*, 1972).

While the shear force per tow, stress relaxation and elasticity of casein–alginate fibres are independent of the concentration of sodium alginate in the spinning solution, increase in the hydrocolloid concentration is accompanied by a marked decrease in fibre breaking strength (see Table 2). This behaviour differs from that reported for the casein–carrageenan system (Downey & Burgess, 1979). Levels of 4% w/v and 2% w/v of sodium alginate (in conjunction with a casein concentration of 15% w/v) represent the upper and lower viscosity limits of casein–alginate solutions from which it was possible to produce fibres in our hands.

Conclusion

As was the case for casein–carrageenan fibres, it was possible to vary certain of the textural properties of casein–alginate fibres by altering some of the condi-

tions of fibre production. Again, the observed changes in stress relaxation and elasticity may not be organoleptically significant, while the degree of change in fibre breaking strength and shear force is more marked. That fibre elasticity and stress relaxation of both casein–carrageenan and casein–alginate fibres are very similar, contrasts with the large differences in fibre shear force and breaking strength between the two fibre types. This suggests that these two groups of measurements (elasticity and stress relaxation on the one hand and fibre shear force and breaking strength on the other) derive from different aspects of fibre structure. If so, different techniques will be required to alter textural parameters within each group as suggested previously by Downey & Burgess (1979).

Acknowledgments

The authors wish to thank Miss N. Hurley for technical assistance and the Irish Dairy Levy Fund for financial support.

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(Received 14 April 1978)

J. Fd Technol. (1979) **14**, 41–48

The stability, thermal dilatation and solidification properties of partially hydrogenated cottonseed oil emulsion in skim milk

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Summary

The stability of partially hydrogenated cottonseed oil in skim milk emulsion depended on the fat content and on the addition of emulsifier, being stable up to 50% fat. The thermal dilatation and the solid to liquid fat ratio were considerably affected by the fat content and the precooling treatment applied prior to dilatation. The total thermal dilatation, from 0 to 45°C, and the dilatation maxima increased with high fat content. Lower precooling temperature caused higher total dilatation, while longer precooling period led to high solid to liquid fat ratio up to 38°C.

Introduction

The use of partially hydrogenated oils as butter fat substitutes in some dairy products is becoming necessary for both economical and nutritional reasons. The stability of the oil emulsion in milk serum is of great importance for satisfactory processing and for the final properties of any milk product. There are different factors which affect this, such as the concentration of the fat in the emulsion and the emulsification properties of the existing constituents, in addition to the physical and chemical properties of the hydrogenated oil itself. Many problems could also arise through characteristic changes in the oil when using cooling or heating processes in food manufacture. Problems of graininess in margarine and bloom in chocolate are related to polymorphic changes on processing (Gunstone, 1967). Polymorphism can be extensively studied from the heating and cooling curves, as dilatometry provides information about the crystal structure of the various polymorphic modifications. Dilatometry is often used for the determination of the solid liquid ratio in commercial fat (Beresteyn, 1972). Polymorphic transitions, however, may also give rise to

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0022-1163/79/0200-0041 $02.00

volume and energy changes, but these are not always accompanied by corresponding changes in the amount of solid fat.

Dilatometry is not only useful in obtaining fundamental physical-chemical information such as phase changes, but has also become one of the few direct practical ways for characterizing and controlling the consistency of commercial fat mixtures, such as margines and shortenings. Melting dilatation varies throughout the melting range and depends to some extent on polymorphic modifications of the crystals (Walstra & Beresteyn, 1975). The maximum proportion of the fat present in the globules that will crystallize is of importance in determining how deep a supercooling is required to ensure complete crystallization or the minimum storage temperature at which crystallization is virtually absent. Accordingly, this study was an attempt to specify characteristics of partially hydrogenated cottonseed oil-in-skim milk emulsion.

Materials and methods

A partially hydrogenated cottonseed oil of 32°C slip melting point and iodine value of 80 was used to form the emulsion in reconstituted skim milk of 8.5% milk-solids-not-fat. Emulsions containing 35, 50, 55 and 60% fat were prepared and 0.5% of glyceryl mono-stearate was used as emulsifier. The oil was emulsified in the milk serum by using a blender for 2 min at 65°C.

To evaluate the stability of the emulsion, the amount of the surface fat or 'cream' layer at 4°C after 24 hr, and of the separated oily surface layer at 60°C, were determined. Graduated 'creammometer' tubes were used to measure the volume of the cream layer at 4°C. Then the tubes were warmed to 60°C to measure the volume of the separated oily surface layer.

To study the change in fat solidification at different temperatures from 0 to 45°C, dilatometric technique was used as described by Mulder & Klomp (1956) for cream.

Results and discussion

When the emulsions of partially hydrogenated cottonseed oil in reconstituted skim milk were kept at 4°C for 24 hr, complete separation of a clear fat layer occurred in the two emulsions containing 55 and 60% fat. In the case of the two emulsions containing 35 and 50% fat, only creamy layers of 8.5 and 10.0% by volume of both emulsions respectively were obtained. When 0.5% of glyceryl mono-stearate was added to the emulsion, the cream line disappeared, but addition of emulsifier had no effect on fat separation in the two emulsions containing over 50% fat.

Table 1 shows the ability of the four emulsions to form separate oily layers on warming up to 60°C. Emulsions containing over 50% fat were unstable, while emulsions with lower fat content appeared to be relatively stable. Addi-

Table 1. The ability of the emulsions with different oil contents to form a separate oily layer on warming up to 60°C

Samples	Fat content (%)	Oily layer (%)	Oily layer fat content
Without emulsifier			
	35	8.1	0.231
	50	13.7	0.274
	55	53.4	0.971
	60	60.8	1.013
With emulsifier			
	35	0	0
	50	6.8	0.136
	55	46.6	0.847
	60	60.3	1.005

tion of the stearate considerably increased the stability of the emulsions with lower than 50% fat, very slightly increased that with 55% fat, had no effect on that with 60%.

The phenomena of additional (delayed) crystallization, polymorphic transitions and recrystallization, occurring in the fat after it has been rapidly cooled and kept at a constant temperature, was distinguished by means of dilatometric technique (Beresteyn, 1972). The occurrence of different crystal modifications is a well known phenomenon associated with the crystallization of long chain compounds.

The dilatation per 2°C, within the temperature ranges 0 to 45°C, was determined for the bulk hydrogenated cottonseed oil (Fig. 1) and for its emulsions of 35 and 50% fat (Fig. 2). All samples were precooled in dilatometers in three different ways, at 0°C for either 15 min or 3 hr, or at 4°C for 24 hr. No dilatation was noticed in the bulk state up to 20°C with the first two treatments, and up to 22°C with the third. At higher temperatures, a sudden very high melting dilatation with a maximum of 63.2‰ at 24°C, 52.6‰ at 26°C and 98.4‰ at 26°C was respectively obtained with the different three precooling treatments. The dilatation was always accompanied by a corresponding drop in the solid: liquid fat ratio. The corresponding maximum drop in percentage solid fat in the fat was 17, 13 and 28.8% respectively. Then the dilatation sharply dropped to reach a constant value of 15.7‰ at 38°C, 16.6‰ at 38°C and 16.4‰ at 34°C respectively. These dilatations phenomena show the melting characteristics of the bulk hydrogenated oil as influenced by the precooling treatments. The differences in dilatation could be attributed to variations in the forms of solidification, crystallization and polymorphism which existed in the fat. The total dilatation up to 45°C differed in the three treatments, being 371.4, 384.8 and 344.5‰ respectively. Holding at 4°C for 24 hr caused considerably the highest maximum dilatation, but caused the lowest total dilatation.

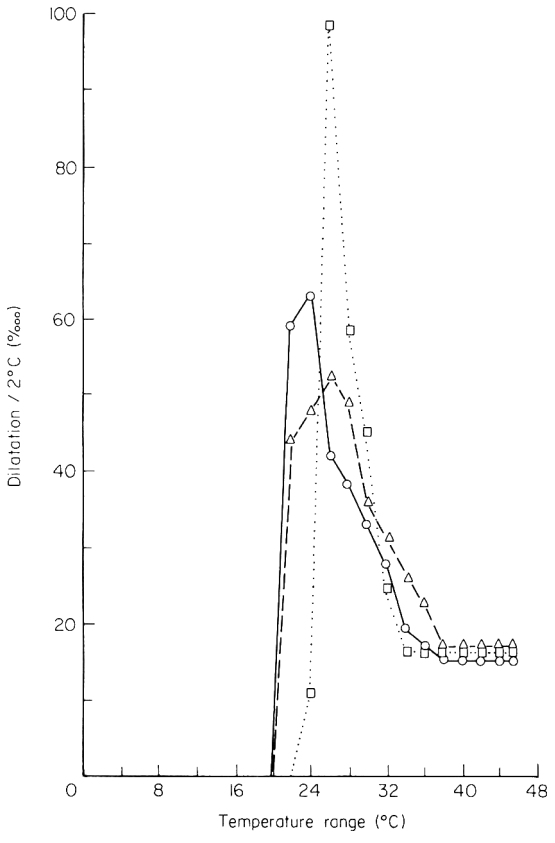


Figure 1. Thermal dilatation of bulk hydrogenated cottonseed oil, held at: 0°C for 15 min, ○——○; 0°C for 3 hr △– – –△; 4°C for 24 hr □·····□.

Figure 2 shows considerable differences in melting dilatation characteristics between the emulsion state and the bulk state, and also between the two emulsions. The maximum dilatation per 2°C was considerably lower in the emulsions than in the bulk, while the emulsion containing 50% fat showed two maxima.

The thermal dilatation per 2°C of the emulsion containing 35% fat, after being kept at 0°C for 15 min, was at its minimum of 5.4‰ up to 6°C, slightly increased at 8°C, remained constant up to 20°C, suddenly reached its maximum of 27.2‰ at 24°C, and then gradually reduced to 10.9‰ at 34°C. It then remained unchanged until fat was completely melted at 45°C.

When kept at 0°C for 3 hr, the dilatation was at its minimum of 5.4‰ up to 4°C, slightly increased up to 10°C, remained constant up to 16°C, reached its maximum of 28.5‰ at 24°C, and then gradually reduced to 11.0‰ at 36°C, to remain unchanged up to 45°C.

When kept at 4°C for 24 hr, the dilatation was at its minimum of 5.4‰ up to 10°C, slightly increased at 12°C, remained constant up to 20°C, suddenly reached its maximum of 31.5‰ at 24°C, and then gradually reduced to

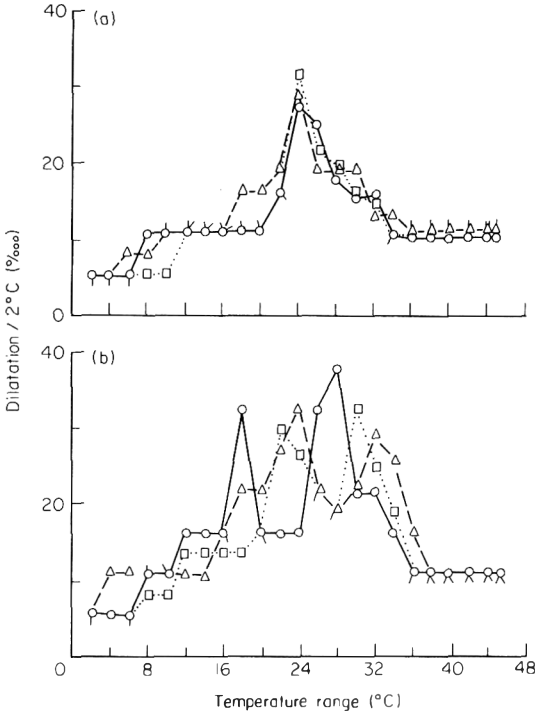


Figure 2. Thermal dilatation of the hydrogenated cottonseed oil emulsion in reconstituted skim milk, held at: 0°C for 15 min, ○——○; 0°C for 3 hr △– – –△; 4°C for 24 hr □.....□. Emulsion contains 35% fat (A); emulsion contains 50% fat (B). ♁, Two different holding treatments; ♁, three different holding treatments.

10.9‰ at 34°C to remain unchanged up to 45°C. The total thermal dilatations were 282.5, 297.1 and 263.5‰ for the three keeping treatments respectively. The corresponding maximum drops in the quantity of solid fat, due to melting dilatation, occurred for all the three treatments at 24°C being 7.8, 8.3 and 10.4% respectively.

The thermal dilatation and solid fat content of the emulsion containing 50% fat were quite different from those of the previous emulsion. The dilatation started at its minimum of 5.5‰ and gradually increased to its first maximum of 32.5‰ at 18°C, 32.9‰ at 24°C and 30.0‰ at 22°C, with the three treatments respectively. In all cases, the dilatation sharply dropped on increasing the temperature, then sharply increased again to reach its second maximum of 37.9‰ at 28°C, 28.5‰ at 32°C and 32.4‰ at 30°C, respectively. The dilatation then dropped to 11.0‰ up to 36°C to remain unchanged till at 45°C. The corresponding maximum drops in solid fat content to these maximum dilatations were 9.1, 8.9 and 8.4% for the first maximum, and, 10.6, 7.5 and 9.2% for the second maximum, due to the three treatments respectively. The total thermal dilatations were 357.7, 378.1 and 327.6‰, respectively.

The dilatation properties and the solidity of fat differed in the bulk fat state and the emulsion state, and were considerably affected by the fat percentage in the emulsion and the precooling treatment applied prior to dilatation. The total thermal dilatation and the dilatation maxima increased with high fat content, being the highest in the bulk fat state. The severe precooling treatment usually caused higher total dilatation.

The dilatometric curve is indicative of the proportion of solid material at any temperature. The proportion of solids is, in turn, a measure of the hardness or consistency of the hydrogenated oil. The melting dilatation varied throughout the melting range. This is said to depend on the polymorphic modification of the fat crystals (Beresteyn, 1972). These polymorphism variations during fat solidification could be widely expected because of the difference in fat content in the emulsion and the difference in precooling treatment. Whether the fat is present in a continuous mass or in a finely dispersed state profoundly affects

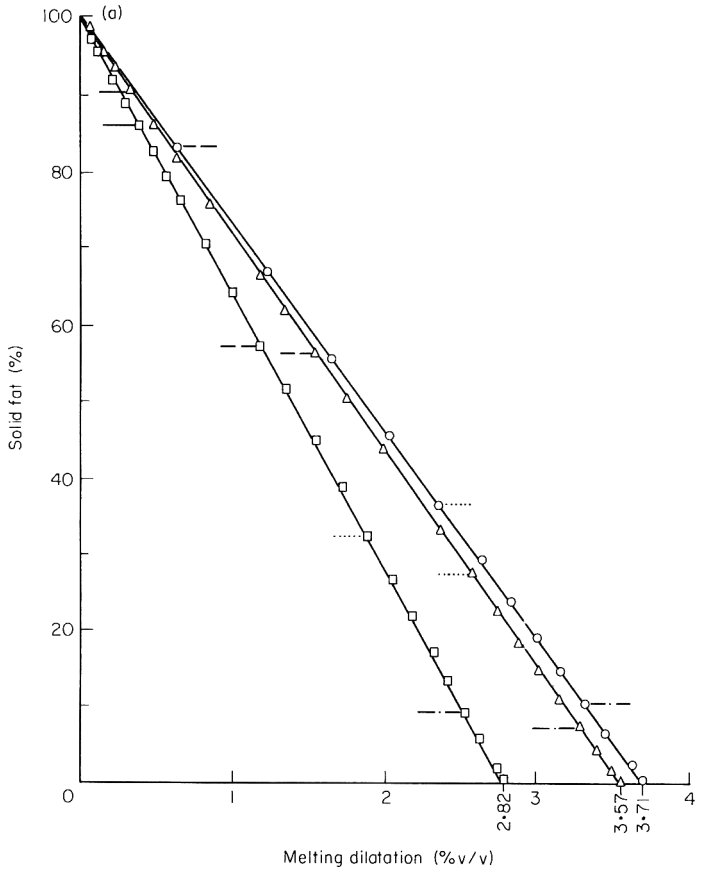
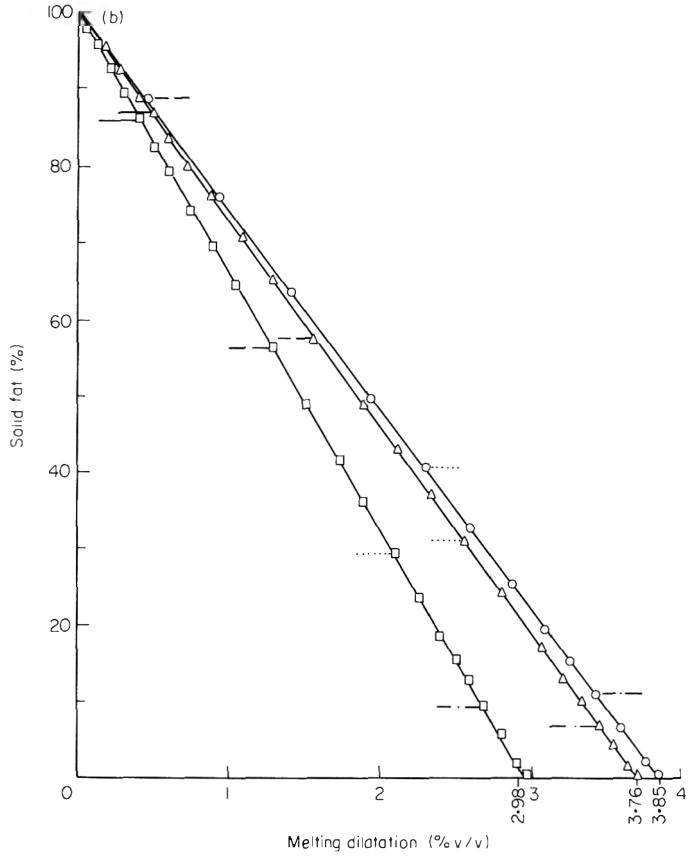
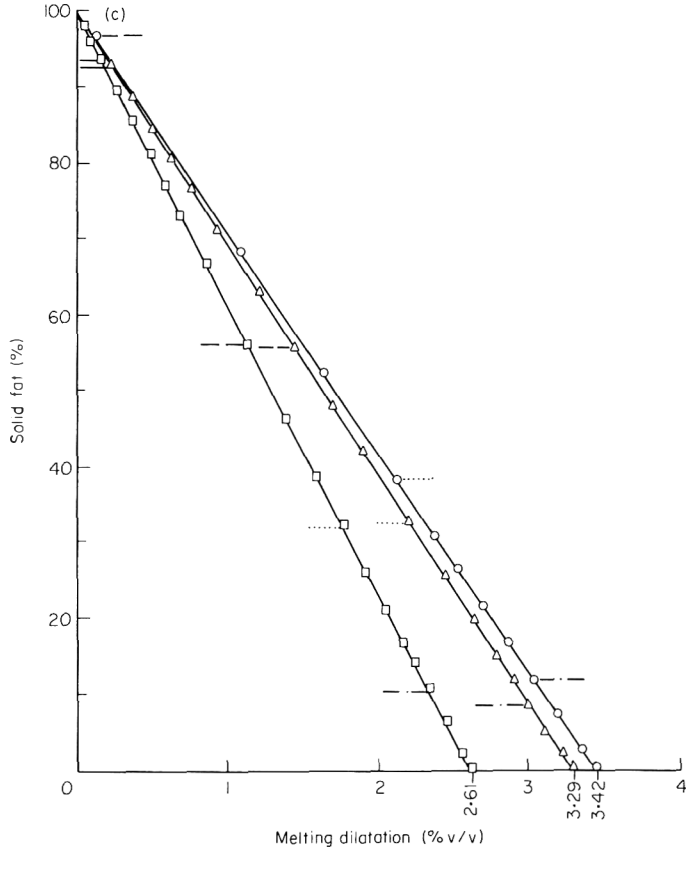


Figure 3. Melting dilatation and fat solidity of hydrogenated cottonseed oil at 0 to 45°C; bulk fat, ○——○; emulsion of 50% fat, △——△; emulsion of 35% fat, □——□; at 10°C, ——; at 22°C, – – –; at 30°C, ...; at 40°C, —·—; complete melting at 45°C; held at 0°C for 15 min (a), held at 0°C for 3 hr (b), held at 4°C for 24 hr (C).


(b)
100
80
60
40
20
0
1
2
3
4
2·98
3·76
3·85
Solid fat (%)
Melting dilatation (% v/v)


(c)
100
80
60
40
20
0
1
2
3
4
2·61
3·29
3·42
Solid fat (%)
Melting dilatation (% v/v)

its crystallization (Beresteyn & Schaap, 1971; Mulder & Walstra, 1974). Deeper supercooling is needed and crystallization proceeds more slowly when the fat is finely divided (Mulder, 1953; Phipps, 1957).

Figure 3a, b and c, shows the melting dilatations expressed as volume changes due to solidification or melting at 2°C intervals, within the temperatures ranged from 0 to 45°C, for the precooling treatments of 0°C for 15 min, 0°C for 3 hr and 4°C for 24 hr, respectively. The subsequent melting dilatation, within the whole temperature range, was considerably low with decreasing fat content in the emulsion. That of bulk fat was always the highest. The magnitude of dilatation depended on the precooling treatment. It is known that temperature history can influence fat nucleation on cooling (Meyering, 1966). The amount of solid fat on warming up was proportional to the melting dilatation. The amount of solid fat was considerably higher in the bulk state than in the dispersed state up to 38°C. The melting rate of both states was the same at 40°C, all the fat being completely melted at 45°C. In the dispersed state, the emulsion of lower fat content was also of lower solid fat content up to 10°C on precooling at 0°C for 15 min or 3 hr. Then up to 45°C, or on precooling at 4°C for 24 hr, the solid fat content was independent of the fat content. It can also be seen that the solid fat content up to 38°C, was higher in both states on precooling at 4°C than at 0°C. The dispersion rate of the fat and the precooling history considerably affected the thermal melting dilatation and the amount of solid fat of the hydrogenated cottonseed oil. According to Walstra & Beresteyn (1975), marked differences were found in the crystallization of milk fat when in bulk and as an emulsion, with various globule sizes, the most striking being the deeper supercooling needed and the lower crystallization rate for a more finely dispersed fat. The authors concluded this was due to differences in nucleation and permits prediction of the crystallization process in emulsified fat.

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(Received 5 May 1978)

J. Fd Technol. (1979) **14**, 49–55

Measurement of the colour of milked tea liquors*

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Summary

A matrix of ninety-five different teas was assigned brightness and colour scores by tea tasters, and the reflectance characteristics of the milked tea liquors were measured on an Agtron colorimeter. Computer analysis of the results, using linear multiple regressions, gave good correlations between the tea tasters' scores and the colorimeter readings.

Introduction

Each day, tea buyers and tea blenders in a large tea company may taste and evaluate up to 1500 different teas and tea blends. No equipment has yet been devised to carry out a task of this magnitude at the speed required, but we have carried out some work with an Agtron colorimeter to measure the colour of milked tea liquors objectively. Such measurements provide (a) an anchor, to prevent drift in the standards and (b) a deeper insight into the meaning of tea tasters' colour descriptions.

Tea tasters in Lyons Tetley evaluate six different characteristics of tea liquors, each on a numerical scale (Theobald, 1977). Two of these characteristics are visual and are known as 'brightness' and 'colour' (i.e. hue), and the other four are taste parameters known as 'thickness', 'softness/coarseness', 'briskness' and 'flavour'.

Brightness in milked tea liquors is judged on a six-point scale varying from 'very dull' (1) to 'very bright' (6), while colour is also judged on a six-point scale varying from weak yellow or greyish colour (1) through golden yellow (2), yellowish-brown (3), brown (4), brownish-red (5) and pink-reddish (6). The characteristics are judged to one decimal place and the maximum

*Presented at a symposium on 'Food Colour & Appearance' organized by The Colour Group at the University of Surrey, 3rd–5th April, 1978.

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0022-1163/79/0200-0049 $02.00

variation within a retail blend is usually of the order of ± 0.3 in brightness and ± 0.2 in colour.

Materials and methods

A matrix of ninety-five blended teas was constructed from four original teas, of different origin, of which two differed markedly in brightness and two in colour. The four teas were carefully selected so that the leaf sizes were compatible and so as to cover a range of brightness and colour, within which the vast majority of teas fall. The scores for these four teas are given in Table 1.

Table 1. Original teas used for construction of a matrix of blended teas

Tea	Brightness score	Colour score
A	1.0	4.0
B	6.7	4.0
C	4.0	1.0
D	4.0	4.6

Tea B was an exceptionally bright tea and was allotted a score of 6.7. Teas A and B were blended in suitable proportions to form a range of bulk teas with brightness varying from 1.5 to 6.5, and C and D were blended to form a range with colour varying from 1.5 to 4.0. Teas in the brightness series were then blended with those in the colour series to form the matrix of ninety-five teas.

The individual teas in the matrix were not judged separately, but were allotted their brightness and colour scores on the principle that the characteristics combine in a linear manner so that, for example, a tea of brightness (2), blended with a tea of brightness (6) produces a tea of brightness (4). This in fact is how the tea tasters' subjective scales are built up and defined.

The Agtron colorimeter, model M-500-A (Fig. 1) is essentially an abridged reflectance spectrophotometer. It is manufactured in America by Magnuson Engineers Inc., and marketed in the U.K. by Henry Simon Ltd. The colorimeter basically measures the percent reflectance of samples in four regions of the visible spectrum, namely blue (λ = 436 nm), green (λ = 546 nm), yellow (λ = 585 nm) and red (λ = 640 nm). The liquid sample is placed in a cylindrical, plastics sample cup, 2½ in diameter, with an optically clear glass bottom (Fig. 2). A spacer ring supports a standard white disc (reflectance 90%) and ensures that a constant depth of liquid is inspected. The sample volume required is 50 mls. The sample cup is illuminated from below by an annular Hg/Ne gas discharge tube, and the reflected colour is measured at right angles to the base of the sample cup by means of a photocell. The major advantage of the instrument is that the scales can be expanded, so that the sensitivity to small differences can be increased. For the measurement of milked tea liquors, the scales were

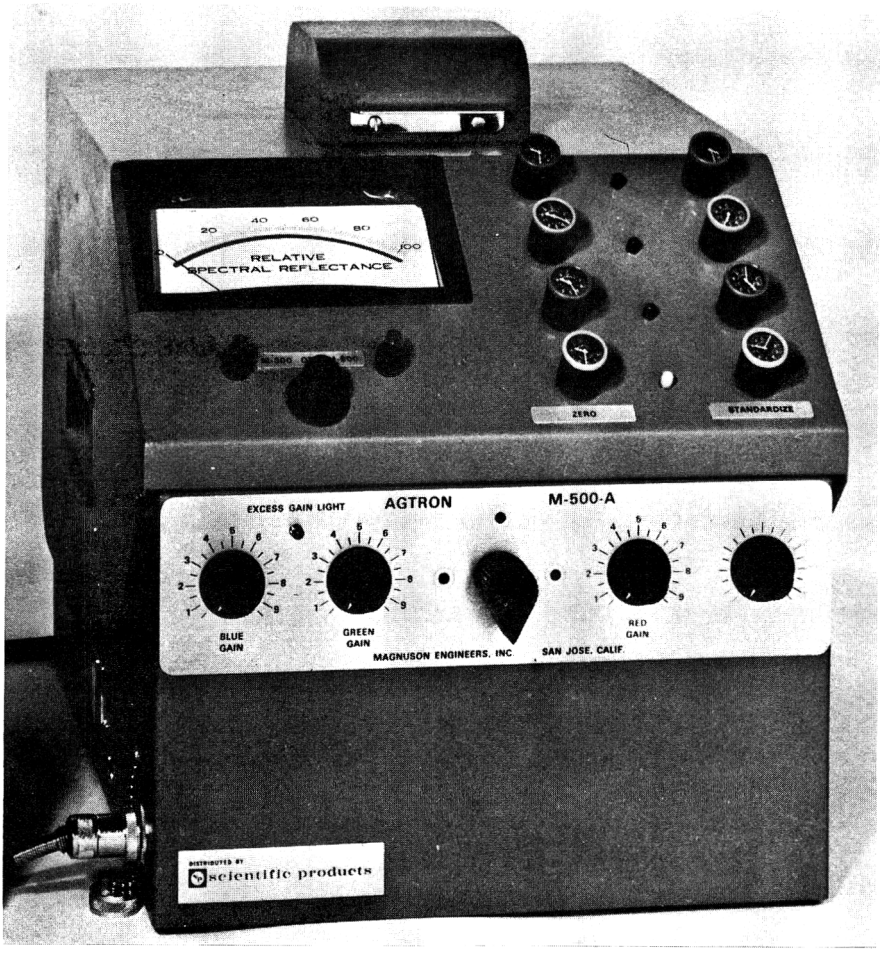

Figure 1. Agtron reflectance spectrophotometer. Model M-500-A.

expanded so that the 0 and 100 readings on the scale corresponded to neutral reflectance discs of 0 and 10 (blue), 7 and 16 (green), 12 and 19 (yellow) and 16 and 33 (red).

In order to study the way in which tea tasters operate, the traditional apparatus was used by us for tea brewing. This consists of a large china brewing mug with partly serrated rim, and provided with a lid, the skirt of which fits loosely inside the mug (Fig. 3). A small off-centre hole is provided in the lid to permit the escape of steam and allow air to enter when the liquor is being poured. Also part of the equipment is a large bowl into which the infusion is poured. In our procedure, $3^{5}/_{8}$ dr. tea (6.42 g) was weighed on an accurate scale balance, and transferred to the mug, which was then filled with freshly boiled tap water (~300 ml). The lid was placed on and the tea allowed to brew for exactly 6 min. The tea liquor was then poured through the serration into the bowl, into which an accurately measured quantity of pasteurized milk (12.5 ml in our case) was first placed. The accuracies achieved in this tea-making proce-

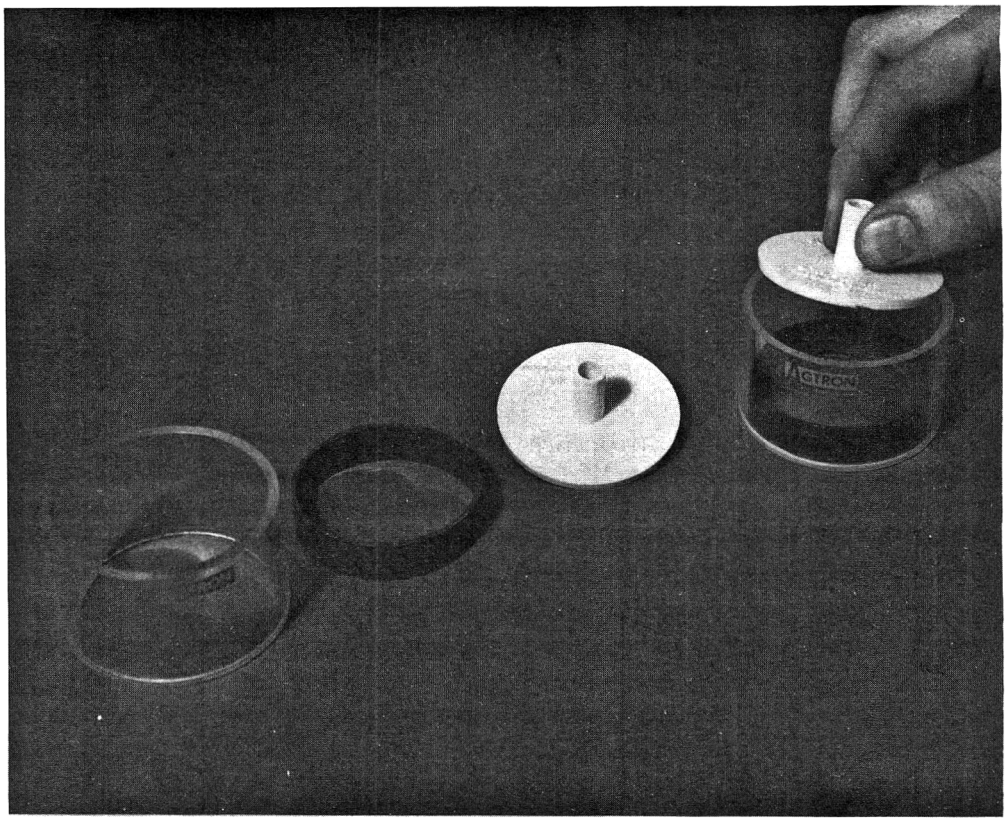

Figure 2. Sample cup, spacer ring and top disc.

dure were: tea weighing ± 0.3%, water dispensing ± 2.0% and milk dispensing ± 1.0%. Milked teas were made up twelve at a time, and the expanded readings in the blue, green, yellow and red regions of the spectrum were measured on the Agtron colorimeter.

Results

Trial graphical plots suggested that linear multiple regressions should be tried between the tea tasters' scores and the colorimeter readings. Regression analyses, therefore, were carried out on a computer, using the results on all ninety-five teas, to investigate the following relationships:

$$\text{(brightness)} = p + q \times \text{(colour)} \quad (1)$$

$$\text{(brightness)} = a + bB + cG + dY + eR \quad (2)$$

$$\text{(brightness)} = a + bB + cG + eR \quad (3)$$

$$\text{(brightness)} = a + dY \quad (4)$$

$$\text{(colour)} = a + bB + cG + dY + eR \quad (5)$$

$$\text{(colour)} = a + bB + cG + eR \quad (6)$$

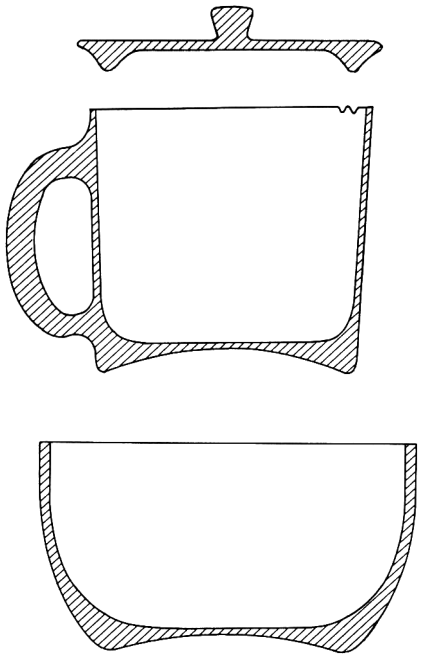

Figure 3. Traditional tea tasters' brewing pot and bowl.

where p, q and a, b, c, d and e are constants, and B, G, Y and R are the colorimeter readings.

The first equation was to test for any interaction between brightness and colour scores, and gave the following result:

$$(\text{brightness}) = 4.894 - 0.360 \times (\text{colour}) \tag{7}$$

with a coefficient of determination (r^2) of only 0.036. The F-ratio was 4.52, which is only barely significant at the 5% level, and should not cause any difficulty. This simply means that small changes in colour can be mistaken for brightness changes, and vice versa – a well-known interaction between the subjective variables of brightness and hue.

The other five equations gave coefficients of determination (r^2) of 0.982 (equation 2), 0.982 (equation 3), 0.916 (equation 4), 0.937 (equation 5) and 0.935 (equation 6).

The best equations for brightness and colour were:

$$\text{brightness} = 3.3735 - 0.14064\,B - 0.025716\,G + 0.11332\,R \tag{8}$$

$$\text{colour} = -1.761 + 0.18888B - 0.14524\,G - 0.03359\,Y + 0.14039R \tag{9}$$

Predicted brightness and colour scores were computed by application of these two equations to the ninety-five sets of instrumental readings. The predicted scores were compared with the brightness and colour scores assigned to the teas on the basis of position in the matrix.

It was found that for brightness:

in 32.6% of the cases, brightness score was predicted correctly, in 42.1% to within ± 0.1, in 17.9% to within ± 0.2 and in 7.4% to within ± 0.3,

while for colour:

in 32.6% of the cases, colour score was predicted correctly, in 37.9% to within ± 0.1, in 21.1% to within ± 0.2, in 6.3% to within ± 0.3 and in 2.1% to within ± 0.4.

Thus in 93% of the cases, brightness score was predicted to $\leqslant 0.2$, and in 92% of the cases, colour score to $\leqslant 0.2$. Subsidiary experiments with a standard plastics disc of 16% reflectance showed that the instrument stability was equivalent to ± 0.2 in terms of brightness score.

Discussion and conclusions

There is very little literature on the colour of *milked* tea liquors. Standard textbooks, such as Harler (1963, 1964) state that tasters judge tea liquors in terms ranging from bright to dull, and in terms ranging from bright red or amber to greyish yellow. The Lyons contribution to this aspect of tea assessment has been to put the judgement of brightness and colour on to a quantitative basis by assigning to teas brightness and colour scores ranging from 1 to 7.

Previous investigations by Smith & White (1965) on the colour of tea infusions have used transmission and reflection spectrophotometry, and also visual colorimetry for quantitative measurements. Clydesdale & Frances (1971) have summarized some of the published work on the colour and turbidity of unmilked tea solutions. All previous colorimeters that we have investigated for tea have been slightly less sensitive to small differences than the eye, but the Agtron colorimeter, with its facility for expanding the scales, has a sensitivity comparable to the eye. Evidence collected during the present work has shown that with such a sensitive colorimeter, small effects, previously hidden, can be picked up. Thus, small but definite colour differences due to day-to-day variation in the milk supplies can just be detected, and it has been found that adding the tea to the milk gives a liquor 0.4 brightness units higher than adding the milk to the tea, confirming a previously reported finding (Smith & White, 1965).

The colour of milked tea liquors depends on a large number of factors such as the type and amount of tea, mineral salts present in the water, time and temperature of the infusion, pH, type and amount of milk, the lighting and the observer. The tea taster aims to keep everything constant except the type of tea, so that true judgements of tea quality can be made. The present investigation has shown that the tea tasters' terms brightness and colour, when quantified, are largely independent of each other and meaningful in physical terms. The term brightness appears to be related to the luminance of the sample, and 'colour' appears to be a judgement of dominant wavelength (i.e. hue). Although

in most cases, the brightness and colour of milked teas provide the tea taster with a clue to quality, this can be misleading, and it is emphasized that brightness and colour are minor quality parameters, the four taste variables – thickness, softness/coarseness, briskness and flavour – being the major ones.

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(Received 11 May 1978)

J. Fd Technol. (1979) **14**, 57–67

The time-temperature tolerance of frozen foods: sensory methods of assessment

R. L. McBRIDE AND K. C. RICHARDSON

Summary

Five commercial frozen foods (fish fingers, beefburgers, green peas, green beans and potato chips) were stored for periods ranging from 4–90 days at temperatures of −8°C and −12°C. Sensory quality was assessed by both an experienced analytical panel and a laboratory consumer panel using a testing format which allowed all samples of a given foodstuff to be evaluated over four consecutive days. Products differed in their susceptibility to deterioration during storage, but the observed deterioration was slight and none of the samples was considered unacceptable. The performances of the two panels were similar indicating that the differences in their sensitivity may be less than is commonly believed.

Introduction

Investigations into the time temperature tolerance (TTT) of frozen foods are concerned with the effect of events which occur in the cold chain between food processing and consumption. The substantial amount of work done in this area has been reviewed by Van Arsdel, Copley & Olsen (1969).

Many workers have sought means of measuring quality changes assumed to occur in frozen foods during storage. Their task has been complicated by variation in quality of raw foodstuffs, and differences in processing treatments. Olson & Dietrich (1971) reported that as much colour could be lost in processing as would be lost in two years at 0°F (−18°C).

As yet, no completely satisfactory objective methods have been devised for quality assessment. Loss of ascorbic acid in stored frozen vegetables and chlorophyll conversion to pheophytin in stored frozen green beans have been used (Olson & Dietrich, 1969). However, they are not sufficiently sensitive to be of much use. Sensory tests have therefore been the primary means of quality

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0022-1163/79/0200-0057 $02.00

assessment. Because 'quality' is elusive, emphasis has been on the development of quantitative rather than descriptive, sensory techniques. Partial success has been achieved by adapting, for use in TTT work, the concept of 'just noticeable difference' (JND) used in classical psychophysics (Van Arsdel *et al.*, 1969).

The determination of the JND (sometimes referred to as the 'first perceptible difference') involves the use of a sensory difference test, e.g the triangle test. Trained panelists are required to detect in one or more selected attributes, such as colour or flavour, a difference between a 'control' sample and a 'test' sample. In TTT studies the control sample, after processing, is stored under conditions (e.g. −30°C or colder) which permit negligible change over the time period involved. The test sample is identical to the control in every way except for the temperature at which it is stored. By using sensory methods it is possible to determine quantitatively if a given storage regime has changed the test sample sufficiently for it to be reliably detected as different from the control, i.e. if the JND has been reached.

The term 'high quality life' is also used in TTT work. 'High quality life' of a product is defined as the time for which it can be stored at a given temperature before the JND is reached (Guadagni, 1969).

These concepts have limited value. They are not reliably related to the practical storage life of a frozen food i.e. the period for which it is acceptable to consumers. In fact the 'practical storage life' will usually exceed the 'high quality life' by a substantial amount (Gutschmidt, 1974). Thus the paradoxical situation arises where the JND is measurable, but of little practical use, whereas the 'practical storage life' which is more difficult to quantify would be of great practical benefit to the frozen food industry. As Olson & Dietrich (1971) observed, the 'first perceptible difference' is a very small quality change detectable by a trained panel under controlled conditions, whereas the consumer evaluates frozen foods when they are presented at meal times under conditions far from ideal for evaluation. Due to the many diversions during a meal most quality assessment is incidental and the only comparison possible is against a remembered level of quality.

Most investigators appreciate that discrepancies exist between a small trained panel and a consumer population, but little has been done to resolve them. Dietrich *et al.* (1959) attempted to bridge the gap by using small panels of food industry personnel ('industry panels') to judge consumer acceptability. However direct assessment of consumer acceptability is not well documented in the literature.

The present experiment, which was both product and panel oriented was designed to:

(i) determine quantitatively, by sensory techniques, the quality changes in commercial packs of selected frozen foods, produced by storage for different periods (4–90 days) at temperatures (−12°C and −8°C) warmer than recommended (International Institute of Refrigeration, 1972).

(ii) investigate the relationship between JND and consumer acceptability by comparing the performance, under controlled conditions, of small

analytical panels with the performance, under conditions designed to stimulate ordinary domestic consumption, of large consumer type panels.

Materials and methods

Materials

The frozen foods selected for inclusion in the experiment were: fish fingers (cod in breadcrumbs and batter), beefburgers (minced beef patties), potato chips (french fries), green peas and green beans. The fish fingers were produced in Great Britain and stored at an air temperature of about −30°C before shipment, at −18°C, to Australia. The other foods came from one major Australian processor. Each was premium grade from the same production batch, and had been stored at temperatures colder than −18°C before arriving at the CSIRO Food Research Laboratory. The fish fingers and beefburgers were packaged in standard retail waxed paper cartons; the peas, beans and chips in standard white pigmented polyethylene bags (55 μm film for peas and beans, 65 μm for chips).

Storage regimes

Two surveys of frozen food distribution in Australia (Middlehurst, Richardson & Edwards, 1972; Sharp & Irving, 1976) revealed that during delivery to retail outlets, product temperatures can rise from the recommended −18°C to −12°C and occasionally as high as −8°C. These findings were instrumental in −12°C being proposed in draft legislation in Australia as the maximum product temperature permissible during transport of frozen foods. Therefore temperatures of −12°C and −8°C were selected for experimental investigation since they would simulate conditions encountered commercially. The surveys also provided information on the range of storage times which occurred in the retail distribution segment of the cold chain and this led to selection of experimental storage periods of 4, 16, 32, 48 and 90 days. The two storage temperatures × five storage periods gave a total of ten experimental treatments.

Sensory evaluation – general

At the laboratory, the frozen food samples were stored initially at −30°C. In conventional shelf-life testing, samples are assessed at appropriate intervals over the entire range of storage times. However, in sensory testing, simultaneous evaluation of all storage treatments is preferable. This was achieved in this study by transferring samples from −30°C to either −12 or −8°C at such times as would provide concurrent completion of all storage treatments. All testing

was carried out in the sensory evaluation laboratory which has been described previously (Christie, 1966).

Testing for JND

The JND determinations were conducted as described earlier in this paper. Twelve assessors were selected. None had any special knowledge of frozen foods, but all were experienced in analytical sensory testing and were skilled in the detection of small differences. Each of the five products was subjected to sensory difference testing by the triangle test, one product each week for 5 consecutive weeks. Within each of the 5 weeks all ten of the experimental treatments were tested in random order against a control – one treatment per session for a total of ten sessions. The method was sensitive in that assessors were able to use all sensory dimensions (i.e. appearance, flavour, texture) in their difference judgements.

Laboratory consumer assessment

Normal 'industrial type' feeding was simulated by serving a hot lunch in the laboratory canteen. Although assessments of food quality were required, panelists were instructed to be no more attentive or critical of the food than would be customary in such a situation. Conversation with neighbours was permitted provided that the food was not discussed and assessments were made independently.

Fish fingers, green beans and beefburgers were assessed by the panel in three series of tests which were held on separate occasions but were of identical experimental design. Each series consisted of four lunchtime sessions (replicates) over consecutive days. Forty laboratory employees attended each session, but the panel composition was not necessarily the same each day. The only qualification for inclusion in the panel was a willingness to participate. All meals consisted of three components. In the first series these were fish fingers, green peas and potato chips. In the second and third series they were beefburgers, green beans and potato chips. Unbeknown to the panelists only one meal component was varied experimentally within each series (viz. fish fingers in the first, green beans in the second and beefburgers in the third).

Within each series, the ten experimental treatments of the variable component were served in random order at each session. Therefore at any one session there were four replicates of each experimental treatment. Although the two unvaried components of each meal were simply 'blanks' necessary to complete the serving, assessors were required to evaluate all three constituents so that the experimental component was not given undue attention. Panelists rated each constituent for colour, flavour and texture on standard nine point hedonic scales (9 = like extremely, 1 = dislike extremely).

Sample preparation

Green peas and green beans were prepared by immersing the still frozen samples in an equal mass of boiling brine, (2% w/v) returning solutions to the boil, then simmering for 5 and 6 min respectively. Frozen potato chips were deep fried in commercial vegetable oil for 3 min at 180°C. Unthawed fish fingers and beefburgers were heated in convection ovens for 10 min at 180°C, and this obviated the possibility of other influences, e.g. frying oil. All products were cooked in batches so that panelists always received hot, freshly prepared samples.

Statistical analysis

The significance of the JND triangle testing was determined by consulting appropriate statistical tables (ASTM, 1968). Analysis of consumer responses was more complex. In each series of consumer tests the experimental product was evaluated with two other components (blanks). Analyses of covariance on the scores given these blanks allowed the ratings of the experimentally manipulated third component to be adjusted for panelists' scoring idiosyncracies. After adjustment for covariates, scores for each experimental treatment were subjected to separate analyses of variance for colour, flavour and texture. In these analyses, variance due to storage effects was partitioned three ways; a linear component, a quadratic component and residual error. Data were analysed using the GENSTAT statistical package on a CDC Cyber 76 computer.

Results and discussion

Table 1 shows those samples which the analytical panel detected as being significantly different from their controls (i.e. JND had been reached).

Table 1. Determination of JND. The number of correct detections made by the trained panel, N = 12. Treatments with asterisks are significantly different from their respective controls (***$P < 0.001$; **$P < 0.01$; *$P < 0.05$)

Storage time (days)	Fish fingers Storage temp. (°C)		Beefburgers Storage temp. (°C)		Green beans Storage temp. (°C)		Green peas Storage temp. (°C)		Potato chips Storage temp. (°C)	
	−8	−12	−8	−12	−8	−12	−8	−12	−8	−12
4	10***	5	7	3	1	8*	7	6	3	5
16	5	7	4	3	4	2	7	8*	7	0
32	6	4	4	7	10***	4	7	1	7	3
48	9**	5	7	5	10***	4	3	2	4	3
90	8*	5	10***	4	12***	8*	4	4	7	3

Table 2. Adjusted mean hedonic scores assigned by the consumer panel (a), for the five storage periods regardless of storage temperatures and (b), for both storage temperatures regardless of storage time

		Fish fingers			Beefburgers			Green beans		
		colour	flavour	texture	colour	flavour	texture	colour	flavour	texture
(a)	Storage (days)	NS	**	**	*	*	*	NS	NS	NS
	4	7.6	7.4a	7.5a	6.9a	7.3a	7.2a	6.8	6.6	6.9
	16	7.5	6.8abc	7.1ab	6.8ab	6.8ab	6.8ab	7.2	6.4	6.6
	32	7.7	7.1ab	7.2ab	7.1a	7.0ab	6.8ab	7.4	7.1	6.7
	48	7.4	6.5bc	6.2c	6.6ab	6.9ab	6.7ab	7.0	6.4	6.5
	90	7.3	6.4c	6.6bc	6.3b	6.5b	6.4b	6.8	6.7	6.5
	Mean	7.5	6.8	6.9	6.8	6.9	6.8	7.0	6.6	6.7
	lsd†		0.7	0.7	0.6	0.6	0.6			
(b)	Temp. (°C)	NS	NS	NS	NS	NS	NS	NS	NS	NS
	−8	7.4	6.9	6.9	6.7	7.0	6.8	7.1	6.5	6.6
	−12	7.6	6.8	6.9	6.8	6.8	6.8	7.0	6.7	6.7
	Mean	7.5	6.8	6.9	6.8	6.9	6.8	7.0	6.6	6.7

** Treatments significantly different beyond the 1% level ($P < 0.01$).

* Treatments significantly different beyond the 5% level ($P < 0.05$).

NS No significant difference.

† lsd least significant difference.

a, b, c Where there are significant treatment effects within columns, scores without a letter in common are significantly different ($P < 0.05$).

Table 2 gives adjusted mean hedonic scores assigned by the consumer panels for all storage times regardless of storage temperature. The significant differences indicated are due to significant linear trends. Texture scores for fish fingers provided the only significant ($P < 0.05$) curvelinear effect. Table 2 also contains adjusted means for both storage temperatures irrespective of storage time. Overall there was no significant difference between products stored at $-12°C$ and $-8°C$. None of the storage time × storage temperature interactions was statistically significant. Effects of the interrelations of storage times and temperatures on colour, flavour and texture are shown in Figs 1–3.

Table 1 shows that frozen foods vary in their susceptibility to storage deterioration. For example, green beans were perceived as different from the control after 32 days at $-8°C$ whereas green peas stored at the same temperatures were not assessed as different even after 90 days storage. However, despite the variation in product stability, there is a preponderance of asterisks toward the bottom of Table 1 indicating a distinct storage effect. Confirmation is provided by Table 2 and Figs 1–3 which show a general decrease in hedonic scores with increasing storage time.

Three anomalies are apparent in Table 1. Fish fingers and green beans stored for only 4 days and green peas stored for 16 days were all detected as different from their respective controls. Because members of the analytical panel were carefully selected for their sensitivity and reliability, and because the consumer panel evaluation showed these samples not be anomalous, it is more likely that these differences indicate sample variability rather than inconsistencies in panel performance. It is suggested that in future work further precautions be taken to ensure that within sample variability is minimized.

An unexpected outcome of the experiment was the similarity in the performances of the analytical panel and the consumer panels. The experimental

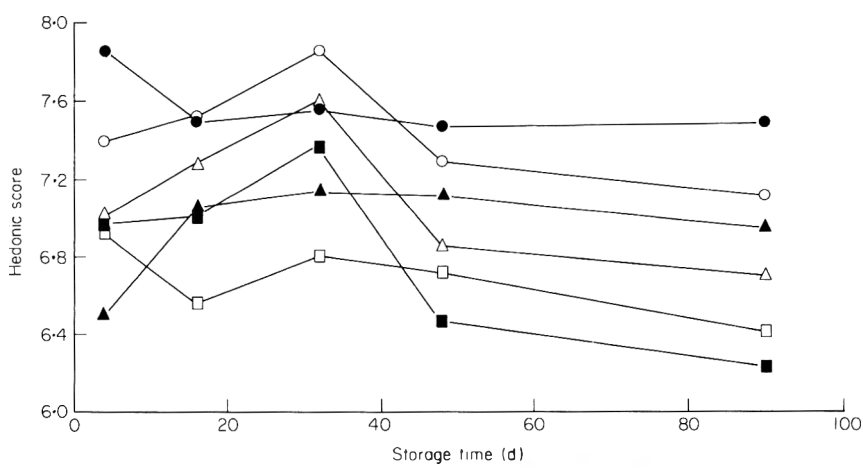

Figure 1. Adjusted mean hedonic scores for colour of fish fingers, beefburgers, and green beans stored from 4–90 days at $-8°C$ and $-12°C$. ●, Fish fingers at $-12°C$; ○, fish fingers at $-8°C$; ▲, green beans at $-12°C$; △, green beans at $-8°C$; ■, beefburgers at $-12°C$; □, beefburgers at $-8°C$.

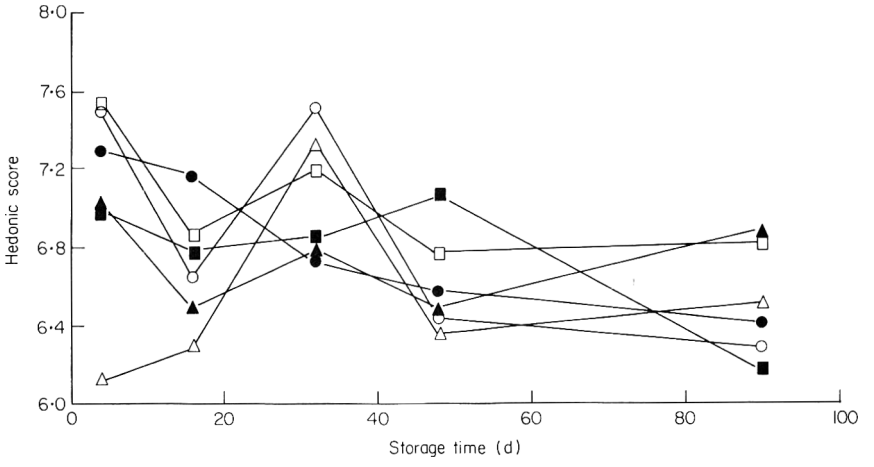

Figure 2. Adjusted mean hedonic scores for flavour of fish fingers, beefburgers and green beans stored from 4–90 days at −8°C and −12°C. ●, Fish fingers at −12°C; ○, fish fingers at −8°C; ▲, green beans at −12°C; △, green beans at −8°C; ■, beefburgers at −12°C; □, beefburgers at −8°C.

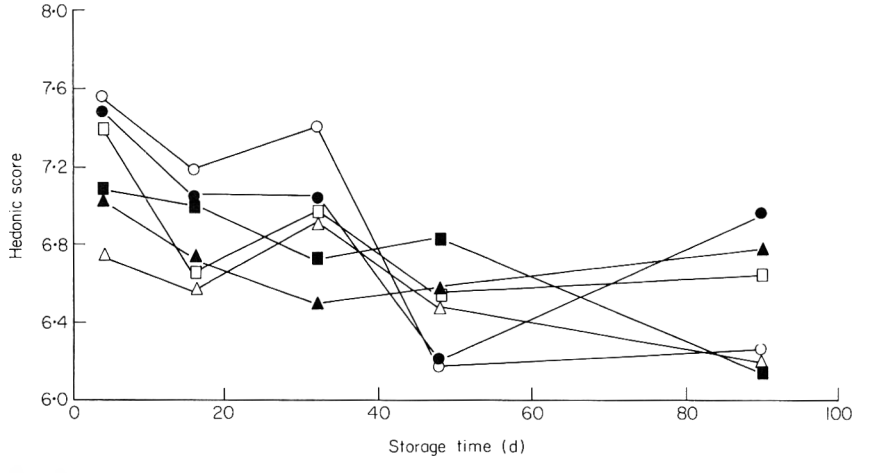

Figure 3. Adjusted mean hedonic scores for texture of fish fingers, beefburgers and green bean stored from 4–90 days at −8°C and −12°C. ●, Fish fingers at −12°C; ○, fish fingers at −8°C; ▲, green beans at −12°C; △, green beans at −8°C; ■, beefburgers at −12°C; □, beefburgers at −8°C.

design used for the consumer assessment was, in all respects, less conducive to the detection of small differences. For instance, the consumers could not make direct quality comparisons at each session. Notwithstanding, statistical analysis revealed that consumers did discern small quality changes (Table 2).

Fish fingers

Samples stored at −8°C for 48 and 90 days were both perceived by the trained panel as having reached the JND. Consumers also gave lower flavour

and texture scores to products stored for 48 and 90 days. Both the 48 and 90 days storage treatments were significantly different ($P < 0.01$) from the 4 days storage treatment. If it is assumed that neglible deterioration had occurred in samples stored for 4 days, they could be regarded as 'controls' the agreement between the results from the analytical panel and consumer panel is then obvious.

Most of the previous work on TTT of frozen fish has been done on fish fillets, not processed products such as fish fingers. However, storage times recommended by Lane (1964) for frozen cod fillets of 105–120 days at 10°F (−12°C approximately) and 45–60 days at 15°F (−9°C approximately) are similar to the present findings.

Beefburgers

The beefburgers evaluation also showed good agreement between trained and consumer panels. Only samples stored for 90 days at −8°C were detected as different by the trained panel. The 90 days treatments were also the only ones which consumers scored significantly ($P < 0.05$) lower than the 4 days storage 'controls'. Jul (1969) reported similar findings for the JND determination of ground hamburger meat: 80 days at −12°C and 50 days at −8°C.

Green beans

Olson & Dietrich (1969) observed that green beans have relatively low stability under commercial conditions of handling. They estimated storage time to the JND to be 94 days at 10°F (−12°C) and 30 days at 20°F (−70°C). These findings compare well with those of 90 days at −12°C and 32 days at −8°C (Table 1).

The consumer panel did not detect any significant differences between samples. This may have been due to large intra-sample variability which made experimentally induced changes appear insignificant. Alternatively, it may reflect lack of panel sensitivity, but this is unlikely in view of performance on the previous two products. The most likely explanation is in terms of the perceptual strategy employed by panelists. Although consumers were advised to give equal attention to all three meal components during assessment, in reality the item considered to be the 'main component' (i.e. fish fingers or beefburgers) was probably given greater attention at the expense of the others (beans or peas and potato chips). This attitude could account for differences in the quality of green beans going unnoticed.

Green peas

The results show that frozen green peas are more stable than frozen green beans. The analytical panel picked only the samples stored for 16 days at

−12°C as being significantly different from the control and as already discussed, this finding is spurious.

Potato chips

None of the experimental treatments effected changes which could be detected by the trained panel. This result is in accord with that of Feustel & Kueneman (1967) who found frozen potatoes to be more stable than most other frozen vegetables.

Conclusion

The findings indicate that the temperature of −18°C is not justified as a legal requirement for the retail distribution of frozen foods and support Ware (1973) who commented: 'existing legislation in some countries sets a product temperature so cold as to be neither technically necessary nor economically feasible'. While it remains necessary to store frozen foods at a sufficiently cold temperature to prevent bacteriological growth and to minimize enzymic and chemical activity (−12°C; Mitchener & Elliot, 1969), there does not appear to be a case for short term temperature fluctuations to constitute an offence in law.

The present experiment could not establish any relationship between the JND and 'practical storage life' because the limit of 'practical storage life' was not reached. Regardless of product type and irrespective of whether they were for colour, flavour or texture, mean scores given the most adverse storage treatment were still in the 'like slightly' to 'like moderately' region of the hedonic scale (Table 2). The 'practical storage life' is, and always will be, an arbitrary judgement, just as all acceptability measures are arbitrary and subjective. Similarly, precise specification of the JND may be at best only of academic interest. This is supported by Olson & Dietrich (1971) who reported that the amount of chlorophyll converted to pheophytin during blanching of various commercial lots of green beans varied between 6 and 24%, and that colour lost in processing is indistinguishable from that lost during storage. They state also that during domestic cooking of green beans, colour loss can be two to four times that lost in 3 months at −12°C.

This study opens to question the traditional belief that laboratory consumer panels are inevitably less sensitive than experienced sensory panels. The consumers' apparent lack of sensitivity may simply reflect the inadequacy of the conventional means of measuring their response.

Acknowledgments

We wish to thank Margot Ferris for her assistance with the statistical analyses, also Christabel Craker and Arthur Kuskis for their help in the experimental work.

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(Received 22 May 1978)

J. Fd Technol. (1979) **14**, 69–74

Effect of processing on ascorbic acid in different varieties of cassava (*Manihot esculenta*, Crantz)

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Summary

The ascorbic acid content of cassava tubers decreased during storage at room temperature. Pressure cooking led to a loss of 20% in ascorbic acid. Steaming resulted in a loss of 50% in ascorbic acid. The loss in the ascorbic acid content in fermented products was about 95%. There was a conversion of a large part of the ascorbic acid to dehydroascorbic acid during processing.

Introduction

Cassava or manioc supplies much of the carbohydrate in the Nigerian diet. It is estimated that it supplies 14% of the total calories. Cassava tuber is rich in vitamin C (Jones, 1959). Loss of vitamin C has been associated with many vegetables on processing. In view of the high level of vitamin C in cassava tuber, this study is undertaken to determine how much of this vitamin is available in the diet after processing.

Materials and methods

Plant materials

Cassava tubers were obtained from the Department of Agricultural Research and Training, Moor Plantation, Ibadan. The local varieties (white and red) were obtained from the Faculty of Agriculture, Research Farm, University of Ife, Nigeria.

Preparation of samples

Cooking studies: cassava roots were cut into cubes of 6 cm in a Hallide automatic slicing/dicing machine and the slices were subjected to the following

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0022-1163/79/0200-0069 $02.00

treatments: boiling, steaming and pressure cooking in small pressure cookers at 15 psi and 121°C.

Fermented samples

The samples were fermented into 'gari' and cassava flour ('lafun') using traditional methods.

The production of gari. The cassava tuber is harvested, peeled and washed. It is then grated and packed into coarsely knit bags. A weight is put on the bag to express some of the juice. It is then heated with constant turning over a heated steel pan. This process has been named 'garifying'. The surface temperature of the oven is about 120°C (Akinrele, 1962). During the process, the grated cassava is dried to about 10% moisture content and the starch is probably partially dextrinized. (Akinerele, 1962). At this stage a little palm oil may be added to give it colour. The final product is gari. When it is soaked in water the grains swell to several times their original size.

The production of cassava flour (lafun). Another product made from the cassava tuber is flour (lafun). This is prepared by soaking whole tubers in water, usually running. They are left for a few days during which the flesh becomes soft. During soaking, the tuber undergoes natural fermentation. It is then peeled and the tubers are broken up into small chips which are dried on hot rocks (an oven) until it becomes dry – with about 13% moisture content. It is then ground into flour and sieved to remove coarse fibre particles.

Storage studies

The fermented samples were stored in polyethylene bags at room temperature or in the refrigerator. Samples were taken for analysis at the beginning, after one month and after two months of storage.

Moisture content was determined by an air oven method (A.O.A.C., 1970).

Measurement of pH. 10 g of cassava tuber were blended with 100 ml distilled water in a Waring blender. The pH of the decanted supernatant was measured using a pH meter.

Determination of ascorbic acid and dehydroascorbic acid. Ascorbic acid was determined by the visual titration method using 2,6, dichloroindol phenol dye. Dehydroascorbic acid was reduced to ascorbic acid with H_2S and the total ascorbic acid was determined. The difference between this level and the level of ascorbic acid before reduction is the measure of dehydroascorbic acid. (A.O.A.C. (1970); Bessey, 1938).

Sampling procedure. Preliminary studies showed that there was variation in the levels of ascorbic acid within the tubers. In general there was a decrease in the level of ascorbic acid from the base to the tip of the root. The level of dehydroascorbic acid increased from the base to the tip. In view of the

variation within the cassava tuber, whole peeled cassava roots were minced and thoroughly mixed together. A sample of 10 g was taken for analysis in duplicate.

50 mg of ascorbic acid was added to the prepared sample. Analyses showed a recovery of 98% of the added ascorbic acid.

Results

Analysis of fresh tubers

Four samples of each variety were analysed. The results are shown in Table 1. The moisture contents of the samples were 60% except 6044 which had a moisture content of 71%. The ascorbic acid contents ranged from 120–150 mg/100 g moisture-free basis (mfb). The dehydroascorbic acid content varied from 56–60 mg/100 g on a moisture-free basis. Analysis of variance showed that there was no significant difference between the varieties. The pH values were similar in all the varieties examined.

Table 1. Levels of ascorbic acid and dehydroascorbic acid in different varieties of cassava

		Local white	Local red	53101	60444
Moisture content (%)		60	59	60	71
pH		6.3	6.4	6.1	6.3
Ascorbic acid	Mean	148	120	120	150
(mg/100 g, mfb)	sd	11	16	16	20
Dehydroascorbic acid	Mean	61	56	57	61
(mg/100 g mfb)	sd	9	4	5	9

mfb = moisture-free basis.
sd = standard deviation.

Storage of cassava tubers

The levels of ascorbic acid (AA) and dehydroascorbic acid (DAA) after 5 days and 8 days of storage are shown in Table 2. At room temperature there was a drastic reduction in the ascorbic acid level to about 25–30% of the original value in 5 days. After 8 days of storage the ascorbic acid content dropped to insignificant levels. There was a greater retention of ascorbic acid in the refrigerated samples although the level of ascorbic acid decreased by about 50% in 5 days and to about 20% of the original value after 8 days. There was a general increase in the level of DAA in the stored samples indicating that some of the AA was converted to DAA.

Table 2. Changes in the levels of ascorbic acid in cassava tubers during storage

		Room temperature		6°C	
Variety	Days	AA (mg/100 g mfb)	DAA (mg/100 g mfb)	AA (mg/100 g mfb)	DAA (mg/100 g mfb)
White local variety	0	147	75	147	75
	5	49	104	108	103
	8	12	122	71	124
Red local variety	0	143	70	143	70
	5	38	99	82	110
	8	2	114	34	138
Variety 53101	0	110	82	110	82
	5	34	87	59	100
	8	10	93	20	111
Variety 60444	0	120	85	120	85
	5	19	105	61	106
	8	–	115	28	113

mfb = moisture-free basis.

Cooked samples

Only the sweet varieties were cooked as they are the only varieties eaten this way. The following methods of cooking were employed:

(i) Normal boiling – some samples were placed in cold water and the temperature was brought to boiling. Other samples were placed directly into boiling water.
(ii) Pressure cooking.
(iii) Steaming.

The results are shown in Table 3. Pressure cooking gave the highest retention of ascorbic acid whereas steaming gave the poorest retention. In each case there is a slight increase over the original level of DAA in the fresh sample. It appears that the longer the cooking period the less the level of ascorbic acid retained but this trend is not evident with DAA.

Fermented samples

The results are shown in Table 4. There is a large loss of ascorbic acid in the fermented samples. Only about 5% of the ascorbic acid is retained, whereas up to 50% of the original DAA was present at the end of fermentation. The red local variety showed significant higher retention of DAA. There was no loss of ascorbic acid in the fermented samples after 2 months of storage.

Table 3. The effect of cooking on levels of ascorbic and dehydroascorbic acids

Variety	Cooking method	Cooking time (min)	Retention percentage		Original level in fresh samples	
			AA	DAA	AA	DAA
60444	Placed in cold (28°C) water and brought up to boiling point	50	56	108	107	93
Original levels AA 107 mg/100 mg DAA 91/100	Placed directly into boiling water	25	78	116		
	Pressure cooked (15 psi, 121°C)	15	82	86		
	Steamed	95	45	105		
Local red	Placed in cold (28°C) water and brought up to boiling point	40	72	121	103	72
Original levels AA 103 mg/100 mg DAA 71/100 mg	Placed directly into boiling water	22	80	119		
	Pressure cooked (15 psi, 121°C)	14	86	108		
	Steamed	98	49	111		

mfb = moisture-free basis.

Table 4. Retention of ascorbic and dehydroascorbic acids in fermented cassava products

Variety	Initial levels (mg/100 g–mfb)		Gari			Lafun		
	AA	DAA	pH	AA retention (%)	DAA retention (%)	pH	AA retention (%)	DAA retention (%)
60444	165	96	4.0	5.1	42	6.0	4.2	41
53101	122	67	4.3	3.8	54	5.8	4.9	57
Local red	156	67	4.1	6.0	70	5.9	4.9	66
Local white	150	68	4.2	5.2	57	6.2	4.1	62

mfb = moisture-free basis.

Discussion

The results showed that cooking, especially pressure cooking, leads to a high retention of ascorbic acid and dehydroascorbic acid. For maximum retention of ascorbic acid cassava tubers should be processed immediately after harvesting, as the ascorbic acid decreases rapidly during storage. A large percentage of ascorbic acid is lost during the production of gari or lafun. However, adequate levels of total vitamin C may be available in gari and lafun because a large amount of dehydroascorbic acid was retained. Dehydroascorbic acid retains about 70% of vitamin C activity (Mills, 1949). An adult eating about 100 g of gari (which is not unusual) meets at least half of his vitamin requirement by so doing.

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(Received 22 May 1978)

J. Fd Technol. (1979) **14**, 75–80

The extraction of haem pigments from fresh meat

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Summary

Different extracting media were compared for the estimation of total pigment concentrations in meat. Acidified 80% acetone and 0.04 M phosphate buffer pH 6.8 extracted the maximum amount of pigment. Water and buffers of low pH resulted in non-extraction of up to 45% of the pigments.

Introduction

The colour of fresh meat is due largely to the concentration and chemical state of the haem pigments, myoglobin and haemoglobin. Various techniques have been described to measure the concentration of these pigments in muscle using extraction into solution and spectrophotometric measurement of one of several derivatives. Hornsey (1956) employed acidified 80% acetone to convert both pigments to acid haematin, while other workers have used water or various buffers. The buffers range in pH from 4.5 to 7.0, one of the principal factors determining choice appearing to be ease of clarification of the final extract. Water was used by Poel (1949), Ginger, Wilson & Schweigert (1954), Wierbicki *et al.* (1955), Fleming, Blumer & Craig (1960) and Rickansrud & Henrickson (1967) despite the finding of Whipple (1926) that this did not extract myoglobin from muscle completely. Watson (1935) confirmed this and found that while alkaline phosphate buffers would fully extract the pigment, the solutions were difficult to clear, but this could be circumvented by using a slightly acid buffer – 0.067 M phosphate at about pH 6.5. This was subsequently used by Lawrie (1950) and a similar buffer, 0.04 M phosphate pH 6.6, by Reynafarje (1963). Bendall (1975) employed 0.1 M phosphate pH 7.0 and Akeson, Biorck & Simon (1968) used 0.02 M phosphate pH 7.2. Considerably lower pH buffers have been used however, Bowen & Eads (1949) using 0.025 M phosphate at pH 5.9 and De Duve (1948) 0.01 N acetate pH 4.5. The principal advantage of this latter buffer is that the extracts are always clear, as is the case with water

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0022-1163/79/0200-0075 $02.00

extracts of *post-rigor* muscle which has achieved a pH of less than 6.3 (Rickansrud & Henrickson, 1967). Because of this, acetate at pH 4.5 has been used more recently by Fleming *et al.* (1960) and Romans, Tuma & Tucker (1965); however, a serious disadvantage is De Duve's (1948) finding that 5–30% of the total pigment remains unextracted in the muscle residue.

De Duve (1948), Fleming *et al.* (1960) and Akeson *et al.* (1968) used exhaustive extraction of the soluble pigments by washing the residue from the first extraction with further buffer. Poel (1949) used a single extraction, assuming total solubilization of the pigments and their equal partition between supernatant and solution trapped in the residue. This was confirmed for dog and cat muscle by Reynafarje (1963). Whilst the ionic strength of the buffers used by different authors has varied, this has little effect below a value of 0.2 (Szent-Gyorgi, 1969).

It has been shown that 0.04 M phosphate pH 6.8 gives complete recovery of haemoglobin (Warriss, 1976) and myoglobin (Warriss, in press) added to meat extracts. In the work reported here the ability of this buffer to extract pigments from meat has been compared with some of those described by different authors, including the buffer used by Helander (1961) for the complete extraction of sarcoplasmic proteins from muscle (0.025 M phosphate pH 7.4). The validity of using a single extraction procedure has also been demonstrated.

Materials and methods

Partition of pigments between supernatant and residue in a single extraction

Samples (5 g) of *post-rigor* pig and sheep *M. longissimus dorsi* (ld) which had been minced through a 4.5 mm plate were extracted with 25 ml ice-cold 0.04 M phosphate buffer pH 6.8. They were homogenized for 20 sec using a Silverson Laboratory homogenizer with microtubular head (Silverson Machines Ltd., Chesham). After standing for 1 hr at 4°C, the homogenates were centrifuged at 6500 *g* for 10 min, and the residues re-extracted twice. After adding a few micrograms of potassium ferricyanide and sodium cyanide to convert the pigments to the cyanmet forms the supernatants were centrifuged at 30 000 *g* and 15°C for 1 hr and the absorbances read at 540 nm. Total pigment concentrations were calculated by multiplying the absorbances by 1.45, a factor derived from the extinction coefficient of cyanmet myoglobin given by Drabkin (1950) and a dilution factor based on the water content of meat taken as 75% wet weight. From the volume and pigment concentration of the supernatants, the measured amounts of pigment in the residues could be compared with the calculated amounts and the efficacy of a single extraction assessed.

Comparison of the extraction efficiency of various buffers

Pig and ox ld and sheep *M. semitendinosus* were obtained *post-rigor*, minced twice through a 4.5 mm plate and mixed. Samples of 2 g were frozen

for not more than 14 days at −20°C until analysed. They were homogenized with 20 ml ice-cold extracting solution as described above but using only one extraction. The extracting media used were water, 0.01 N acetate pH 4.5, 0.025 M phosphate at pH 5.9 and 7.4 and 0.04 M phosphate at pH 6.6, 6.8 and 8.0. All extractions were carried out in duplicate and the extract pH was recorded. The extracts were clarified and the pigments converted to the cyanmet forms and quantified as described above. pH did not affect the extinction coefficient of the cyanmet pigments between 4.9 and 8.0. Protein in the clarified extracts was determined by the microbiuret method (Bailey, 1967). In a separate experiment, Hornsey's (1956) method was compared with extraction using 0.04 M phosphate buffer pH 6.8 for eleven muscles from a single sheep carcass, pigment concentrations being calculated according to De Vore & Solberg (1974).

Results

Validity of single extraction

The results of the experiment with pig and sheep ld are shown in Table 1. These support the hypothesis that pigment is partitioned equally between the supernatant and the liquid phase of the residue. Thus a single extraction under the stated conditions is sufficient to extract all soluble pigment.

Comparison of different buffers

The pig, sheep and ox muscle contained respectively 1.86, 5.03 and 5.03 mg pigments per g wet weight determined using 0.04 M phosphate pH 6.8. Phosphate buffers with pH above and including 6.8 for pig muscle and 6.6 for sheep and ox muscle all extracted maximum pigment, however pig and ox extracts made with phosphate pH 8.0 were impossible to clear by centrifugation (Table 2). The buffering power of the meat caused the pH of extracts to vary from that of the original buffer, thus the necessary pH for full extraction of the pigments was lower than the ostensible value indicated by the buffer. Water extracted less pigment than any buffer, despite the final pH of the extracts being higher

Table 1. The distribution of pigment between supernatant and residue after a single extraction with 0.04 M phosphate buffer pH 6.8 (means ± SD, $n = 4$)

Muscle	Pigment calculated to be in residue (mg)	Pigment found in residue (mg)	% recovery
Pig ld	1.24 ± 0.16	1.16 ± 0.02	94.7 ± 12.2
Sheep ld	4.52 ± 0.30	4.42 ± 0.24	97.9 ± 3.5

Table 2. Final pH of extracts and the amounts of total pigments and protein extracted with various extracting media. Concentrations are expressed as percentages of values for 0.04M phosphate pH 6.8 (each value is the mean of duplicate samples)

	Pig			Sheep			Ox		
	pH of extract	Total pigment	Protein	pH of extract	Total pigment	Protein	pH of extract	Total pigment	Protein
Water	5.60	54.8	40.2	5.60	69.6	64.7	5.78	82.3	66.7
0.01 N acetate pH 4.5	5.01	75.8	66.8	5.07	74.0	70.4	5.20	93.0	84.4
0.025 M phosphate pH 5.9	5.67	67.7	79.0	5.71	79.3	77.9	5.73	90.7	82.8
0.04 M phosphate pH 6.6	6.31	89.2	94.5	6.39	97.0	102.3	6.30	97.6	97.8
0.04 M phosphate pH 6.8	6.50	100.0	100.0	6.59	100.0	100.0	6.51	100.0	100.0
0.025 M phosphate pH 7.4	6.81	100.0	98.8	6.91	98.4	105.0	6.83	98.9	109.5
0.04 M phosphate pH 8.0	7.17	–*	–*	7.27	101.6	107.5	7.19	–*	–*

* Extract opalescent after centrifugation.

than those using acetate. The total protein extracted by different buffers closely paralleled the amount of pigment extracted (Table 2).

In the comparison of acidified 80% acetone and 0.04 M phosphate buffer pH 6.8 for extraction of the eleven sheep muscles, the mean pigment concentration determined as the cyanmet compounds was 5.22 ± 1.05 mg/g, and as acid haematin 5.60 ± 1.01 mg/g, with high correlation between the two sets of results (r = 0.996).

Discussion

The results show that buffers of near neutral pH and of sufficient ionic strength to give a final extract pH of > 6.4 ensure complete solubilization of myoglobin and haemoglobin with a single extraction; water and buffers of low pH do not. This agrees with the finding of Watson (1935).

We have used 0.04 M phosphate pH 6.8 which is similar to the buffer described by Reynafarje (1963). Reynafarje's method was designed for the determination of myoglobin in freshly-excised (*pre-rigor*) muscle which has a pH near neutral. The pH of normal (*post-rigor*) meat is about 5.5 to 6.0 and it has sufficient buffering capacity to lower the final pH of the extract, hence the desirability of using a slightly more alkaline extracting solution of pH 6.8.

The need to clear extracts made with this buffer by high speed centrifugation could be overcome by use of Hornsey's (1956) acetone-HCl extraction followed by determination of the acid haematin. The overestimate of total pigment because of the inclusion of cytochromes is negligible. The average difference of 7.4% found between total pigments measured as the cyanmet compounds and acid haematin in the comparison between the two methods probably largely represents errors in standardization of the haematin procedure. Standardized against haemoglobin of known concentration, the mean pigment concentration as acid haematin was 5.15 mg/g, comparing very favourably with the 5.22 mg/g determined as cyanmet-myoglobin and haemoglobin.

Acknowledgments

I wish to thank Dr D. B. MacDougall and Mr T. M. Leach for helpful criticism of the manuscript.

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(Received 8 June 1978)

J. Fd Technol. (1979) **14**, 81–87

The accumulation of unacceptably high levels of nitrite in vacuum packed back bacon

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Summary

Backs from two bacon weight pigs, one of high ultimate pH and the other of normal ultimate pH, were slice cured with either nitrate free or nitrate containing brines and then stored in vacuum packs. Microbiological conditions likely to occur in chemically similar bacons produced by the traditional Wiltshire method were produced in half the packs using an inoculum from mature Wiltshire bacon sides. Changes in nitrite concentration were followed in the *M. longissimus dorsi* and in the total slice during storage at 5°C. Although the initial levels of nitrite and nitrate (where added) in the total slice were within currently permitted limits, rapid conversion of nitrate, and with little or no nitrite depletion produced 380 ppm $NaNO_2$ within the first week of storage in the inoculated bacon from the high pH_u pig. The implications of these findings with regard to commercial practice are discussed.

Introduction

Current legislation in the U.K. restricts the concentrations of sodium nitrate and sodium nitrite in bacon to 500 ppm and 200 ppm respectively (Statutory Instruments, 1975) and careful attention at all stages of the curing procedure can ensure that these limits are not exceeded. Under certain circumstances nitrate can become reduced during storage to such an extent that unacceptable levels of nitrite may accumulate. In such a case the curer may be considered to be at fault and an understanding of the factors involved in the production of high levels of nitrite is therefore of interest to both industry and legislation.

Materials and methods

Changes in nitrite concentration were followed in back bacon from pigs of normal and high ultimate pH (pH_u) and with and without nitrate using a slice

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0022-1163/79/0200-0081 $02.00

curing technique (Holmes, 1960). Half the slices were stored without further treatment, and the other half were treated with an inoculum from mature Wiltshire bacon sides. Inoculated bacons were therefore comparable chemically and microbiologically to vacuum packed Wiltshire bacon, whilst those not inoculated resembled rapid cured bacon.

Two grade Al pig carcasses were selected from a bacon factory chill-room on the basis of pH_u as described previously (Taylor, Shaw & Jolley, 1976). The first carcass, designated 'high pH', had a pH_u in the *M. longissimus dorsi* (ld) above 6.7. The second carcass, designated 'normal pH', from a similar position in the kill had a pH_u in the ld of 5.7, near the average value previously ascertained for the factory. Colour measurement by fibre optic probe (MacDougal & Jones, 1975) indicated that the normal pH carcass was not PSE. The middles were removed from both carcasses and taken under refrigeration to the laboratory.

Preparation of microbial inoculum

Fifteen matured Wiltshire bacon sides were each wet and dry swabbed with absorbent cotton wool at eight different 100 cm^2 sites on the rind side. Wet swabs were moistened before use with a sterile solution of 4% (w/v) NaCl + 0.1% (w/v) peptone. After use, all swabs were shaken together in 1.2 litres of 4% (w/v) NaCl + 0.1 (w/v) peptone to obtain a suspension of micro-organisms from the sides. This suspension was concentrated for inoculation by centrifuging at 7000 rev/min for 20 min followed by resuspension in one-tenth of the original volume.

Slicing and curing

The four middles were boned out and streaks discarded. The short backs were then sliced (3 mm thickness). The slicer blade and work surfaces were wiped with alcohol before slicing each fresh back. Slices were kept at +1°C until required for curing.

Groups of ten consecutive slices were taken from each back commencing at the head end and the pH determined on the third and eighth slices from each group using a Radiometer pH M63 Digital pH meter with a surface electrode (Russel pH Ltd). Each group of slices was then cured in either a nitrate containing (NO_3 [+]) or a nitrate free (NO_3 [−]) brine. The cure used was alternated between sides and along each back, i.e. the first ten slices from the left sides of both the high and normal pH carcasses were cured in the NO_3(+) brine whilst the corresponding slices from both right sides were cured in the NO_3(−) brine; the treatments were reversed for the second set of ten slices, and so on. The slice curing procedure followed closely that of Holmes (1960) with brines designed to produce 100–150 ppm $NaNO_2$, 400–450 ppm $NaNO_3$, and 4–5%

NaCl in the bacon lean. Brines were discarded after they had been used for twelve curing cycles (five slices per cycle).

Inoculation and packing procedure

After curing, the five odd-numbered slices from each group were inoculated by spreading 0.15 ml of the bacterial suspension on one surface. The inoculated slices from each back were then stacked before packing so that both surfaces of every slice, apart from the first and last in each back, were contaminated with the inoculum. They were then vacuum packed, five to a pack, in Metathene X pouches (Metal Box Company Ltd., London). The even-numbered slices were packed in the same way but without inoculation. All packs were stored at 5°C until sampled.

Initial samples

A pack of high pH bacon and a corresponding pack of normal pH bacon were taken for chemical analysis immediately after curing with the $NO_3(+)$ brine. The ld was removed from each slice, extracted and analysed as below. Total viable counts (TVC) were assessed on total slices from packs corresponding to those chemically analysed, using methods described previously (Taylor *et al.*, 1976).

Sampling during storage

One pack from the same position in each of the eight treatments (2 pH × 2 cures × 2 bacterial levels) was taken for analysis at each sampling time. pH was determined on the third slice which was vacuum packed again and stored at −18°C until analysed as 'total slice'. The ld was removed from the remaining four slices, coursely chopped for moisture determination and analysis of nitrate and nitrite as described previously (Taylor & Shaw, 1975), using N-l-naphthylethylenediamine dihydrochloride. Chloride was determined on the cleared extract using a Radiometer CMT10 chloride titrator. The same procedure was followed for analysis of total slice.

Results

Initial chemical analysis of the ld and TVC of the total slice are shown in Table 1. The TVC of the inoculated bacons were similar to those on Wiltshire bacon reported by Taylor & Shaw (1975). Slice curing produced a fall of about 0.25 pH units in slices of high pH_u but had little effect on the slices of normal

Table 1. Chemical analysis of *M. longissimus dorsi* and total viable count of whole slice at the beginning of storage

	pH	$NaNO_2$ (ppm)	$NaNO_3$ (ppm)	NaCl (%)	NaCl (on water) (%)	Moisture (%)	Log_{10} total viable count g^{-1} Uninoculated	Inoculated
High pH_u	6.55	185	444	4.83	6.61	73.0	3.6	4.4
Normal pH_u	5.63	178	416	4.97	6.61	75.1	3.4	4.0

pH_u. The initial concentrations of nitrate and nitrite in the total slice were estimated using linear regression analysis of the respective values in the lean and the whole bacons during the first 14 days of storage, and substituting the actual analysed values in the lean at the beginning of storage into the equations so derived. The values obtained were as follows: normal pH bacon–146 ppm $NaNO_2$, 318 ppm $NaNO_3$ (where added); high pH bacon–124 ppm $NaNO_2$, 407 ppm $NaNO_3$ (where added), ($P < 0.001$ in all cases).

Changes in nitrite concentration in the l.d. in the bacons from each of the eight treatments are shown in Fig. 1. During the first 5 days storage little

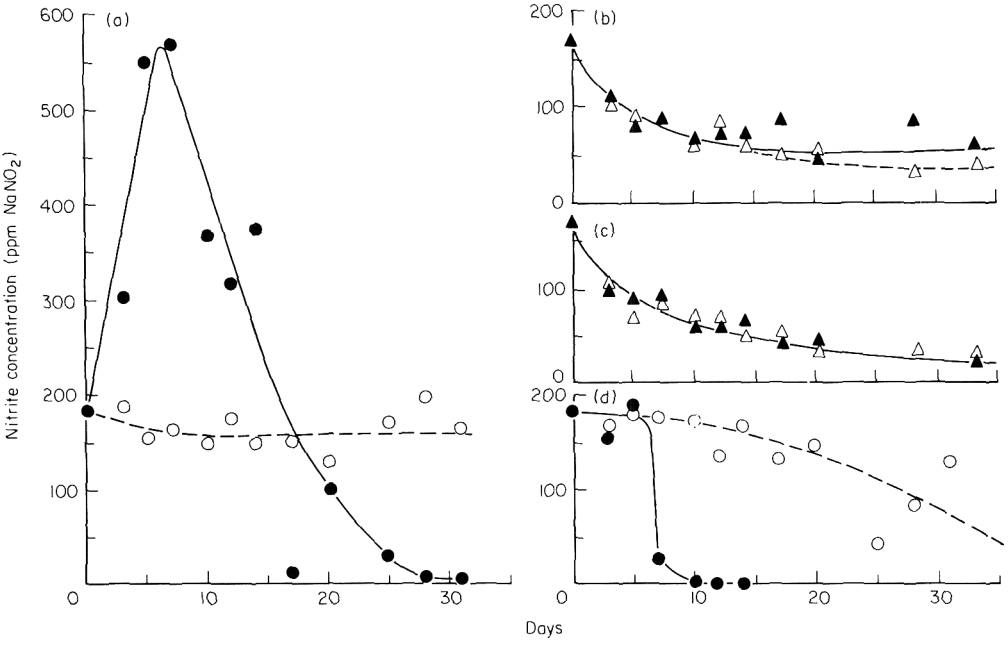

Figure 1. Changes during storage at 5°C in the concentration of nitrite in the *M. longissimus dorsi*. (a), high pH_u, $NO_3(+)$; (b), low pH_u, $NO_3(+)$; (c), low pH_u, $NO_3(-)$; (d), high pH_u, $NO_3(-)$. Circles are from high pH_u bacons, triangles are from low pH_u; filled symbols and solid lines are from inoculated bacons, open symbols and broken lines (if the pattern differs with treatment) are from uninoculated bacons.

nitrite was lost in the l.d. of the high pH bacons (Fig. 1, a and d). In the inoculated high pH_u $NO_3(+)$ bacon this combined with a virtual 100% reduction of nitrate to nitrite during this period to produce a very high level of nitrite (550 ppm or above on days 5 and 7). No nitrate was detected in the ld of this bacon after day 5. Subsequently nitrite was lost rapidly in both the inoculated high pH bacons, but remained relatively constant in the uninoculated bacons until about day 20. At this time, nitrite concentration fell in the absence of nitrate but not in its presence although nitrate analysis showed a distinct loss of nitrate in the $NO_3(+)$ bacon only on day 28.

There was no marked difference in the patterns of nitrite changes in the ld of the four normal pH bacons (Fig. 1, b and c) until the seventeenth day of storage, when nitrate reduction in the inoculated $NO_3(+)$ bacon became apparent. Similar results were obtained from typically processed Wiltshire bacon (Taylor & Shaw, 1975; comparison A), where little difference in nitrite in the lean of back bacon cured with or without nitrate was observed during the first 22 days of storage at 5°.

The pattern of changes in total slice (Fig. 2) was very similar to that in the ld but the level of nitrite was lower and more variable. The maximum level of nitrite analysed in the inoculated $NO_3(+)$ bacon of high pH_u was 380 ppm (day 7).

Discussion

Wiltshire curing involves comparatively lengthy immersion in brine followed by a period of maturation, during which nitrate reducing bacteria can develop and be spread across the rasher on slicing. When this condition was simulated by

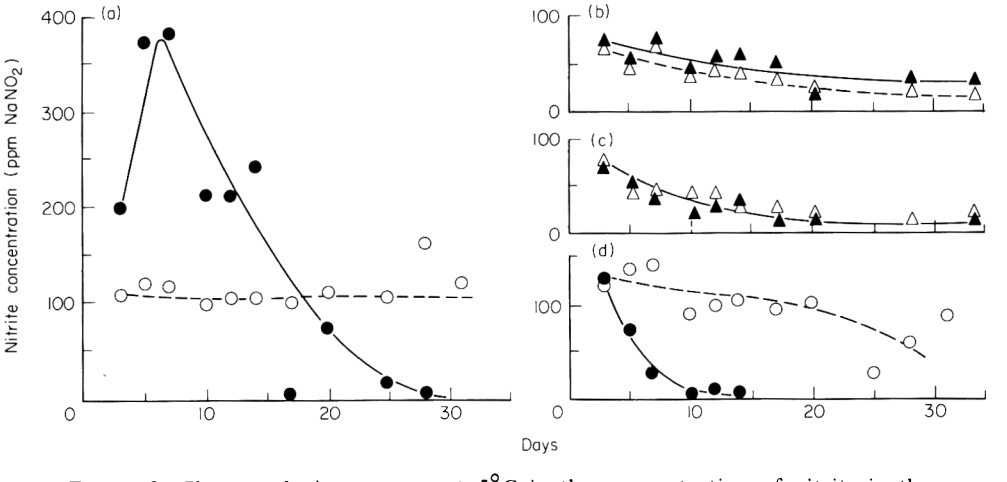

Figure 2. Changes during storage at 5°C in the concentration of nitrite in the total slice. Treatments are distinguished as in Fig. 1.

inoculating slice-cured bacon, nitrite did not accumulate at normal pH. However, in bacon made from high pH_u pigs and with initial nitrite and nitrate satisfying current regulations, high levels of nitrite were produced which would, in a practical case, exceed the permitted maximum. This unsatisfactory level of nitrite occurred well within the expected refrigerated storage life of the bacon. Eddy & Ingram (1962) considered this improbable, but they presumably studied bacon from pigs of normal pH, and the definition of an unacceptable level of nitrite has undoubtedly changed.

The results from the uninoculated bacons show that when bacon is produced rapidly without the opportunity for growth of nitrate-reducing bacteria, as in slice curing, increased levels of nitrite during vacuum packed storage are unlikely. This is true whatever the pH of the bacon, although the effects of sugar, polyphosphates, and other additives are uncertain.

The results in this paper suggest that, in commercial practice, it is only in bacon cured by the Wiltshire (or a closely related) process that an unsatisfactory nitrite concentration is likely to develop. A similar, though less pronounced pattern of nitrite accumulation was observed in the ld and collar lean of typically cured Wiltshire bacon made from pigs with pH_u 6.1–6.3 (Taylor *et al.*, 1976), and by taking these earlier results with those of the present study it can be assumed that Wiltshire cured bacon lean with a pH of 6.50 and above will show increased nitrite production with the possibility of illegal levels accumulating during vacuum packed storage. The fact that less than 5% of pig sides used for bacon manufacture in the British Isles have a pH_u above 6.50 (Kempster & Cuthbertson, 1975; Gallwey & Tarrant, 1977) indicates that the likelihood of this situation occurring is also less than 5%, but the risk will be higher in factories with mean pH_u above the normal, and in cuts where the lean to fat ratio is greater and muscles of generally higher pH_u predominate. In factories where the mean pH_u suggests a high level of risk, the problem can be avoided by omitting nitrate from curing brines and accepting the poorer storage stability of high pH bacon (Taylor *et al.*, 1976). As an alternative, the ultimate pH of sides going for bacon processing could be measured and those with abnormally high values eliminated from Wiltshire curing. Even though unacceptable levels of nitrite may develop in Wiltshire bacon after it leaves the factory, the onus for preventing this must remain with the curer.

Acknowledgments

The author gratefully acknowledges helpful discussions with Dr B. G. Shaw, who also devised the methods of preparation and application of the inoculum, and also Mr A. A. Taylor. He is further indebted to Hilliers Ltd., and the technical expertise of Miss J. C. Waring, Mr N. F. Down and Mr I. R. Chambers of the Meat Research Institute.

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(Received 5 July 1978)

J. Fd Technol. (1979) **14**, 89–93

Technical note: A technique for predicting moisture transfer in mixtures of packaged dehydrated foods

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Introduction

It is well known that in a combination of dehydrated foods there is a transfer of moisture from items of high water activity (a_w) to those of lower a_w; at equilibrium, all items have the same a_w. Salwin & Slawson (1959) developed a procedure to predict moisture transfer, in combinations of dehydrated foods, from the knowledge of the sorption isotherms of the individual components. In this procedure, portions of the isotherm of components are approximated by straight lines and slopes evaluated in order to calculate the equilibrium water activity. A major limitation of this technique is the assumption of a linear isotherm. On the other hand, the procedure does not allow the prediction of 'equilibrium' a_w when there is a simultaneous moisture exchange with the environment, i.e. when a mixture of packaged dried foods gains moisture due to water transfer through the packaging film.

The purpose of the present work is to illustrate the use of a technique based on the classical B.E.T. isotherm equation, to predict equilibrium conditions after mixing dehydrated foods. The technique is applicable to mixtures of dehydrated foods in the range of a_w, 0.05–0.40, which is the range of application of B.E.T. equation (Labuza, 1968). As a matter of fact, the range of a_w, 0.05–0.40, is the one of more practical interest when concerning mixtures of dehydrated foods, i.e. dried soup or sauce mixtures.

Materials and methods

This technique is based on the application of the B.E.T. isotherm equation and the concept of additivity of the isotherms, that is, that the amount of water sorbed at a given activity is derived by the weight percentage of each component times the amount it would sorb alone (Labuza, 1968).

The mixture of dehydrated foods considered is shown in Table 1.

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0022-1163/79/0200-0089 $02.00

Table 1. Dehydrated foods considered

Component	Amount (dry basis)	Initial moisture content (% d.b.)
Rice	300 g	9.8
Chicken, cooked	200 g	8.3
Peas	80 g	6.1
Laurel	1 g	5.5

The sorption isotherms of rice, cooked chicken and peas were measured by Taylor (1961), while that of laurel was reported by Wolf, Spiess & Jung (1973). The B.E.T. constants, $(X_m)_i$ and $(C)_i$ for each individual component were calculated by Iglesias & Chirife (1976), and are as follows in Table 2.

Table 2. B.E.T. constants for dehydrated foods considered

Component	$(X_m)_i$ (% d.b.)	$(C)_i$
Rice	8.1	30.2
Chicken, cooked	6.9	23.2
Peas	5.0	87.7
Laurel	4.5	15.9

Using the above parameters and the B.E.T. equation, the individual isotherms are reconstructed and shown in Table 3 (numbers between brackets are the experimental values for purposes of comparison):

Table 3. Individual isotherms for foods under consideration

	Moisture content (% d.b.)			
a_w	Rice	Chicken, cooked	Peas	Laurel
0.05	5.2 (5.2)	4.0 (4.1)	4.3 (4.3)	2.2 (2.3)
0.10	6.9 (6.9)	5.5 (5.6)	5.0 (5.2)	3.2 (3.1)
0.15	8.0	6.5	5.5	3.9
0.20	8.9 (9.0)	7.4 (7.3)	6.0 (6.0)	4.5 (4.5)
0.25	9.8	8.2	6.5	5.1
0.30	10.7 (10.7)	9.0 (9.1)	7.0 (7.0)	5.6 (5.7)
0.35	11.7	9.8	7.5	6.2

The additive isotherm for the mixture is now calculated; the amount of water sorbed at any a_w is derived by the weight percentage of each component times the amount it would sorb alone. The isotherm shown in Table 4 is thus obtained.

Table 4. Additive isotherm for mixture of dehydrated foods

$(a_w)_a$	$(X)_a$ (% d.b.)
0.05	4.7
0.10	6.2
0.15	7.2
0.20	7.8
0.25	8.8
0.30	9.6
0.35	10.5

The B.E.T. parameters for this isotherm are now calculated and found to be:

$(X_m)_a = 7.2$

$(C)_a = 29.2$

From knowledge of the mixture composition it follows that the moisture content of the mixture is 8.8% (d.b.). Solving B.E.T. equation for the additive isotherm, the equilibrium water activity is found to be 0.252. The final moisture content of the individual components can be now readily obtained and found to be as shown in Table 5.

Table 5. Moisture content of individual foods

Component	Initial moisture content (% d.b.)	Final moisture content (% d.b.)
Rice	9.8	9.9
Chicken, cooked	8.4	8.2
Peas	6.1	6.5
Laurel	5.5	5.1

Verification

A computer program was developed to check the accuracy of the above predictions.

(a) The computer is fed with the B.E.T. equations for each of the individual components, their dry weights and initial moisture contents.

(b) The machine assumes a given low value of a_w (i.e. 0.05) and calculates the moisture content of each component. The summation of calculated moisture contents should be equal to the moisture content of the mixture. If not, the a_w is increased by a small value and calculations repeated until an a_w value is found which satisfies the moisture balance. With that a_w and each individual isotherm equation, the final moisture content of each component is calculated.

Table 6. Calculated final equilibrium moisture content values compared with those obtained by additive isotherm technique (% d.b.)

Component	Additive isotherm	Computer calculated
Rice	9.9	9.8
Chicken, cooked	8.2	8.2
Peas	6.5	6.5
Laurel	5.1	5.1

The calculated values, compared with those obtained by the additive isotherm technique, are as shown in Table 6.

As it can be seen, there is very good agreement.

The rate of transport of water vapour through a flexible film is given by, (Labuza, Mizrahi & Karel, 1972).

$$dw/d\theta = \frac{PA}{e}(p_e - p) \tag{1}$$

where, w = weight of water transferred across the film, θ = time, P = permeability of the film, e = film thickness, A = area of the film, p_e = vapour pressure of water outside of film, p = vapour pressure of water inside of film. If the sorption isotherm of the mixture, or additive isotherm, is approached by B.E.T. equation, then it follows that the rate of water transport through the flexible film is given by (Iglesias *et al.*, 1977).

$$\left(\frac{1}{p_0}\frac{dp}{(1-p/p_0)^2} + \frac{1}{p_0}\frac{(C_a-1)\,dp}{[1+(C_a-1)\,p/p_0]^2}\right)\frac{(X_m)_a}{p_e-p} = \frac{PA\,d\theta}{em_s}$$

where, m_s = dry weight, $p/p_0 = a_w$, and $(X_m)_a$ and $(C)_a$ are the B.E.T. parameters for the additive isotherm. The above equation can be solved to calculate the 'equilibrium' a_w of the mixture at each time; the 'instantaneous' moisture content of each component can be now readily obtained from its sorption isotherm equation.

Acknowledgments

The authors acknowledge the financial support from the Secretaría de Estado de Ciencia y Tecnología de la República Argentina (Programa Nacional de Investigaciones de Tecnología de Alimentos).

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(Received 8 March 1978)

J. Fd Technol. (1979) **14**, 95–100

Technical note: Utilization of citrus wastes for the production of fungal protein

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Introduction

Large quantities of food materials are discarded as waste because they are not in a form suitable for direct human consumption. The use of moulds and other microorganisms appears to be an efficient method of utilizing the present available food energy from such wastes. This communication reports the results of the utilization of citrus wastes as fungal medium by two species of edible mushrooms.

Materials and methods

The following mushroom cultures were used in this study: *Morchella crassipes* NRRL 2686 was kindly supplied by the Fermentation Laboratory of the Northern Regional Research Laboratory, Peoria, Illinois and *Agaricus bisporus* I.A.R.I.p_5 which was supplied by the Division of Mycology and Plant Pathology, Indian Agriculture Research Institute, New Delhi. Stock cultures were maintained on agar slants of potato dextrose medium and transferred monthly. The inocula were prepared by using the method described by Bent & Morton (1964). One ml of spore suspension was used for each test culture. The citrus waste extracts were prepared according to the method described by Janardhanan, Kaul & Husain (1970). The clear filtrate was used as a medium either alone or after addition of carbon and nitrogen sources. The fungal mycelium was collected and dried according to the method described by Litchfield & Overbeck (1965). Reducing sugar content was determined colorimetrically by the method of Neish (1952) using the alkaline copper reagent of Smogyi (1945) and the arseno-molybdate reagent of Nelson (1944). Nitrogen content was determined by the semi-micro-Kjeldahl method described in A.O.A.C. (1970). pH value was determined using a glass electrode PYE pH meter Model 79.

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0022-1163/79/0200-0095 $02.00

Results and discussion

The results in Table 1, give the approximate composition of citrus peel extracts, which were utilized as media both with and without additives for the growth of two cultures of mushroom. It was observed that the pH values ranged from 4.30 in the case of lemon and sweet lime peel extracts to 4.80 in that of mandarin, the reducing sugar content (g/l) ranged from 5.92 in sweet orange to 7.90 in navel orange and the crude protein content (g/l) ranged from 0.78 in baladi orange to 2.15 in lime. The C/N ratios of these extracts ranged between 7.82 in lime to 23.32 in baladi orange. The ash content (g/l) ranged from 0.85 in navel orange to 1.53 in lime and the total solids (g/l) ranged from 13.18 in sweet lime to 21.00 in navel orange.

The results in Table 2, show the mycelium growth and protein production of *A. bisporus* and *M. crassipes* using citrus peel extracts as unmodified media. The mycelium yield (g/l) of *A. bisporus* ranged from 2.25 in baladi orange to 4.83 in lime extracts, while in case of *M. crassipes* it ranged from 1.93 in sweet orange to 4.30 in lime extracts. The economic coefficient (g mycelium/100 g reducing sugar supplied) of *A. bisporus* varied from 39.34 in blood orange to 72.63 in lime extract and that of *M. crassipes* ranged between 32.60 in sweet orange to 64.66 in lime extracts.

The crude protein content of *A. bisporus* mycelium ranged between 21.25% in navel orange to 25.18% in that of the Baladi orange extracts. In case of *M. crassipes* mycelium it ranged from 27.52% in mandarin to 30.57% in lemon extracts. The efficiency of protein production (g/100 g reducing sugar consumed) varied from 11.28% in navel orange to 16.84% in lime extracts. In case of *M. crassipes* the corresponding value ranged from 13.25% in grape fruit to 18.05 in lime extracts. The protein productivity (g/l/day) of *A. bisporus* ranged

Table 1. Proximate composition of the extracts prepared from citrus wastes

Citrus crop	Initial pH	Reducing sugars (g/l)	Crude protein content (g/l)	C/N ratio	Ash (g/l)	Total solids (g/l)
Grapefruit	4.6	6.02	1.15	13.10	1.10	14.90
Lemon	4.3	6.48	0.88	18.38	0.96	16.50
Lime	4.7	6.65	2.15	7.82	1.53	17.96
Sweet lime	4.3	6.19	1.21	12.76	0.92	13.18
Mandarin	4.8	7.12	0.98	14.96	1.35	16.51
Blood orange	4.5	6.10	1.18	12.91	1.17	18.14
Navel orange	4.6	7.90	1.08	18.26	0.85	21.00
Baladi orange	4.7	7.23	0.78	23.32	1.02	18.55
Sweet orange	4.6	5.92	1.23	12.62	1.24	20.30
Sour orange	4.5	6.81	1.27	13.62	1.50	16.53

Crude protein content = N% x 6.25.
All values are averages of duplicate determinations.

Table 2. Mycelium growth and protein production using unmodified media of citrus wastes

Citrus crop	*Agaricus bisporus*				
	Mycelium yield		Protein production		
	g/l of medium	g/100 g supplied reducing sugar	Crude protein content (%)	Protein production efficiency (g/100 g consumed reducing sugar)	Protein productivity (g/l/day)
Grapefruit	2.73	45.24	23.90	12.50	0.09
Lemon	3.50	54.01	21.86	12.42	0.11
Lime	4.83	72.63	23.27	16.84	0.16
Sweet lime	3.42	55.25	24.25	13.61	0.12
Mandarin	3.75	52.67	24.84	14.31	0.13
Blood orange	2.40	39.34	24.36	12.34	0.08
Navel orange	4.15	52.53	21.25	11.28	0.13
Baladi orange	2.25	40.80	25.18	12.99	0.11
Sweet orange	2.52	42.57	24.12	13.56	0.09
Sour orange	3.70	54.33	23.30	12.84	0.12
	Morchella crassipes				
Grapefruit	2.10	34.88	28.85	13.25	0.09
Lemon	3.05	47.07	30.57	14.53	0.13
Lime	4.30	64.66	27.96	18.05	0.17
Sweet lime	3.22	52.02	28.32	14.92	0.13
Mandarin	3.36	47.19	27.52	13.33	0.13
Blood orange	2.13	34.92	28.19	13.33	0.09
Navel orange	3.34	42.28	30.24	15.30	0.14
Baladi orange	2.52	34.85	29.62	15.00	0.11
Sweet orange	1.93	32.60	29.95	14.15	0.08
Sour orange	3.42	50.27	27.89	14.12	0.14

Cultures were incubated in 500 ml Erlenmeyer flasks containing 100 ml of medium for 7 days at 30°C. Crude protein content = N% x 6.25.

All values are averages of duplicate determinations.

between 0.08 in blood orange to 0.16 in lime extracts, and from 0.08 in sweet orange to 0.17 in lime in the case of *M. crassipes*.

One can presume that the limiting factor for obtaining appreciable fungal growth for each species is the level of reducing sugars, provided that other nutritional elements are present in adequate amounts. Comparison between the results obtained using the two mushroom cultures grown on the same unmodified citrus medium demonstrates the vital importance of the species used. This conclusion is reached by several authors in their studies on the effect of medium type on the mycelium yield (g/l) and protein content of mycelium. Sugihara & Humfield (1954), Block *et al.* (1956), Litchfield, Overbeck &

Davidson (1963), Tremato, Taguchi & Yoshida (1966), Janardhanan, Kaul & Hussain (1970) and Labaneiah, *et al.* (1977a) (1977b), found that the mycelium yield and protein content of fungal mycelia depend invariably on the conditions of the culture. The wide variations in mycelium yields and coefficient of substrate conversion are quite obvious. They also found that protein content may vary slightly in single species.

Data shown in Table 3 indicate that the mycelium yield (g/l) of *A. bisporus* ranged between 10.52 in lime to 13.70 in sour orange extracts, while in case of

Table 3. Mycelium growth and protein production using modified media of citrus wastes

Citrus crop	*Agaricus bisporus*				
	Mycelium yield		Protein production		
	g/l of medium	g/100 g supplied reducing sugar	Crude protein content (%)	Protein production efficiency (g/100 g consumed reducing sugar)	Protein productivity (g/l/day)
Grapefruit	11.36	37.87	24.60	14.16	0.40
Lemon	11.75	39.17	23.66	13.37	0.40
Lime	10.52	35.07	24.33	14.14	0.37
Sweet lime	12.16	40.53	23.95	13.79	0.42
Mandarin	12.24	40.80	24.73	15.38	0.43
Blood orange	11.71	39.03	24.50	14.07	0.41
Navel orange	11.47	38.23	25.20	14.24	0.41
Baladi orange	11.53	38.43	23.97	13.53	0.39
Sweet orange	12.08	40.17	25.46	14.46	0.44
Sour orange	13.70	45.67	24.25	14.31	0.47
	Morchella crassipes				
Grapefruit	8.27	41.35	29.36	15.09	0.35
Lemon	9.44	47.20	30.97	16.04	0.42
Lime	9.96	48.80	30.29	15.33	0.43
Sweet lime	8.65	43.25	31.35	16.13	0.39
Mandarin	8.14	40.70	30.82	15.79	0.36
Blood orange	9.06	45.30	29.96	15.40	0.39
Navel orange	8.36	41.80	30.74	15.86	0.37
Baladi orange	8.46	42.30	31.10	16.34	0.38
Sweet orange	9.15	45.75	39.46	15.16	0.39
Sour orange	10.30	51.50	29.71	16.45	0.44

The C/N ratios of 41.0 : 1 and 27.3 : 1 were obtained in the media of *A. bisporus* and *M. crassipes* respectively in the presence of 30 g and 20 g reducing sugars as glucose and 1.38 g $(NH_4)_2SO_4$. KH_2PO_4 (0.5 g/L) was added. Initial pH value was 6.3. Cultures were incubated in 500 ml Erlenmeyer flasks containing 100 ml of medium for 7 days at 30°C. Crude protein content = N% x 6.25. All values are averages of duplicate determinations.

M. crassipes it varied from 8.14 in mandarin to 10.30 in sour orange. The economic coefficient of *A. bisporus* ranged from 35.07 in lime to 45.67 in sour orange and from 40.70 in mandarin to 51.50 in sour orange in the case of *M. crassipes*. This is in agreement with the conclusion reached by Reusser, Spencer & Sallans (1958) and Litchfield *et al.* (1963). By modifying the fungal medium to provide optimal conditions, the mycelium growth (g/l) was approximately tripled. The results also show that the mycelial growth and the economic coefficient for each fungi did not vary appreciably due to the close affinity between the extracts of the various citrus varieties.

The percentages of crude protein content of *A. bisporus* mycelium ranged from 23.66 in lemon to 25.46 in sweet orange, while that of *M. crassipes* ranged from 29.36 in grape fruit to 31.35 in sweet lime. This is consistent with results obtained by Block *et al.* (1956) on orange juice and citrus press water using *A. blazei*. The efficiency of protein production of *A. bisporus* ranged from 13.37% in lemon to 15.38% in that of mandarin and from 15.09% in grapefruit to 16.45% in sour orange in the case of *M. crassipes*. Comparing the values with those obtained with unmodified citrus media shows the stimulating influence of the modified media on increasing the production efficiency of protein of the mycelium. Block *et al.* (1956) reported that the protein production efficiency differed when two citrus media were used for the growth of *A. blazei*. The protein productivity (g/l/day) of *A. bisporus* varied from 0.37 in lime to 0.47 in sour orange and from 0.35 in grape fruit to 0.44 in sour orange in the case of *M. crassipes*.

Conclusion

The two fungi grew well on the unmodified waste extracts of the peels of ten citrus species with known amount of reducing sugars, nitrogen and ash. The highest values of mycelium yield (g/l) and economic coefficient of both fungi were obtained with lime as compared with the lowest values obtained with baladi orange in the case of *A. bisporus* while with sweet orange in the case of *M. crassipes*. The protein content, protein production efficiency (g/100 g reducing sugar consumed) and protein productivity (g/l/day) of the mycelia also varied with the type of citrus extract used. The modification of citrus extracts which include a change in the C/N ratio increased the mycelium yield approximately three-fold. Consequently, the protein productivity of mycelia increased. However, the protein content and efficiency of protein production were also found to differ according to the type of the modified medium used.

Acknowledgments

The authors would like to thank Professor C. W. Hesseltine, Northern Regional Research Laboratory, Peoria, Illinois and the Division of Mycology and Plant

Pathology, Indian Agricultural Research Institute, New Delhi, which provided the mushroom cultures.

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(Received 22 May 1978)

J. Fd Technol. (1978) **14**, 101–104

Book reviews

Microorganisms in Foods. 1. Their significance and Methods of Enumeration (2nd ed). International Commission on Microbiological Specifications for Foods (ICMSF) of the International Association of Microbiological Societies: Toronto: University of Toronto Press, 1978. Pp xvii + 434. Can$ 30.

This is the second edition of the well-known volume of the same name edited by F. S. Thatcher and D. S. Clarke (1968), now revised under the management of a six-person editorial committee with the assistance of a further sixty-two contributors and consultants.

The new edition is some 200 pages longer than the 1968 version. Of this, approximately 125 pages are accounted for by coverage of entirely new subject areas (anaerobic counts, foodborne parasitic and viral agents, foodborne microbial toxins, yeast and moulds, *Vibrio cholerae,* streptococci other than those designated enterococci, other bacteria of possible or occasional food poisoning significance, microbiological examination methods for enteropathogenic *Escherichia coli,* methodology for staphylococcal enterotoxins, a major section entitled 'Important Considerations for the Analyst' and a report on ICMSF collaborative tests for coliform methods). Additions to sections on enumeration of mesophilic aerobes and coliforms, specifications for media, reagents and ingredients, appendices and references account for 45 more pages and the remaining 30 extra pages result from simple elaboration of existing themes throughout the book.

The extended length of the new edition has been kept down to some extent by some minor reductions (for instance, the salmonella methodology subsection), and by two major deletions – the entire section 'Importance of Detection' and the whole sub-section on selection and sampling procedures. Presumably those were considered to have been adequately dealt with later in volume 2 (1974), but it was felt by this reader that the succinct summary in the former section 'Importance of Detection' of (1), the factors influencing the type and numbers of microorganisms present in a processed food and (2), the problems of interpretation of results of sampling, had a continued place in this volume. Deletions in the earlier discussion of coliforms has left the reasons for recording coliform levels and the manner in which to interpret them even less clear than is already the case.

In the sense of updating the earlier edition, the second edition was a necessary and valuable work although inevitably sections such as those on mechanization and automation and on viral gastroenteritis are already in need of further updating. It was clearly essential to have an edition bringing in as this does, such important new topics in food-borne disease as parasites, viruses, yeasts and

moulds, toxins and mentioning other bacteria now coming under suspicion of causing at least occasional food poisoning.

Much of the updating is well balanced, such as the treatment of enterobacteriaceae (with greatly improved Table 1 relating them to their origin, pathogenicity and the coliform test), staphylococci, *Clostridium perfringens* (although serotyping, which has proved such an effective epidemiological tool in the U.K. is given a disappointingly negative treatment) and *Bacillus cereus* in foods. However, a significant proportion of 'updating', it has to be admitted, consists simply of increased numbers of words too often resulting in loss of what was the pleasing conciseness of the earlier edition.

Editorial discipline must definitely be called into question over the greatly disproportionate coverage given to enteropathogenic *E. coli* and haemolytic streptococci (as separate from enterococci) in view of the paucity of their documented involvement in foodborne disease. The relatively newly understood picture of toxigenic and invasive type *E. coli* has correctly replaced the discussion of implicated serotypes in the earlier edition, but 18 pages of *E. coli* isolation and identification techniques seem hardly justified. Likewise, a rather uncritically implied association of haemolytic streptococci in raw milk with the many types of clinical streptococcal infection (just a single reference is given) does not seem to justify the space given to C substance, M, T and R antigens and eight pages of isolation and identification procedures.

Similar criticism can be levelled at (1), 11 pages on *V. cholerae* complete with mode of toxin action at the adenylate-cyclase/cyclic-AMP level in view of the author's own comment that '*V. cholerae* is seldom recovered from (the) incriminated food'; (2), a seven page table listing presumably all known mycotoxins complete with structural formulae, ecology and mycological species producing them and (3), a seven page coverage of paralytic shellfish poisoning in contrast to a single page each for *Staphylococcus aureus, C. botulinum* and scombroid in the section on 'Food-borne Microbial Toxins'.

As a laboratory manual, the book probably suffers most from 'overkill'. Considering that Part II (Recommended Methods for Microbiological Examination) is introduced with the statement that 'It is assumed that the methods will be used by . . . a competent microbiologist', it would seem that 'Apparatus and Materials' subsections could have been frequently simplified by omission of items such as refrigerated cabinets incubators and waterbaths, pH meters, petri dishes, blenders and mixers, centrifuges, graduated and pasteur pipettes, inoculation needles, test tubes and so on – all of which the competent microbiologist would already know as necessary in his laboratory. Similarly, common and well known methods need not have been spelled out; details were only needed where conditions are peculiar to food microbiology. As an example, we are told how to inoculate and incubate triple-sugar iron agar slants in at least four separate places; there are other similar examples.

While it is easy, of course, to criticize, justice dictates the need for a word of appreciation of the enormous undertaking for already busy people to put together a truly international methodology book, satisfactorily combining the

contributions of so many experts with such varied microbiological experience. This volume is certainly a most valuable part of an extremely important effort to standardize methods and interpretations in food microbiology as related to international public health and food trade. The criticisms made here are not intended to play down the excellent quality of the book but are offered as a plea to aim in the third edition at conciseness and simplicity leaving the general microbiology to standard textbooks and the intricate details to appropriate journals while attending solely to the parameters and conditions which need to be standardized specifically for international food microbiology.

P. C. B. Turnbull

Structure-Activity Relationships in Human Chemoreception. By M. G. J. Beets; London: Applied Science Publishers, 1978. Pp. xii + 408. £25.

Dr Beets is one of the Vice-Presidents of International Flavours and Fragrances (Europe). He is well known for his broad, multidisciplinary interest, of which chemoreception provides an excellent example. From 1972–74 he was Vice-President of the European Chemoreception Research Organisation and President from 1974–76. His interest in the present subject, human chemoreception, is long standing as illustrated by his contribution to a Symposium on 'Molecular Structure and Organoleptic Quality' published in 1957 as the first of the well-known series of Monographs of the Society of Chemical Industry.

So much for the man, now what of the book? This deals with the following topics: 'Theoretical aspects: Scope and definition – information in (a) sensory processes and (b) chemoreception. Experimental aspects: The anatomy of the chemosensory system – informational deficiencies – chirality – structural parameters – indirect relationships and response intensity. Structures and modalities in olfaction: The 'Musk' modality – Other informational modalities. Structures and modalities in gustation: The bitter-sweet modalities – the sour and salty modalities'.

No brief synopsis can do justice to the wealth of detailed information summarized and interpreted in this book. Dr Beets uses the term 'modalities' in a special sense to refer to the principal distinctive qualities within taste and olfaction. Traditionally this term is used in sensory physiology to distinguish between the different classes of sensations, (e.g. sight, hearing, touch, taste and smell) rather than to the separate qualities within any one of these senses. No taste or smell, even that associated with single chemical substances of high purity, is considered to represent a single sensation or sensory quality. Particular emphasis is placed upon tastes and odours as complex patterns specified in terms of profiles in which these separate qualities (modalities in Beets' terminology) of 'element' may vary in intensity or degree. These patterns constitute the 'information' communicated by tastes and odours and which is to be explained. The structure-activity relationships which are sought

and formulated are very much concerned with the particular structures and other properties of these taste and odour stimuli and the taste and odour profiles to which they give rise. There is a close relationship in principle between these facts and phenomena and those of drug action.

Dr Beets draws freely upon the available knowledge and experimental data from many different fields, including individual differences in sensitivity which is observed in its extreme form in 'taste and odour blindness' (ageusia and anosmia). Experimental data on fatigue, cross-adaptation, masking and mixture also provide important information on one side of the structure-activity equation. A limited number of sensory qualities are considered to have a special position in such discussions, including the Sweet, Bitter, Salty and Sour modalities. The first two and the last two of these are grouped together in view of the dominant importance of structural considerations for the former and other properties in the case of the latter. As already indicated attention is also given to those tastes/substances showing taste blindness and an increasingly larger group of odours/substances showing selective anosmia. Knowledge concerning the second group is far from complete although Amoore, who has also been greatly involved in the study of 'steric properties' in relation to odour qualities, has identified over thirty chemicals in this group. These include certain substances with odours in the fishy, 'urinous' and musk quality groups. The musk odour and related chemical structures represents one of the most intensively investigated groups. In the case of taste, Shallenberger's AH-B System is well-known as an attempt to explain differences in sweetness and attention is being turned to the corresponding approach in connection with bitterness. The scope for further investigations relating to both tastes and odours must be practically unlimited, but above all these should be conducted within some clearly defined theoretical framework such as Dr Beets so effectively provides.

The question arises, what is there in this book for readers of this journal? Although not explicitly concerned with the creation of flavours and perfumes, Dr Beets obviously deals with many of the underlying scientific problems. Certainly some disciplines are dealt with more fully than others, but this is inevitable in any single author text. However, with a bibliography of over 500 items, many of them published in the last 5–10 years, the reader also has access to most of the contemporary knowledge and speculation. The work must be described as a *magnum opus*. Although individual experts may argue about points of detail *Structure-Activity Relationships in Human Chemoreception* constitutes the most comprehensive and integrated statement on the subject and will remain so for a number of years to come.

Roland Harper

Books Received

The children of Santa Maria Cauqué. By L. J. Mata
Cambridge, Mass: MIT Press, 1978. Pp. xvii + 395. £14.00.

A detailed study, extending over 9 years, of the nutritional state and the growth and development of forty-five children in a rural settlement in Guatemala.

Food Packaging
Food Technology Review No. 47. By N. D. Pintauro.
New Jersey: Noyes Data Corporation, 1978. Pp. xv + 415 US$39.

An appraisal of the American patent literature relating to food packaging since 1966.

Dairy Products and Eggs
Food Technology Review No. 48. By M. Gutcho.
New Jersey: Noyes Data Corporation, 1978. Pp. xii + 360. US$39.

This review covers the American patent literature since January 1966 under the headings cheese, whey, milk, yoghurt, ice cream, whipped topping, margarine and eggs.

Report of the Government Chemist 1977
London: Her Majesty's Stationery Office, 1978. Pp. 173 £3.00.

Besides general comments on the work of the Laboratory of the Government Chemist the report contains a wealth of interesting accounts of problems of food analysis dealt with during the year.

JOURNAL OF FOOD TECHNOLOGY: NOTICE TO CONTRIBUTORS

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Typescripts (two complete copies) should be sent to the Editor, Dr H. Liebmann, c/o Research and Development Department, Metal Box Ltd, Twyford Abbey Road, London NW10 7XQ. Papers should be typewritten on one side of the paper only, with a 1½ inch margin, and the lines should be double-spaced. In addition to the title of the paper there should be a 'running title' (for page headings) of not more than 45 letters (including spaces). The paper should bear the name of the author(s) and of the laboratory or research institute where the work has been carried out. The full postal address of the principal author should be given as a footnote. (The proofs will be sent to this author and address unless otherwise indicated.) The Editor reserves the right to make literary corrections.

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

References. Only papers closely related to the authors' work should be included; exhaustive lists should be avoided. References should be made by giving the author's surname, with the year of publication in parentheses. When reference is made to a work by three authors all names should be given when cited for the first time, and thereafter only the first name, adding *et al.*, e.g. Smith *et al.* (1958). The '*et al.*' form should always be used for works by four or more authors. If several papers by the same author and from the same year are cited, a, b, c, etc. should be put after the year of publication, e.g. Smith *et al.* (1958a). All references should be brought together at the end of the paper in alphabetical order. References to articles and papers should mention (a) name(s) of the author(s) (b) year of publication in parentheses; (c) title of journal, underlined, abbreviated according to the *World List of Scientific Publications*, 4th edn and supplements; (d) volume number; number of first page of article. References to books and monographs should include (a) name(s) and initials of author(s) or editor(s); year of publication in parentheses; (b) title, underlined; (c) edition; (d) page referred to; (e) publisher; (f) place.

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

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

SI UNITS

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

NON SI UNITS

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

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

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

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Journal of Food Technology

Volume 14 Number 1 February 1979

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Printed by Adlard and Son Ltd, Bartholomew Press, Dorking