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Aqueous extraction of black leaf tea.
III. Experiments with a stirred column*

V. D. LONG

Summary

Stirred batch aqueous extractions of black tea at 5:1 water-to-tea mass ratios showed stirring reduced yield of solubles in extract and that tea solubles may be grouped into three classes: effectively instantaneously soluble, rapidly soluble and slowly soluble, apparently following independent first-order solution processes. Rate equations are given for dissolution of soluble tea solids from leaf at 60, 80 and 100°C and their application to continuous extraction discussed.

Introduction

During the development of extraction processes for instant tea manufacture, work with a column extractor (Long, 1978) suggested that increasing the degree of convective mixing in a column reduced the final extract concentration, and it was predicted that the lowest possible extent of extraction should occur with vigorous stirring. The present experiments were carried out to test this hypothesis, and to investigate for stirred batch extractions the time-dependence of extract concentration, liquid uptake by leaf and yield of solubles in free extract to establish overall rate equations for stirred dissolution.

Materials and methods

The leaf extracted was a large-leaf orthodox tea taken from the batch previously used. The extraction apparatus, a steam-jacketed glass cylinder, about 80 mm internal diameter, with a conical bottom, differed slightly from the former

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model in having an aluminium bed support specially constructed to expedite drainage of extract while reducing the 'dead space' available to seepage, a separately-jacketed detachable conical bottom to facilitate discharge of spent leaf and a slotted lid to allow top-insertion of coaxial stirrers.

The bed support was a 60° frustum, 60 mm diameter at the broad end tapering to 12 mm diameter at the narrow end, having eight equally-spaced, square channels 3 mm wide milled along the side from top to bottom. Nine holes 3 mm diameter were drilled perpendicularly through the base of the frustum, one along the axis and the remaining eight at a radius of 12 mm from the axis, equally spaced to connect with the channels.

Two stirrers were used: for deep beds, a helical stirrer 64 mm overall diameter comprising two aluminium strips 12 mm wide and 530 mm long twisted to produce a double helix of axial length 460 mm and pitch 300 mm attached to a tubular shaft 10 mm o.d. by seven equally-spaced cross-pieces, and for shallow beds, a six-bladed disc turbine of standard proportions (Holland & Chapman, 1966) 50 mm diameter. Both were driven at speeds of 2–3 rev/sec by a ¼ hp series-wound electric motor. Despite the appreciable power of the stirrer motor, the stiffness of the leaf-water mixture restricted extractions to water-to-tea ratios of 5:1. Even so the helical stirrer required manual help to start and could not effectively handle more than 200 g tea. Extractions with the turbine were restricted to 50 g tea.

The extraction technique followed with stirring was similar to that employed with the static column. After raising the temperature of the column by passing steam or water from a constant temperature supply through the outer jacket, a weighed amount of leaf was introduced with the stirrer at rest. Next water at the chosen extraction temperature was added in one stage for unconditioned extraction or two stages for conditioned extraction. The stirrer was started after complete addition of all water and switched off immediately before discharge of extract. The nominal extraction time was taken as the time between completion of the first or only water addition (as appropriate) and the opening of the discharge valve. For estimating rates of solution a corrected extraction time was calculated by adding to the nominal time the average time of addition of water and the average time of drainage of extract. Since the total time of addition was a few seconds only, it was considered sufficiently accurate to take the average as half the total time but because drainage was lengthy, the average drainage time was found by numerical integration of the volume-time record of discharge. Typically, the average drainage time was between 0.5 and 2.5 min, the longer times tending to be associated with longer extraction times, possibly because the time-dependent swelling of leaf gave less voidage under these conditions.

Using the helical stirrer in 200 g batches of tea at 100°C, four runs were made at each of five nominal extraction times: 1, 2, 5, 10 and 20 min. Where the latter was greater than 3 min, half the runs were made with conditioned leaf. This involved two-stage addition of water, in which 40% of the total extraction water was added initially and the remainder added 3 min later. Using the turbine stirrer in 50 g batches of tea, single runs were made with unconditioned leaf at

both 80 and 60°C for eight nominal extraction times: 0.25, 0.5, 1, 2, 5, 10, 15 and 20 min. For comparison and to extend the range of investigation a number of unstirred extractions was also undertaken. These involved: two runs with conditioned leaf and two runs with unconditioned leaf at 100°C for nominal time 10 min at each of four levels of leaf content, 100, 200, 300 and 400 g, two runs with 100 g leaf at 100°C for each of three nominal-plus-addition times of 0.25, 0.5 and 1 min, and controls for all turbine-stirred extractions.

Measurements made were volume of extract at room temperature (13°C) and total solids content of extract determined gravimetrically in duplicate by vacuum evaporation of 10 ml aliquots. Because of comminution of leaf by attrition in turbine-stirred extractions, extracts from these were centrifuged at the extraction temperature before determining total solids content; similar centrifugation of extracts from unstirred extractions was found to make negligible difference to the total solids content.

Results

All results presented relate to a water-to-tea mass ratio of 5:1 with the tea in its as-received state containing 6 mass % of moisture. The specific net liquid uptake reported below is the difference per unit mass of as-received tea extracted between the volume of water added to the tea and the volume of extract separated by gravity drainage.

Average results for extract concentration and specific net liquid uptake for nominal ten-minute extractions at 100°C with various amounts of conditioned and unconditioned leaf with and without stirring are given in Fig. 1 and Table 1 respectively. In the former, concentration is plotted against the mass of tea used in unstirred extraction with the values for stirred extraction of 200 g tea arbitrarily shown for comparison at zero mass. The points relating to unstirred

Table 1. A comparison of specific net liquid uptake under stirred and unstirred conditions at 100°C for a nominal ten-minute extraction

Mass of leaf extracted (g)	Specific net liquid uptake (ml/g)			
	Conditioned leaf		Unconditioned leaf	
	Average	Standard deviation	Average	Standard deviation
200 (S)	2.87	0.08	3.15	0.05
100 (U)	2.51	0.24	2.66	0.18
200 (U)	2.72	0.09	2.58	0.09
300 (U)	2.71	0.04	2.53	0.07
400 (U)	2.75	0.24	2.69	0.19

S = stirred U = unstirred

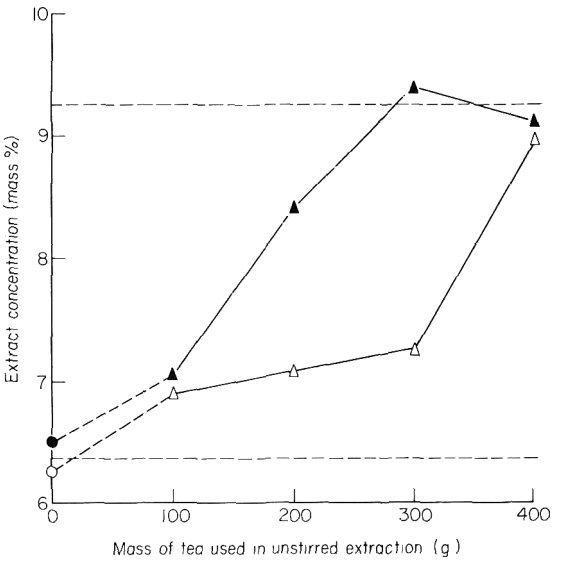

Figure 1. Comparison of extract concentrations obtained in stirred and unstirred extractions at 100°C using a 5:1 water-to-tea mass ratio with a nominal extraction time of 10 min. ●, stirred extraction of 200 g conditioned leaf; ○, stirred extraction of 200 g unconditioned leaf; ▲, unstirred extraction of conditioned leaf; Δ, unstirred extraction of unconditioned leaf.

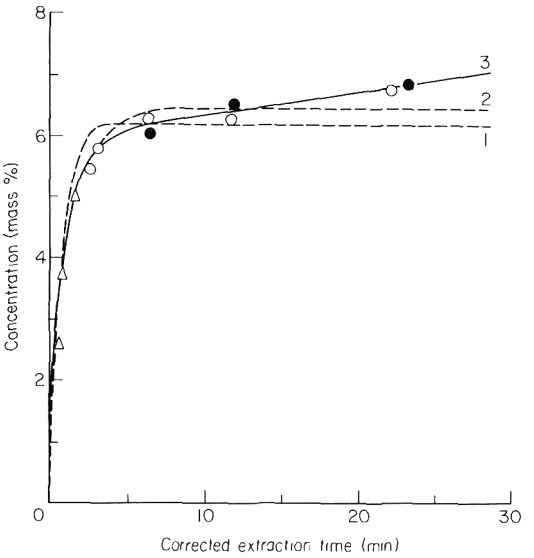

Figure 2. Extract concentration as a function of average contact time at 100°C using a 5:1 water-to-tea mass ratio. Symbols as Fig. 1 but with unstirred extraction relating to 100 g leaf; 1, 2 and 3 relate to the number of soluble components modelled by the curves shown.

extraction have an average standard error of 0.25 mass % and are connected by straight lines merely to illustrate the separate trends for conditioned and unconditioned leaf. The broken horizontal lines represent possible average values for upper and lower limits of performance. The standard error for the stirred extractions was 0.03 mass %.

Values of extract concentrations and specific net liquid uptake for stirred extractions at 100°C averaged for each nominal extraction time and condition of leaf are plotted as a function of corrected extraction time in Figs. 2 and 3. The lines drawn on the concentration graph relate to three mathematical models to be discussed later. Figs. 2 and 3 also contain points relating to unstirred extractions of short duration at 100°C. These had nominal extraction times not exceeding 1 min for which it was impracticable to run the helical stirrer and employed 100 g leaf to approach nearer to stirred conditions (Fig. 1).

Extract concentrations measured for stirred and unstirred extractions at 80 and 60°C are shown as a function of corrected extraction time in Fig. 4 and corresponding results for specific net liquid uptake are shown in Fig. 5. In both figures the results have been averaged for each nominal extraction time and condition of leaf.

Discussion

The extract concentrations in Fig. 1 obtained with 200 and 400 g of leaf agree within statistical error with values reported in part II (Long, 1978) in a comparable column viz: with 200 g leaf, 6.8 and 8.5 mass %, with 400 g leaf, 9.1 and 9.4

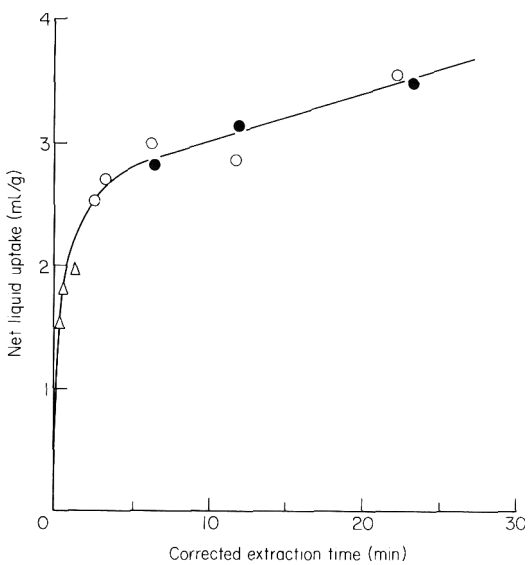

Figure 3. Net liquid uptake of leaf as a function of average contact time at 100°C using a 5:1 water-to-tea mass ratio. Symbols as Fig. 2.

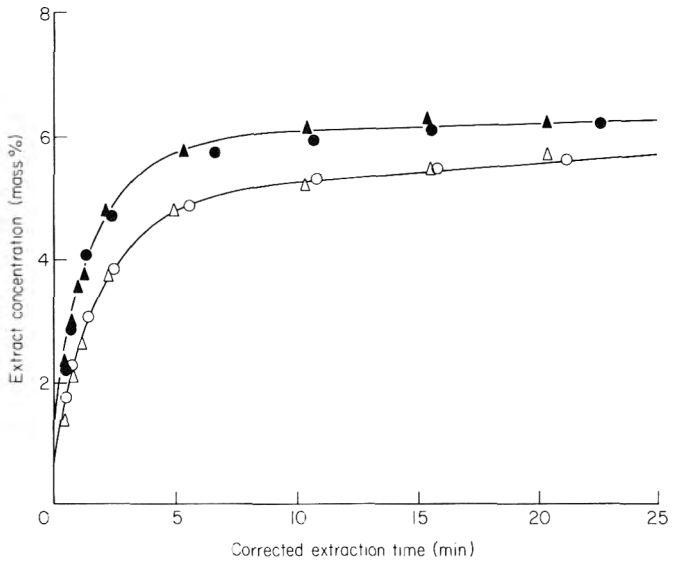

Figure 4. Extract concentration as a function of average contact time at 80°C and 60°C using 5:1 water-to-tea mass ratio. ●, stirred extraction 80°C; ▲, unstirred extraction 80°C; ○, stirred extraction 60°C; Δ, unstirred extraction 60°C.

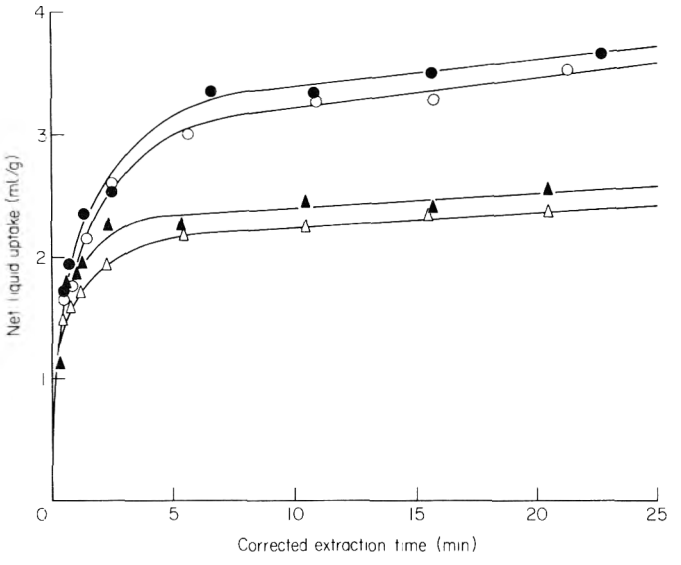

Figure 5. Net liquid uptake of leaf as a function of average contact time at 80°C and 60°C using a 5:1 water-to-tea mass ratio. Symbols as Fig. 4.

mass % for the unconditioned and conditioned states respectively. Taken in conjunction with the latter results Fig. 1 shows:

(i) for comparable extractions conditioned leaf always gave higher average concentrations than unconditioned leaf, although statistically this appears to be fortuitous where the points are close,
(ii) the difference between conditioned and unconditioned leaf was most marked for unstirred extractions using the middle range of leaf mass,
(iii) stirred extraction gave average concentrations lower than any for the unstirred extractions,
(iv) the graph of extract concentration plotted against mass of tea used in unstirred extraction appears to be approximately S-shaped, the curves for conditioned and unconditioned leaf differing in the middle portion, but more or less coinciding at the extremities,
(v) the presence of nearly coincident extremities suggests the existence of upper and lower limits to extractor performance, the lower limit corresponding approximately to stirred extraction.

The above observations agree completely with the starting hypothesis that the greater the degree of mixing in the extractor, the lower the final extract concentration. The effect of conditioning is to reduce bed voidage and convectional mixing of extract in the present apparatus when it is used below full capacity without stirring. It may also be noted from Fig. 1 for future reference that concentrations obtained with 100 g of unstirred tea differ from the stirred values by less than 10%.

Results for net liquid uptake given in Table 1 show the average for stirred extractions was 3.01 ml/g compared with 2.64 ml/g for unstirred extractions. Since the overall standard deviations of both groups were identical at 0.17 ml/g, this difference is statistically highly significant, showing greater absorption of extract during stirred extractions probably because of increased freedom for leaf expansion. There is also a suggestion that conditioned leaf retained more extract than unconditioned leaf. This could be a genuine difference, particularly in unstirred extraction, where reduced voidage arising from internal compression of the bed during conditioning could give poorer drainage, but the effect remains unproven at present. Finally, it may be pointed out that the average net water uptake found for unstirred extractions agreed fairly well with the value 2.70 ml/g reported previously (Long, 1978). It will be obvious from the size of the net liquid uptake that over half the potentially extractable solids was retained by the residue, indicating the need for residue washing stages in any commercial process.

Because of difficulty in getting the helical stirrer to start and the cumbersome nature of the experimental procedure it was not possible to carry out stirred extractions of 200 g tea for times less than 1 min. Results for stirred extractions of 200 g tea at 100°C presented in Figs. 2 and 3 are therefore supplemented by results for unstirred extraction of 100 g unconditioned leaf having extraction times down to 0.26 min. From the comparative work above the results so

obtained should approximate those of stirred extraction fairly well, slightly overestimating extract concentration and slightly underestimating net liquid uptake. With regard to concentration the results for both methods of extraction lay on or about a single continuous curve so for convenience they were treated as a single set and tested for conformity to a multicomponent first-order dissolution law of the form:

$$dc/dt = \sum_{j=1}^{n} k_j (c_{\infty j} - c_j) \quad (1)$$

where c is the total concentration of tea solubles in solution, t is time, c_j and $c_{\infty j}$ are the instantaneous and equilibrium concentrations respectively of the jth component, k_j is its rate constant and n is the number of components considered in the model. The above equation is essentially a multicomponent differential rearranged form of eqn 21 in Part II. Preferred concentrations for use in such expressions are the theoretically significant mass/volume rather than the practically determined mass % (their interrelation has been considered in Part II).

Three models relating to one, two and three soluble components, fitted by minimizing variance of the scatter in concentration before averaging, are depicted in Fig. 2 and detailed in Table 2. The simplest model is a straightforward application of the first-order solution equation. This assumes all soluble tea solids behave in the same way and gives only moderate accuracy but is sufficiently close to be useful in preliminary studies of extraction systems where simplicity of manipulation may be advantageous. The two component model corresponds to the addition to the first model of a component which dissolves instantaneously. To retain a formal relation with eqn (1) this model may be considered as two first-order processes one of which has an infinite rate constant. Agreement is markedly improved in the early stages by inclusion of such a component but to be complete over the whole range investigated requires the further addition of a slowly soluble component which over the time span of

Table 2. Models describing the rates of solution of tea leaf in water at a water-to-leaf mass ratio of 5:1

Number of components in model	Equation for total concentration	Constants Concentrations: g/100 ml Rate constants: min^{-1}	60°C	80°C	100°C	Standard deviation of scatter (g/100 ml) 60°C	80°C	100°C
One	$c = c_\infty(1-\exp(-kt))$	c_∞	5.40	6.11	6.32	0.29	0.32	0.47
		k	0.62	0.90	1.52			
Two	$c = c_1 + c_{\infty 2}(1-\exp(-k_2 t))$	c_1	0.98	1.45	2.17			
		$c_{\infty 2}$	4.55	4.79	4.41	0.14	0.16	0.33
		k_2	0.41	0.56	0.68			
Three	$c = c_1 + c_{\infty 2}(1-\exp(-k_2 t)) + c_{\infty 3} k_3 t$	c_1	0.84	1.37	1.88			
		$c_{\infty 2}$	4.26	4.69	4.18	0.10	0.14	0.26
		k_2	0.50	0.60	0.94			
		$c_{\infty 3} k_3$	0.026	0.012	0.039			

these experiments dissolved linearly with time and may be regarded as following the early stages of a first-order process. Thus as far as rates of solution are concerned tea solids appear to fall into three groups: effectively instantaneously soluble, rapidly soluble and slowly soluble. In view of the heterogeneous nature of tea solids each group must contain a wide range of different compounds and the distinction between groups is largely one of physical availability. For instance the instantaneously soluble component must be immediately accessible to water, so it is quite possible that much of it was already on the outside of the leaf as the dried residue of plant juices expressed during manufacture. From its amount most, if not all, of the rapidly soluble component must have been extracted from inside the leaf where a complex structure of crushed and initially desiccated cells retards both penetration of solvent and outward diffusion of solute thereby slowing down the overall mass transfer however fast the internal solution process may be. Finally the slowly dissolving component may be either higher molecular weight matter which diffuses more slowly through the leaf matrix or a slowly formed product of hydrolysis; qualitatively it was observed that its increase in concentration coincided with increasing foaming tendency of the extract.

From Fig. 3 it will be seen that the specific net liquid uptake behaves like extract concentration in that most of the uptake occurs in the first few minutes with a slow linear increase with time thereafter. Even a small instantaneous contribution may be expected from the filling of any surface cavities capable of retaining extract on drainage. One practically interesting distinction between Figs. 2 and 3 however is that the initial rate of uptake is slightly slower than the rise in concentration giving a peak yield of solubles in solution after about two minutes. Also the later stages of uptake proceed proportionately faster than the corresponding dissolution so that after about ten min the yield falls markedly and in twenty-five min is not much different from the instantaneous value. From Figs. 2 and 3 it will be seen there is no evidence of difference in behaviour between conditioned and unconditioned leaf in stirred extraction of 200 g leaf, in striking contrast to the unstirred cases of Fig. 1 and the previous publication.

The results in Figs. 4 and 5 relating to extractions of 50 g leaf at 80°C and 60°C are broadly similar in form to those found for stirred extraction at 100°C, but with decreasing temperature extraction is naturally slower and smaller in extent. The curves shown in Fig. 4 relate to the appropriate three-component models detailed in Table 2 and clearly are a good description of the results. Parameters for other less precise models fitted to the experimental points are given in the same Table. This series of runs permits direct comparison of unstirred and stirred extraction. Apart from very short extraction times of less than 1 min, stirring seems to have made negligible difference to extract concentration but considerably increased net liquid uptake thereby greatly reducing yield in free extract. The indifference of concentration to stirring once again underlines the rapidity and ease of solution of tea solids and the surprising degree of convective mixing of extract possible in shallow layers of tea leaf having height less than diameter.

Finally brief consideration will be given to applying the present results for batch extraction to continuous processes having greater industrial interest. From the viewpoint of solution kinetics the main differences between batch and continuous processing lie in the residence time distribution of elements of slurry and the effects of recirculation on local extract concentrations. Both these differences arise from the system flow characteristics which lie between two simple theoretical extremes: plug flow of leaf and extract and perfect mixing. In plug flow corresponding elements of extract and leaf remain together and all such elements have the same residence time giving a spatial variation in extract concentration through the extractor corresponding to the application of batch kinetics over the elapsed time since entry. By contrast in the steady state operation of a perfectly stirred extractor with continuous input and output there is no spatial variation in extract concentration and only the concentration within the leaf changes through the extractor giving a 'one-sided' approach to equilibrium. Moreover the equal probability of outflow for all elements of slurry gives a wide distribution of leaf residence times following a simple exponential law which shows that the fraction of leaf insolubles having a residence time not less than n times the average is $\exp(-n)$. The effect of the residence time distribution and to a smaller extent the 'one-sided' concentration change is to retard extraction relative to a batch process having the same average residence time. Now since any practical continuous process will be somewhere between the above extremes it follows that continuous extraction will proceed more slowly than for a batch process having the average residence time. Qualitatively this is because relatively unextracted particles having a high solubles transfer rate are allowed to escape while some overextracted particles are retained. Quantitatively it is shown in the Appendix that in most practical situations continuous extraction in a single stirred tank followed by pipe flow to a separator will give extract having a concentration about $90 \pm 5\%$ of that found in a batch extraction having the same residence time.

Conclusions

(i) In batch extractions at 5:1 water-to-tea mass ratio, followed by gravity drainage, stirring increased the liquid uptake of leaf giving less free extract and in deep beds also reduced extract concentration, both factors adversely affecting extraction yield in a single stage.
(ii) In stirred batch extractions of up to 20 min duration extract concentration increased continuously with time but at a decreasing rate giving an averaged curve which could be exactly matched by a mathematical model of a system of three soluble components each obeying a first-order solution law, one extracted instantaneously, one fairly readily extracted and one slowly extracted.
(iii) Applying the above results to a theoretical study of continuous extraction shows that a continuously operated stirred tank in the steady state gives lower concentrations for a given residence time than with flow of tea-water slurry

through a pipe or with a batch-operated stirred tank, the best performance in a continuous stirred tank extractor occurring when the tank is merely a collecting reservoir and initial mixer, with as much as possible of the extraction done in the pipeline to the separator, when the extract concentration will approach that found in a batch extraction having the same residence time.

Acknowledgments

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Appendix

Continuous stirred extraction

The time over which dissolution of tea solids can occur is clearly the residence time of the insoluble solids from which the solubles are leached.

(i) *Residence time distribution in a perfectly stirred tank*. If the steady flow of insolubles through the system is $\dot{m}$ and the average residence time t_r, the mass of insolubles held is $\dot{m}\bar{t}_r$. Thus for equal probability of outflow, the fraction of the total outflow originating from a small mass Δm remaining at time t from a small addition Δm_0 at arbitrary zero time, is $\Delta m/\dot{m}\bar{t}_r$, and by conservation of mass it follows:

$$-\mathrm{d}(\Delta m)/\mathrm{d}t = \Delta m/\bar{t}_r \tag{2}$$

Hence on separating the variables and integrating between limits $\Delta m = \Delta m_0$, Δm for $t = 0, t$:

$$\Delta m/\Delta m_0 = \exp(-t/\bar{t}_r) \tag{3}$$

from eqns 2 and 3 the fraction $f\mathrm{d}t$ of the total insolubles flow in the steady state with a residence time between t and $t+\mathrm{d}t$ is then given by:

$$f = -\mathrm{d}(\Delta m/\Delta m_0)/\mathrm{d}t = (1/\bar{t}_r)\exp(-t/\bar{t}_r) \tag{4}$$

(ii) *Rates of extraction of one component by 'first-order' mass transfer*. In a perfectly stirred vessel in the steady state, the concentration of free extract (c_e) will be constant. Thus each element of leaf extracted will be uninfluenced by the history of any other and will behave as though it were in isolation with its appropriate mass of extract. If the fall in concentration of tea extract held within an element of leaf (c_r) may be represented by:

$$-dc_r/dt = K(c_r - c_e) \tag{5}$$

it follows that the fractional completion of the possible mass transfer of that element, conveniently designated the 'extractedness' (E), will be given by:

$$E = 1 - \exp(-Kt) \tag{6}$$

where from eqns (18) to (22) of part II the coefficient K is related to the rate constant k of eqn (1) by:

$$K = k/(A + 1) \tag{7}$$

where A is the ratio of volume of extract in leaf to volume of free extract.

The mean 'extractedness' for all elements of slurry leaving the vessel is the weighted mean over all available residence times, viz:

$$\bar{E} = \int_0^\infty f(1-\exp(-Kt)dt/\int_0^\infty f dt \tag{8}$$

Substituting for f from eqn (4) and integrating gives:

$$\bar{E} = K\bar{t}_r/(K\bar{t}_r + 1) \tag{9}$$

(iii) *Extract concentration*. Now if the mass of solubles in unit mass of tea available for solution is S, then the mass of solubles extracted from unit mass of tea is $\bar{E}S$. This extracted solid is used for two purposes: first to provide free extract in the output stream and secondly to replace extract adsorbed by freshly added leaf. The total volume of extract produced in these two cases will equal the volume of water (W) added per unit mass of tea plus the volume of tea solids extracted. Hence the outflowing extract concentration will be given by:

$$c_e = \bar{E}S/(W + \bar{E}S/\rho) \tag{10}$$

where ρ is the density of soluble tea solids.

The above concentration may for convenience be expressed as a fraction of the equilibrium concentration (c_∞) for batch extraction viz: $S/(W + S/\rho)$ when it is seen:

$$c_e/c_\infty = F\bar{E} \tag{11}$$

$$\text{where } F = (W/S + 1/\rho)/(W/S + \bar{E}/\rho) \tag{12}$$

Clearly from eqn (12) the 'extractedness' factor F is never less than, nor greatly exceeds, unity. Over the range of practical interest ($W \lesssim 4, S \gtrsim 0.4$) it is

less than 1.06 and the use of an average value 1.03 or even its total neglect cannot incur much error.

(iv) *Comparison with batch extraction*. Now the concentration ratio predicted by eqn (11) may be compared with that for batch extraction viz:

$$c_{e,b}/c_\infty = 1 - \exp(-kt) \tag{13}$$

and from eqns (9), (11) and (13) the ratio of concentrations of outflowing extracts for continuous and batch well-stirred extractions having the same average residence time ($\bar{t}_r$) is:

$$c_e/c_{e,b} = F/[(1 - \exp(-P))\,(1 + 1/BP)] \tag{14}$$

where $P = k\bar{t}_r$ and $B = 1/(A + 1) < 1$

It will be seen that B is the ratio of free extract to total extract. Inspection of eqn (14) shows:

(1) apart from the trivial cases of infinite residence times for all extractions and zero residence time at infinite dilution (i.e. $B = 1$), batch stirred extraction always gives higher concentrations than continuous stirred extraction,
(2) for short residence times (i.e. as $P \to 0$) the concentration ratio tends to BF,
(3) when there is at least as much extract inside leaf as outside (i.e. $B \leqslant 0.5$) the lowest value of the concentration ratio occurs at zero residence time and equals BF,
(4) when there is more extract outside leaf than inside (i.e. $B > 0.5$) the concentration ratio has a minimum value less than BF but greater than $0.77\,BF$ and occurs at P less than 1.8 (numerical values appropriate to infinite dilution).

Applying the results of the 5:1 extractions at 100°C reported earlier for which the net liquid uptake (V_n) was 3.0 ml/g and according to the one component model $c_\infty = 6.32$ g/100 ml and $k = 1.52\ \text{min}^{-1}$, taking additionally $\rho = 1.7$ g/ml and 5% moisture in leaf before extraction, gives results shown in the first two columns of Table 3.

(v) *Extension to a three component model of solution*. Extension of theory to a system of three soluble components of the kind given in Table 2 is quite simple because instantaneously and linearly soluble components will behave identically in batch and continuous processes having the same average residence time. Any difference between batch and continuous processes arises solely from the rapidly soluble component. Hence from eqn 11 and Table 2 it follows for the same residence time:

$$e_e/c_{e,b} = (c_{\infty 2}\,F\bar{E} + C)/[c_{\infty 2}(1-\exp(-k\bar{t}_r)) + C] \tag{15}$$

where $C = c_1 + c_{\infty 3}k_3\bar{t}_r$

Values of the concentration ratio calculated from eqn (15) using the parameters from Table 2 appropriate to extraction at 100°C are given in the final column of Table 3. These show some improvement for short residence times, largely due to the effect of the instantaneous component but at longer times are relatively

Table 3. Comparison of concentrations calculated for batch and continuous stirred extractions at 5:1 water-to-tea mass ratios and 100°C followed by gravity drainage

Average residence time (min)	Concentration ratio, continuous/batch	
	One component	Three components
1	0.49	0.69
2	0.58	0.69
4	0.71	0.75
6	0.79	0.80
10	0.86	0.87
15	0.90	0.91
20	0.93	0.93

unaffected. Although not shown up well by the results presented, the ratio for the three-component model passes through a minimum between 1 and 2 min residence time, again attributable to the instantaneously soluble part. It will be seen that under the least favourable conditions the concentration of extract from a continuous process using a 5:1 water-to-tea mass ratio will be about two-thirds of that from a batch process having the same residence time.

(vi) *Application to actual processes*. In practice a better performance will be achieved than indicated above because no extractor is perfectly stirred and more importantly the mixer is used in conjunction with a non-integral separator, most likely a centrifuge. Under these conditions extraction continues in the pipeline between the extraction vessel and the separator and in the latter some of the high concentration extract held by the leaf will be expressed. After such treatment the net liquid uptake is typically reduced to around 2.0 ml/g so about one third of the intra-leaf extract is made available to bridge the disequilibrium gap between leaf and free extract. If it is assumed that the tea-water slurry will spend about 1 minute in the pipework undergoing plug flow extraction before separation in a centrifugal separator to the dryness indicated above then it is unlikely that the concentration ratio for continuous to batch extractions of the same residence time will fall below 0.9.

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Effect of simple processing on the properties of protein and polysaccharide from black gram

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Summary

Autofermentation of black gram (or dhal) flour for 20 hr was not deleterious to either the surface activity of the proteins or the viscosity of the arabinogalactan polysaccharide. Dispersion of freshly prepared black gram flour showed higher batter volume than that of stored flour (12–16 months at 22–26°C). In the extracted fractions during germination (in the dark for 7 days at 22–25°C), surface activity and viscosity decreased continually, but the latter more rapidly and to a greater extent after the first day. Similar effects were noted after heat treatment (100/155°C).

Introduction

The commonly employed simple processing steps such as fermentation, germination and heat treatment as applied to legumes are known to improve their nutritional value and digestibility. They also improve the taste and flavour of the finished products. In this respect fermented soy bean and wheat products have been studied extensively but other products have received little attention.

Idli, a steamed pudding, is a popular Indian breakfast food made out of finely ground black gram and coarsely ground rice semolina batter after fermentation. The nutritional (Ananthachar & Desikachar, 1962; Steinkraus, Van Veen & Thiebau, 1967; Van Veen & Steinkraus, 1970) and fermentative (Desikachar *et al.*, 1960; Radhakrischnamurty *et al.*, 1961; Mukherjee *et al.*, 1965) aspects of idli have been studied. Subsequent investigations carried out in our laboratory (Kadkol, Desikachar & Srinivasan, 1961; Susheelamma & Rao, 1974, 1978a, b, c) indicated that black gram has two constituents: (1) surface active (foam forming) proteins (globulins) and (2) an arabinogalactan which gives highly viscous solutions, play an essential role in imparting the typical porous and spongy texture to the leavened food (idli). Therefore it was of interest to

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find out the effect of the above mentioned simple processing steps on the functional properties of these two constituents. Changes brought about in the surface activity of the protein and the viscosity of the polysaccharide after autofermentation of black gram flour dispersion for 24 hr and germination of the seeds for seven days at room temperature (22–25°C) and heating the bulk of the grains to 100 or 150°C were studied.

Materials and methods

Preparation of protein and polysaccharide

The major portion of surface active proteins could be extracted from black gram flour with NaCl and precipitated by $(NH_4)_2SO_4$ (Susheelamma & Rao, 1978a). The arabinogalactan polysaccharide is also extracted and coprecipitated with the protein, mostly with the 40–75% $(NH_4)_2SO_4$ precipitate. Therefore these fractions, i.e., (1) the 5% NaCl extract of black gram (2) and (3) the protein fractions precipitated at 0–40% and 40–75% (w/v) $(NH_4)_2SO_4$ respectively and (4) the 40–75% $(NH_4)_2SO_4$ supernatant were prepared and used for the estimation of total protein, surface activity and carbohydrate content to follow the changes in these constituents and their functional properties, after subjecting the seeds to various treatments. Arabino galactan polysaccharide free from protein was obtained by trichloroacetic acid (TCA) extraction of the flour followed by acetone precipitation (Susheelamma & Rao, 1978b) and used for viscosity and other measurements.

All these experiments were carried out in triplicates and the average values were recorded.

Analytical procedures

Protein was estimated according to Lowry *et al.* (1951) and carbohydrate using orcinol–H_2SO_4 reagent (Winzler, 1955). Surface activity was measured using a Stiepel type foam meter (Susheelamma & Rao, 1974). Viscosity was measured in an Ostwald Viscometer.

Fermentation

Fifty g lots of black gram flour prepared as described earlier (Susheelamma & Rao, 1974) were dispersed in 125 ml of water to get uniform and thick batters and allowed to undergo autofermentation under conventional conditions at room temperature (22–25°C) for 24 hr. Samples were removed at different intervals of time and used for the extraction of protein and polysaccharide. In another batch freshly prepared and stored flour dispersions were fermented as described above. Batter volumes were recorded at different intervals of time and a photograph was taken at the end of 24 hr.

Germination

Black gram seeds in 5 g lots were washed with water and then with 0.1% mercuric chloride for surface decontamination. They were then thoroughly washed with water, soaked for 10–12 hr at room temperature (22–25°C) and spread on filter paper placed on absorbent cotton in petri dishes and allowed to germinate for seven days at room temperature (22–25°C) in the dark. Samples taken out at intervals of 24 hr were used for the extraction of protein and polysaccharide.

Pre-heat treatment of seeds and dhal

Black gram seeds or dhal (dehusked and split seeds are referred to as dhal) were heated in an open pan on a flame for 4–5 min. The temperature attained by the bulk of grains was measured with a thermometer (0–360°C) immediately after transferring them to a beaker. The temperature was 100–110°C. In another test batch, seeds were heated for 12–15 min to attain a temperature of 155°C. (These are the temperature ranges to which the seeds or dhal are subjected in the conventional preparation of savoury foods). They were cooled to room temperature by spreading in a thin layer exposed to air, powdered and used for the extraction of protein and polysaccharide. To determine the solubility of proteins, flours from unheated and preheated dhal were extracted with water, followed by 5% NaCl and 0.1 M NaOH. The water extract, dialysed NaCl extract and neutralized and dialysed NaOH extracts were freeze dried and their weights determined. As majority of the surface active proteins could be extracted with 5% NaCl, a direct saline extraction of these flours was carried out to determine the surface activity.

Flours from unheated and preheated black gram or dhal were used for the extraction of the TCA-polysaccharide. The isolated polysaccharides were taken in two sets in glass-stoppered test tubes. One set was kept in a water bath at 90°C for 30 min and the other set in an oven at 150°C for 30 min. They were cooled to room temperature, and 0.3% aqueous dispersions of all these preparations were made for viscosity determination.

These flours were also used to obtain test idli preparations. Bulk density of steamed puddings was determined as described earlier (Susheelamma & Rao, 1974).

Results and discussion

Fermentation

The solubility of protein in 5% NaCl and the yield of 40–75% (w/v) $(NH_4)_2SO_4$ precipitate from this extract containing the majority of surface

active protein (80–90%) did not change significantly during the fermentation period. The amount of surface active protein extracted in 5% NaCl increased from 60–100% up to 12 hr of fermentation (Fig. 1a), probably due to increased hydration of the meal and hence better extractability of the surface active protein. It remained constant between 12–20 hr and then declined. The changes in the carbohydrate content of NaCl extract and the $(NH_4)_2SO_4$ fractions are shown in Fig. 1b. The decrease in total carbohydrate was found to be associated only with 40–75% $(NH_4)_2SO_4$ supernatant indicating that only the free sugars were utilized during fermentation and no significant degradation of the polysaccharide occurred until 20 hr. This was further supported by the observation that the viscosity of the isolated TCA-polysaccharide dispersion did not change over the 20 hr period of fermentation.

The effect of storage on the fermentabilities in terms of batter volumes is shown in Table 1. Fresh flour dispersion gave 40% higher batter volume as compared to the stored flour dispersion after 24 hr. It was also observed that the raising of the batter was continuous and uniform in the fresh flour dispersion (Fig. 2). In the stored flours the surface activity of the NaCl soluble proteins was found to have decreased by 25–30% while the yield as well as viscosity of the TCA-polysaccharide was decreased by 35–40%.

Germination

The surface activity of proteins in the NaCl extract and the $(NH_4)_2SO_4$ precipitates decreased during germination (Fig. 3a). The change in carbohy-

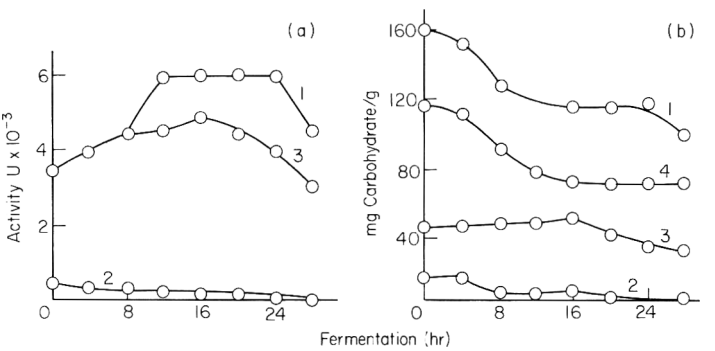

Figure 1(a) Variation of surface activity during fermentation. (1), 5% NaCl extract of fermented black gram flour; (2), 0–40% $(NH_4)_2SO_4$ precipitate from 1; (3), 40–75% $(NH_4)_2SO_4$ precipitate from the supernatant of 2.

Figure 1(b). Variation of total carbohydrate during fermentation. (1), 5% NaCl extract of fermented black gram flour (containing free sugars and the polysaccharide); (2), 0–40% $(NH_4)_2SO_4$ precipitate from 1 (containing negligible amounts of carbohydrates); (3) 40–75% $(NH_4)_2SO_4$ precipitate from the supernatant of 2 (containing arabino-galactan polysaccharide); (4), 40–75% $(NH_4)_2SO_4$ supernatant (containing the free sugars).

Table 1. Batter volumes of stored and fresh flours of black gram dhal during fermentation

Time (hr)	Batter volume (ml)	
	Fresh flour*	Stored flour†
0	200	200
14	800	700
17	850	750
21	900	650
24	960	650

* Black gram stored in sealed containers in the cold, until use, was dehusked and powdered afresh.

† Flour prepared as above was stored for 12–16 months at 22°–26°C.

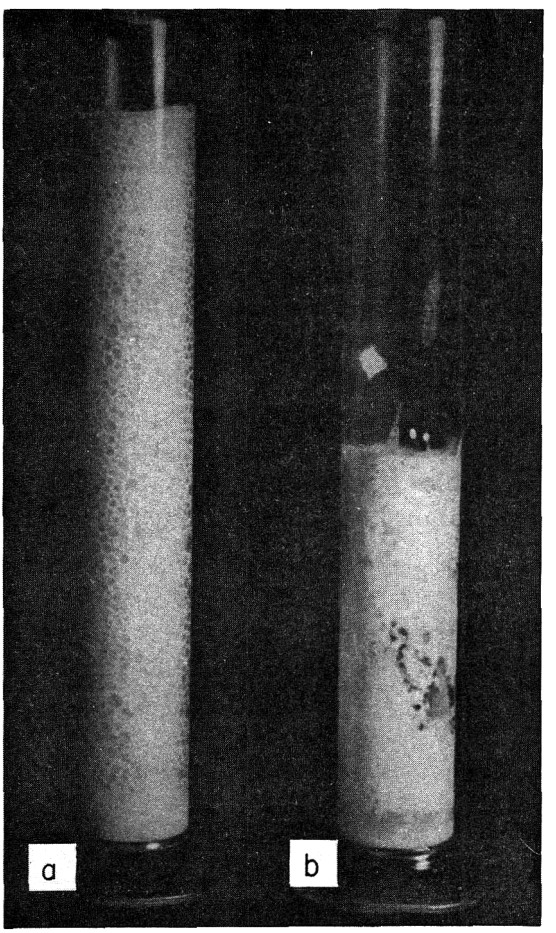

Figure 2. Batters of fresh and stored flours of black gram after fermentation (a) fresh flour, (b) stored flour.

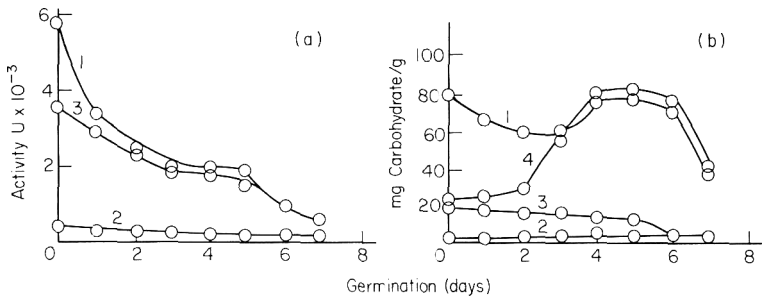

Figure 3(a). Variation of surface activity during germination. (1), 5% NaCl extract of germinated black gram; (2), 0–40% $(NH_4)_2SO_4$ precipitate from 1; (3), 40–75% $(NH_4)_2SO_4$ precipitate from the supernatant of 2.

Figure 3(b). Variation of total carbohydrate during germination. (1), 5% NaCl extract of germinated black gram (containing free sugars and the polysaccharide); (2), 0–40% $(NH_4)_2SO_4$ precipitate from 1 (containing negligible amounts of carbohydrates); (3), 40–75% $(NH_4)_2SO_4$ precipitate from the supernatant of 2 (containing arabino-galactan polysaccharide); (4), 40–75% $(NH_4)_2SO_4$ supernatant (containing the free sugars).

drate content of the extracts and the precipitates is shown in Fig. 3b. The NaCl extract showed a decrease up to three days, then increased up to six days and again decreased. The 40–75% $(NH_4)_2SO_4$ precipitate (which contained the majority of surface active protein and the polysaccharide) progressively decreased in quantity. The 40–75% $(NH_4)_2SO_4$ supernatant showed an increase up to six days and then decreased. The initial decrease in the NaCl extract and 40–75% $(NH_4)_2SO_4$ precipitate corresponded to the increase in the supernatant, indicating the degradation of the polysaccharide. The subsequent increase in the NaCl extract as well as the supernatant may be due to the degradation of reserve polysaccharide (starch). After coleoptile formation all fractions showed a decrease in carbohydrate.

The yield of TCA extractable polysaccharide and its viscosity during germination is shown in Table 2. It is seen that the reduction in viscosity of the polysaccharide dispersion is much larger compared to its yield. These indicate

Table 2. Yield of TCA-polysaccharide and its viscosity during germination of black gram in the dark

Germination days	Yield (%)	Viscosity* (%)
0	5.1	100
1	4.9	55
2	4.0	41
3	3.4	30
4	2.8	9
5	1.9	8
6	1.8	1.7
7	1.1	0

* Specific viscosity of 0.3% aqueous dispersion of polysaccharide from ungerminated seeds as determined by Ostwald viscometer was 15 cps units and taken as 100%.

that some significant change occurs during this period (first day) of germination, probably due to some enzymatic activity, which modifies or degrades the polysaccharide.

Heat treatment

After heating the seeds to 110°C the solubility of protein in water or 5% NaCl decreased by 30% while the alkali solubility slightly increased (20%). Heating to a much higher temperature (150°C) decreased the solubility in all the extracts by 65%. Reduced solubility after heat treatment has been observed with other food proteins also. Heat treatment has been reported to reduce the solubility of soy bean (Beckel, Bull & Hopper, 1942; Mecham & Olcott, 1947) and cotton seed (Thurber *et al.*, 1954; Olcott & Fontaine, 1942) proteins almost completely. Solubilities of pea seed (Evans & St John, 1948) and wheat gluten (Mecham & Olcott, 1947; Pence, Mohammad Ali & Mecham, 1953) were reduced considerably, but peanut proteins (Fontaine, Samuels & Irving, 1944; Srikanta & Narasinga Rao, 1974) were less sensitive.

The results in Table 3 indicate that at the lower temperature surface activity of the protein decreased by 30%, while the viscosity of the polysaccharide was reduced by 70%. At higher temperatures surface activity decreased by 65% while viscosity was reduced by 98%. The change in the yield of the TCA-extracted polysaccharide was not significant. Unwashed and washed seeds (washed and air dried dhal is preferred in some savoury and snack dishes) showed a similar behaviour.

The effect of heat treatment on the isolated polysaccharide is shown in Table 4. Heating the polysaccharide from unheated dhal at 90°C for 30 min caused a

Table 3. Effect of heat treatment on the proteins and polysaccharide from black gram dhal

No.	Sample	Protein		TCA-polysaccharide	
		Yield (g)	Activity* (%)	Yield (g)	Viscosity† (%)
1.	Flour from black gram	10	100	4.9	100
2.	Flour from pre-heated black gram dhal (110°C)	4.0	70	5.0	30
3.	Flour from pre-heated black gram dhal (155°C)	3.2	35	5.0	2.3

Fifty grams of flour was used for extraction of protein and 100 g for extraction of TCA-polysaccharide.

* Surface activity of unheated flour was 5000 units/g on dry weight basis.

† Specific viscosity of 0.3% aqueous dispersion of polysaccharide from ungerminated seeds as determined by Ostwald viscometer was 15 cps units and taken as 100%.

Table 4. Effect of heat treatment on the viscosity of TCA extracted polysaccharide from black gram

No.	Source of TCA-polysaccharide	Viscosity (%)* No heat treatment	Solid heated at 90°C for 30 min	Dispersion heated at 90°C for 30 min	Solid heated at 150°C for 20 min
1	From black gram flour	100	95	81	2.7
2	From pre-heated seeds (110°C)	20	19	18	2.7
3	From pre-heated dhal (110°C)	18	17	15	2.5
4	From pre-heated seeds (150°C)	10.5	10.4	7	2.3
5	From pre-heated dhal (150°C)	10.7	10.5	8	2.5

* Specific viscosity of 0.3% aqueous dispersion of polysaccharide from ungerminated seeds as determined by Ostwald viscometer was 15 cps units and taken as 100%.

slight decrease in the viscosity of the dispersion, which when heated again at 90°C for 30 min showed a further decrease. On the contrary heating black gram or dhal to temperatures above 100°C (110°–150°C) was highly deleterious as viscosity of the polysaccharide dispersions reduced by 80–90%. Further heating of these polysaccharides or their dispersions caused only marginal decrease. Aqueous dispersions of polysaccharides from preheated black gram had slightly higher viscosity (90%) compared to that from preheated dhal (80%). The presence of husk (which is removed to get the dhal) may be responsible for this difference. Heating the isolated polysaccharide (from all the samples) to 150°C almost completely reduced the viscosity of aqueous dispersions. These results indicate the high thermal sensitivity of the viscosity of the polysaccharide.

The bulk densities of steamed puddings wherein flours from pre-heated seeds were incorporated are shown in Table 5. The bulk densities of test idli preparations were as high as that of rice semolina control and the products were very hard.

Conclusions

Based on these observations it may be stated that overnight fermentation does not adversely affect the functional properties of the surface active protein and the arabino galactan polysaccharide of the black gram. Seeds probably stored in the cold in sealed containers until powdering would help the protein and polysaccharide to retain the functional properties unaltered and leavened food

Table 5. Bulk densities of steamed puddings containing flours from pre-heated black gram dhal

No.	Sample	Weight (g)	Bulk density (g/ml)
1	Rice semolina (RS)	7.5	0.77
2	RS + flour from black gram dhal	5 + 2.5	0.56
3	RS + flour from pre-heated black gram dhal (110°C)	5 + 2.5	0.67
4	RS + flour from pre-heated black gram dhal (155°C)	5 + 2.5	0.77

The values represent averages of three independent experiments. The mean deviation was found to be ±0.03.

preparations with satisfactory texture could be obtained. Although preparations containing germinated black gram are rare, optimal soaking of the seeds is an important factor as the viscosity of the polysaccharide shows a tendency to decrease after soaking the dhal for longer periods (18–20 hr). Heat treatment, however, drastically affects the functional properties of both the protein and the polysaccharide and should be avoided in the preparation of foods with special textural qualities.

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A comparison of the texture of expanded milk protein meat extenders and TVP

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Summary

The Minnesota texture method (MTM) was used to compare the texture of expanded protein meat extenders produced from milk proteins, by microwave expansion and thermoplastic extrusion, and commercial TVP products with raw and cooked minced beef. The texture of the expanded TVP products was found to resemble that of cooked minced beef. Microwave textured whey protein had considerably higher texture parameter values than commercial TVP products. When half the whey protein was replaced by casein the texture values of the resulting microwave expanded product were decreased below those of the commercial TVP products. The extruded skim milk protein product was also found to have lower texture parameter values than the commercial expanded TVP products.

Introduction

Textured vegetable protein (TVP) is now widely used as a meat extender in comminuted meat products. The term TVP is generic and has been applied to a broad class of products which vary considerably in chemical composition and physical properties, including texture. Two distinct types of TVP are commercially used as meat extenders; edible protein fibres and expanded protein products. Wet spinning is the most important method for the production of protein fibres while expanded protein products are most often produced by thermoplastic extrusion (Gutcho, 1973).

Although the use of expanded TVP products has increased in the processed meat industry, relatively little has been published regarding their basic textural characteristics. Maximum shear force and work done during shearing have been

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used to evaluate the texture of extruded TVP products using a single blade shear cell (Iles & Elson, 1973) and a modified Warner-Bratzler shear instrument (Cumming, Stanley & de Man, 1972 and Maurice, Burgess & Stanley, 1976). Tensile properties such as breaking strength, break elongation and stress relaxation were determined by Maurice *et al.* (1976) and they also used the Ottawa Texture Measuring System (OTMS) multiblade shear cell to measure compression and stress relation of extruded TVP. Because of the particulate and irregular nature of many expanded protein products shear tests and tensile property measurements are of limited value in defining texture. The Minnesota Texture Method (MTM), developed by Barker (1974), was used by Breene & Barker (1975) to separate commercial TVP products into textural classes. The MTM can also be used to evaluate the texture of ground beef and for this reason was selected for texture measurement in this investigation.

Attempts to produce expanded protein products from casein (Poznanski *et al.*, 1975) and skim milk protein (Tuohy, 1979) using conventional thermoplastic extrusion techniques were unsuccessful. With a modified twin screw extruder Lahousse (1978) was able to texturize skim milk protein by thermoplastic extrusion. A thermoplastic method was used to texturize milk protein in Poland (Poznanski *et al.*, 1977) and Smietana *et al.* (1978) concluded from structural analysis, by scanning electron microscopy, that the product, to some extent, showed a structural resemblance to meat tissue. A microwave expansion technique has been used in this laboratory to texturize whey protein and whey protein-skim milk protein blends (Burgess *et al.*, 1978).

The purpose of this work was to compare the texture of microwave textured whey protein, a microwave textured milk protein product (50% casein/total protein), extruded skim milk protein and 6 commercial TVP products with minced beef the material they would commonly extend.

Materials and methods

Materials

The textured whey protein (TWP) was produced by microwave expansion as outlined by Burgess, Downey & Tuohy (1978) and Tuohy (1979) and comprised 52% protein and 14% moisture.

The textured milk protein (TMP) was also produced by microwave expansion and consisted of 51% protein (25.5% casein, 25.5% whey protein), 15% moisture.

The textured skim milk protein was produced by Creusot Loire Ltd. (42701 Firminy, France) using a modified twin screw thermoplastic extruder (Model B.C.72). The product contained 58% protein and 11% moisture.

The 6 commercial TVP products which were examined are listed in Table 1.

Table 1. Commercial TVP products tested

Code	Product	Supplier
A	Arkady TVP mince	British Arkady Co. Ltd.
B	Bontrae TVP mince	GMB Proteins Ltd.
C	Centex TSP mince	Hypak Division, Oppenheimer Casing Co. Ltd.
D	Solus TVP mince	Solus Soya Foods Ltd.
E	Itona TVP chunks	Itona Products Ltd.
F	Temptein (spun TVP)	Miles Laboratories, Marshall Division.

Samples A, B, C, and D were mince size, sample E was in the form of chunks and sample F was a product consisting of edible protein fibres. Commercial TVP products contain approximately 50% protein and less than 10% moisture.

Minced beef was purchased locally and evaluated raw (RM) and cooked (CM). Cooking comprised of heating 100 g quantities for 2 min in a microwave oven followed by shallow fat frying for 10 min. The raw minced beef was used as a standard.

Preparation of samples for texture measurement

The TVP mince size samples were rehydrated in tap water until an equilibrium moisture content was reached. The excess water was then drained off. All the other textured protein samples were rehydrated, drained and minced through a plate containing holes of 8 mm diameter using a Kenwood Chef mincer attachment. The cooked minced beef was also ground prior to texture measurement.

Texture measurement – Minnesota Texture Method (MTM)

Samples were compressed-extruded in a 50 cm^2 Ottawa Texture Measuring System (OTMS) cell with the 8 wire grid (Voisey, 1971) affixed to the Instron Universal Testing Machine Model 1112. A 500 kg compression cell, calibrated in tension with a 2 kg weight, was used to measure force. The crosshead speed was 5 cm min^{-1} and the chart speed 10 cm min^{-1}. The gauge length was set to stop the piston 0.5 cm from the wires. A minimum of 5 replicates of each sample were tested.

Seven texture parameters were determined from the force distance curves, obtained on the Instron recorder, as defined by Breene & Barker (1975). A typical force distance curve is shown in Fig. 1.

A. Maximum force is the maximum force in kg attained during extrusion.

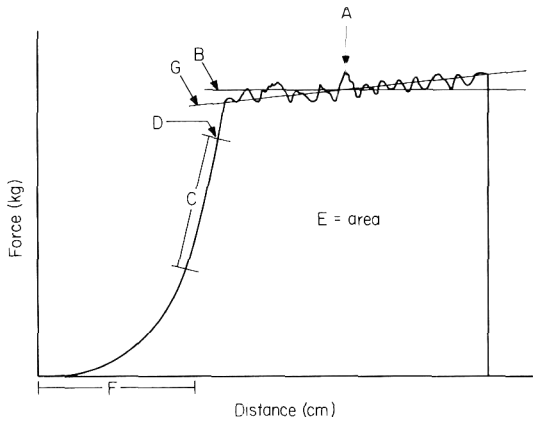

Figure 1. A typical force – distance curve for textured protein products obtained on the Instron UTM using the OTMS wire grid cell. A, maximum force; B, average maximum force; C, hardness; D, cohesiveness; E, chewiness; F, packability; G, extrudability.

B. Average maximum force is the average force in kg required to maintain a constant extrusion rate.
C. Hardness is defined as the force necessary to attain a given deformation. The average slope of the initial (approximately linear) portion of the curve in kg cm^{-1} can be interpreted to indicate hardness.
D. Cohesiveness relates to the strength of the internal bonds making up the body of the product. It is quantified as the force in kg at which shearing and extrusion are initiated.
E. Chewiness is the energy required to masticate a solid food product to a state ready for swallowing. It is interpreted as the area under the curve. The area represents the energy used to compress, shear and extrude the sample.
F. Packability is the distance (cm) travelled by the plunger before an average linear slope is reached.
G. Extrudability is a function of hardness and cohesiveness. It is taken as the average slope of the curve in kg cm^{-1} after the onset of shearing and extrusion.

Results

Mean values and 95% confidence limits for maximum force, average maximum force, hardness, chewiness and cohesiveness of the eleven samples outlined in the materials and methods section are given in Fig. 2. The values are plotted in order of decreasing magnitude. The correlation coefficients between the mean values of the seven texture parameters were calculated on the basis of the eleven samples evaluated (Table 2).

Table 2. Correlation coefficients for the seven MTM texture parameters obtained with the eleven samples tested

Texture parameters X	Y	Correlation coefficient (r)
Maximum force	Average maximum force	0.9904
"	Hardness	0.9546
"	Chewiness	0.9706
"	Cohesiveness	0.9784
"	Packability	0.5919
"	Extrudability	0.2490
Average maximum force	Hardness	0.9820
"	Chewiness	0.9610
"	Cohesiveness	0.9963
"	Packability	0.6661
"	Extrudability	0.1913
Hardness	Chewiness	0.9058
"	Cohesiveness	0.9874
"	Packability	0.7775
"	Extrudability	0.1581
Chewiness	Cohesiveness	0.9457
"	Packability	0.4632
"	Extrudability	0.0795
Cohesiveness	Packability	0.6957
"	Extrudability	0.1677
Packability	Extrudability	0.1763

($n = 11 : P < 0.01, r = 0.7347; P < 0.05, r = 0.6021$)

The maximum force parameter values (Fig. 2a) of the commercial TVP samples A, C, D, E, the microwave textured milk protein (TMP) and the cooked minced beef (CM) are not significantly different from the raw minced beef standard (RM), which had a mean value of 60 kg. The mean values of this group ranged from 50 to 73 kg. The microwave textured whey protein (TWP) and the commercial TVP sample F had considerably higher values of 168 and 165 kg respectively. The commercial TVP sample B and the thermoplastic extrusion textured skim milk protein (TSMP) had significantly higher and lower values than the standard (RM) respectively.

The average maximum force parameter (Fig. 2b) correlated highly with the maximum force parameter (0.9904; $P < 0.01$) but was more sensitive in distinguishing differences between the products. Only the TMP and the TSMP gave values similar to the standard (RM), which had a mean average maximum force of 36 kg. The commercial TVP samples had higher values than the standard but samples D, E were not significantly different from the cooked minced beef (CM), which had a mean value of 62 kg.

An even greater separation of the products can be seen from the hardness parameter results (Fig. 2c). All the samples, with the exception of the TSMP,

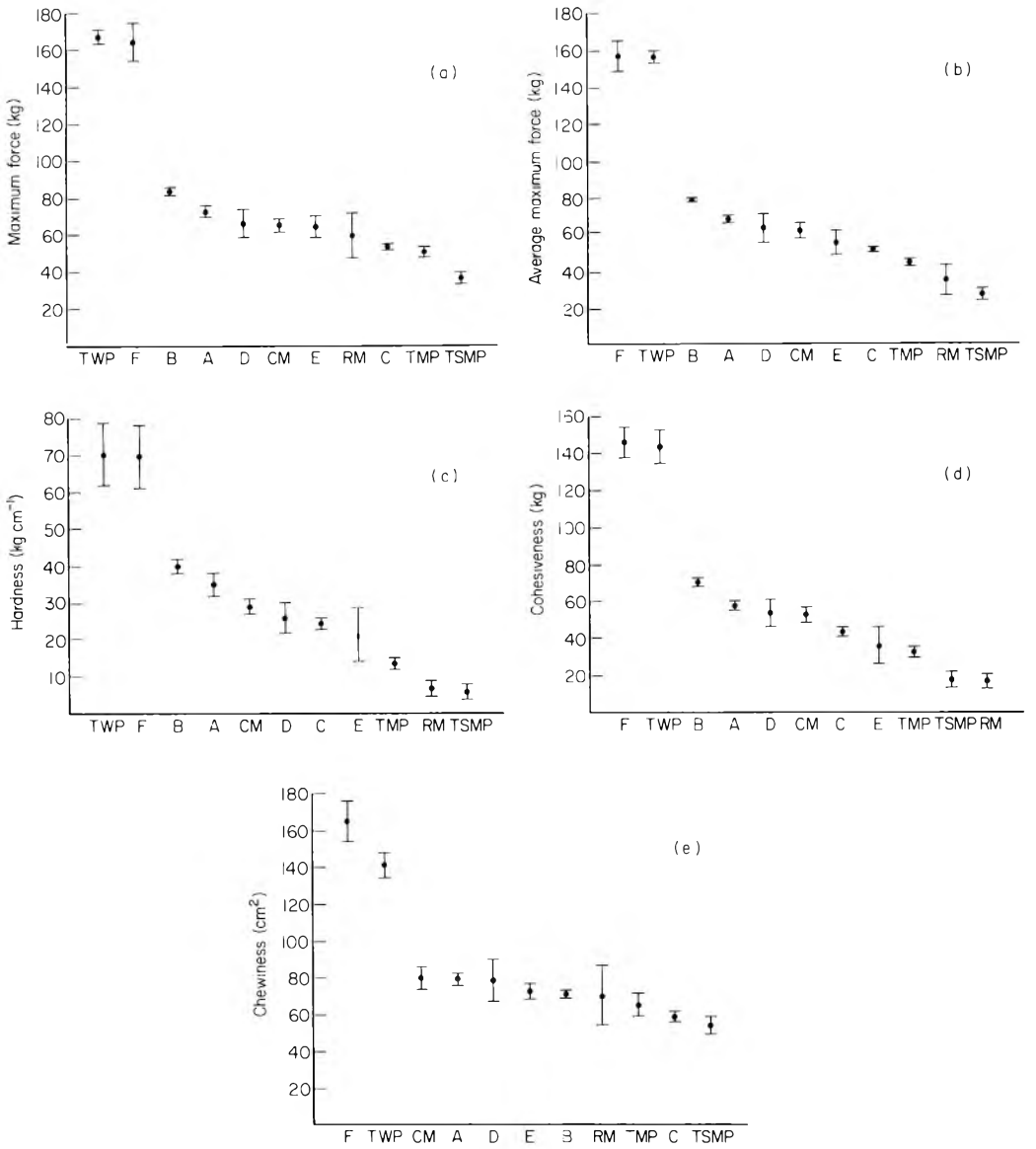

Figure 2. Mean values and 95% confidence intervals for five texture parameters of the eleven samples tested, as described in 'Materials and methods'.

had significantly higher hardness values than the raw minced beef standard (mean 6.9 kg cm^{-1}). The expanded commercial samples A, B, C, D, E, had hardness values, approximating more closely to the cooked minced beef sample (mean 29 kg cm^{-1}) and higher than the value for the TMP (mean 14 kg cm^{-1}). Cohesiveness values (Fig. 2d) were very highly correlated with the hardness values (0.9874, $P < 0.01$) and segregated the products in a similar manner.

Table 3. Mean values and range ($0.05 > P > 0.01$) of the packability and extrudability parameters for the eleven samples tested

Products	Packability Mean	Range ($P<0.05$)	Extrudability Mean	Range ($P<0.05$)
A	15.5	15.4–15.7	0.52	0.04–1.00
B	17.9	17.7–18.1	1.23	0.65–1.81
C	16.2	15.8–16.6	0.25	−0.03–0.53
D	14.1	13.8–14.5	0.31	−0.35–0.97
E	13.2	12.5–13.8	1.49	0.61–2.37
F	15.4	15.2–15.7	−1.82	−3.00–(−0.64)
TWP	18.7	18.1–19.3	5.55	4.61–6.49
TMP	12.6	11.3–14.0	0.81	0.51–1.01
TSMP	8.6	7.3–10.0	0.91	0.77–1.05
RM	8.8	5.9–11.7	1.80	1.45–2.15
CM	15.1	14.8–15.4	−0.44	−1.61–0.73

Based on the chewiness parameter (Fig. 2e) there was no significant difference between the expanded protein products, (with the exception of the TWP and TVP sample F), whose mean values ranged from 54–80 kg, and the raw minced beef standard (mean 71 kg). This parameter was highly correlated with maximum force (0.9706, $P < 0.01$).

The packability parameter values (Table 3) were very similar for all the commercial TVP products and the extrudability parameter values were too variable to allow a meaningful comparison of samples.

Discussion

Five of the texture parameters, maximum force, average maximum force, chewiness, hardness and cohesiveness correlated significantly with each other for the eleven samples evaluated ($0.01 > P > 0.005$). This is in agreement with the findings of Breene & Barker (1975). However hardness, cohesiveness and average maximum force were found to be more sensitive in detecting differences between samples than maximum force and chewiness. This differs from the order average maximum force, maximum force, chewiness, cohesiveness and hardness found by Breene & Barker. This difference may be due to the greater friction from the walls of the larger OTMS cell (50 cm^2) used for this work compared with the 10 cm^2 OTMS cell used by Breene & Barker.

The compression – extrusion force (maximum force), the work done during compression–shearing–extrusion (chewiness) and the ratio of force to displacement during compression (hardness) are based on well defined reheological properties of food materials (Mosenin, 1970). For this reason and because of the high correlation between five of the parameters (Table 2) the maximum

force, chewiness and hardness values would probably be sufficient and most meaningful in characterizing the texture of expanded protein products.

Based on the results for these three parameters the texture of the microwave textured whey protein (TWP) and the commercial edible protein fibre type TVP product (sample F) are considerably different from that of the raw minced beef standard and the other eight samples. From the ratio of force to displacement during compression results (hardness) it also appears that the texture of the commercial expanded TVP products (A, B, C, D, E) approximates more closely to the texture of the cooked minced beef than that of the raw minced beef. This is not surprising since expansion texturization is equivalent to a cooking step, because of the high temperatures used, and this result suggests that cooked minced beef is a more realistic standard with which to compare expansion textured protein products.

The five commercial expanded TVP products A, B, C, D, E (Table 1), as a group, had reasonably similar texture parameter values, although individual products differed significantly from each other. Inter product differences most likely arise from different texturization processes and/or operating conditions used in their manufacture. Overall, products D and E had a texture most similar to cooked minced beef, while samples, A, B had higher texture values and sample C slightly lower values. The numerical values of the texture parameters are not directly comparable with the results obtained by Barker (1974) and Breene & Barker (1975) because of the different size OTMS cells used.

When 50% of the whey protein was replaced by casein the texture values of the microwave textured product (TMP) were greatly decreased compared with the TWP. This was due to a dilution effect of the heat labile whey proteins, which are primarily responsible for the structure of expanded whey protein, with heat stable casein protein. The caseins do not appear to undergo much crosslinking in the microwave expansion process and hence contribute little to the texture. At an intermediate casein/total protein ratio a microwave expanded product with a texture similar to that of cooked minced beef can be obtained (Tuohy, 1979).

The textured skim milk protein (TSMP) produced by thermoplastic extrusion had significantly lower texture parameter values than comparable TVP products and cooked meat. It must be concluded that the thermoplastic extrusion of skim milk protein requires further investigation in order to obtain a product texturally similar to cooked minced beef. In this respect the MTM is a useful instrumental method for texture measurement in textured protein product development work.

In order to determine the practical significance of the differences found between products it would be necessary to correlate these instrumental results with sensory evaluation. This was not undertaken here but the MTM parameters have been correlated positively with sensory chewiness, cohesiveness, springiness, juiciness and negatively with sensory tenderness and mealiness (Breene, 1977 and Loh, 1975).

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Crossover electrophoresis with indirect immunofluorescence in the detection of soy protein in heated meat products

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Summary

A new method based on crossover electrophoresis with indirect immunofluorescence for the identification of soy protein in heated meat products is described. The procedure demonstrates specific precipitation reactions between the antisera and soy protein, even when these are present at concentrations of 2.5% in meat products heated to the level of commercial sterility (125°C × 25 min).

Introduction

In the world market there are in commerce different meat products which contain a certain amount of non-meat proteins, particularly soy and lactoproteins.

While the addition of 25–30% soy protein is permitted in some countries, in others such protein does not appear either in the ingredients or in the allowed additives lists and therefore its addition constitutes a commercial fraud for lower price and inferior quality in regard to meat protein.

For this reason there is a need for methods which allow qualitative-quantitative determinations of soy protein which may be present in fresh and heated meat products. In essence, if the soy is present at very low concentrations, it is not possible to taste it in the flavour of the food; however, for some products, such as sausages, the presence of vegetable protein allows an increase in the quality of the product in regards to consistency and digestibility.

In the last 10 years numerous analytical procedures have been proposed which mainly utilize electrophoretic, immunological and histological techniques to reveal the presence of soy protein in meat products.

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Electrophoresis has allowed the analysis of soy protein in the presence of meat protein by means of their solubilization and separation in an electric field on different support media; of these polyacrylamide gel has been favoured by most researchers (Llewellyn & Flaherty, 1976; Parsons & Lawrie, 1972; Persson & Appelqvist, 1977); while starch gel (Olsman, Houtepen & Leeuwen, 1969) and cellulose acetate membrane (Gils & Hidskes, 1973) have been used more rarely.

Gel electrophoresis has been conducted in both columns and thin layers (Guy, Jayaram & Willocox, 1973; Hofmann, 1973; Homayounfar, 1977), together with a variety of extractants and buffer systems. Those based on urea alone, urea and 2-mercaptoethanol (Olsman, Houtepen & Leeuwen, 1969; Guy, Jayaram & Willocox, 1973), sodium dodecylsulphate (Hofmann, 1973; Hofmann, 1977; Lacourt, Malicrot & Dauphant, 1977), or tris-(hydroxymethyl-) methylamine and glycine systems have been demonstrated more valid for the detection of soy protein.

A quantitative method to determine the soy protein content in fresh and cooked meat-soy blends was developed by Lee *et al.* (1975) using the stacking SDS (sodium dodecylsulphate) – acrylamide gel electrophoresis method. The method, equally applicable for soy flours, soy concentrates and soy isolates, is able to determine the soy protein content within $\pm$ 2% of actual content. The same results are obtained if the product contains animal origin non meat extenders such as milk powder, casein and egg white proteins (Lee *et al.*, 1976).

To obtain an even more accurate electrophoretic resolution, even more sophisticated extraction procedures have been successively reported (Homayounfar, 1977); for example, the migration of the protein has been performed on a gel which has an internal gradient maintained by a system of amphoteric buffers, according to the isoelectric focusing technique (Llewellyn & Flaherty, 1976). Soy protein has also been found through the analysis of its amino acid components (Fischer & Belitz, 1976).

All these methods allow detection of soy protein in raw meat products or those heated to the level of commercial sterility (125°C $\times$ 25 min). The procedures utilized are sensitive enough to reveal the presence of soy at levels corresponding to an addition of $\geq$ 1%; on the other hand, these procedures, because they are very laborious and time-consuming to conduct, are not useful for routine laboratory analysis. An alternative to the electrophoretic technique is represented by immunological methods which utilize soy-specific rabbit antisera. Among these, the indirect haemagglutination (Smith, 1974), Ouchterlony's double gel diffusion technique (Hargreaves, Jarvis & Wood, 1974), Mancini's radial gel diffusion (Mancini *et al.*, 1964); Grabar's qualitative electrophoresis and Laurell's quantitative electrophoresis (Koie & Djurtoft, 1977) have been successfully used.

The immunological procedures have been conducted using antisera prepared by immunization with soy protein either unheated, moderately heated (Peter, 1970) or preheated (autoclaved for 30 min at 121°C) (Llewellyn & Sawyer, 1977; Hammond *et al.*, 1976). Particularly, the problems of antisera prepara-

tion and variability were reviewed by Kruger & Grossklaus (1970) and Herrmann *et al.* (1973).

In any case, both the immunodiffusion and the electroimmunodiffusion methods have been shown to be rapid and easy to perform and useful to detect soy protein in fresh and heated meat products. Detection limits vary in relation both to heat treatment and kind of soy added to the meat products (Hammond *et al.*, 1976). The experiments conducted have also demonstrated that the antisera prepared with preheated soy protein are more suitable for use in the detection of soy in heated products. However, none of the methods reported above permits an effective quantitative evaluation of the soy content (Llewellyn & Sawyer, 1977).

It is necessary to recall that some soy-specific antisera give cross-reactions with other vegetable proteins (onion powder, coriander, cinnamon and others): their responses are negative if such ingredients are assayed at those concentrations at which these would normally be present in foods (Llewellyn & Sawyer, 1977; Hammond *et al.*, 1976). Along with the electrophoretic and immunological methods, a histological technique has also been proposed that provides for microscopic examinations of sections of the product to be examined (Flint & Meech, 1978; Bergeron & Durand, 1977).

This method is called 'stereological' because it enables information about three-dimensional quantities, including volume ratios, to be obtained from the study of two-dimensional sections. Though the preparation of the samples in this method is not difficult, the microscopic examination calls for a higher specialization in the reading. By superimposing particular grids on the field of view and following with the appropriate staining technique, it is possible to make an accurate quantitative determination of soy protein in comminuted meat products (soy concentration levels $\geq 1\%$) (Flint & Meech, 1978; Bergeron & Durand, 1977).

None of the three methods previously described seems able to satisfy the need of a control laboratory for the routine detection of soy protein, especially in countries like Italy, where the addition of soy protein in meat product is not permitted and where commercial antisera to heated proteins are not available. In fact, the immunological procedures that are most used today allow detection of soy protein in meat products at levels corresponding, for heated products ($115°C \times 60$ min), to an addition of $\geq 3\%$ meat equivalent* only using antisera prepared with preheated soy isolate (Hammond *et al.*, 1976). On the other hand the electrophoretic and stereological methods, while more sensitive (a possibility of revealing concentrations $\geq 1\%$), due to their complexity, consumption of time or need of expertised people (stereological method), are not useful in routine laboratory examinations which have to be performed on a great number of samples. For these reasons, we have evaluated the validity of a new extremely

*This is defined for products with a protein content of not less than 90% as the percentage weight of the fully hydrated product (assuming a degree of hydration of 66%); and for products with a protein of between 50 and 90% as the percentage weight of the fully hydrated product reduced in the ratio (actual per centage protein content in dry fat-free product)/90.

sensitive immunological method, crossover electrophoresis with indirect immunofluorescence, in its ability to rapidly detect even very low quantities of soy protein that may be present in meat products, using commercially available antisera.

In addition the presence of soy protein in the same kinds of samples has been revealed utilizing the classic electrophoretic procedure on polyacrylamide gel, as a comparison.

Materials and methods

Samples of minced, fresh bovine meat are prepared containing respectively, 1, 2.5, 5 and 10% soy flour.

The different preparations are then subdivided in aliquots to be submitted to different thermal treatments for varying times according to the following scheme:
exposure to 60, 70, 80 and 90°C for 60 min;
exposure to 105, 120, and 125°C for 25 min.

Extracts are then prepared for the immunological and electrophoretic procedures, using the method most suitable for each case.

Crossover electrophoresis with indirect immunofluorescence

Extraction of proteins. Five gram samples are homogenized in enough 0.143 M sodium chloride to obtain a 1:4 dilution (w/v). After shaking at room temperature for 30 min the samples are centrifugated at 1000 ***g*** for 20 min and the supernatants are filtered under vacuum.

Antisera. Crossover electrophoresis is performed using soy-specific rabbit antisera; sheep anti-rabbit γ-globulin fluorescent sera are used for the indirect immunofluorescence. All the products are obtained from Behringwerke AG, Marburg.

Method. The procedure first used by Kohn (1968) and perfected by Vergani (1971), calls for Ouchterlony's double diffusion technique, but performed in an electric field, so that the antigen and the antibody migrate against each other in an electrophoretic system. Gelatinized cellulose acetate strips (6 × 14 cm) (Cellogel-Chemetron, Milan) previously hydrated in a tris-glycine buffer solution, pH8.6, for 10 min, are then dried between two sheets of filter paper to remove excess liquid and then stretched on the electrophoresis bridge.

Thirty μl of each of six extracts to be examined are deposited towards the cathode at 1 cm from the middle of the strip; in front of each sample, towards the anode, at a distance of 2 cm, 15 μl of soy-specific rabbit antisera are deposited (the quantity of the 2 reagents can be reduced to 10 μl by working with samples that have not been exposed to temperatures higher than 90°C for 60 min).

The electrophoresis is performed in tris-glycine buffer, pH 9.0, by applying 200 V for 40 min.

At the end of the migration the strips are washed in 0.85% saline solution for 1 hr, whilst continually shaking the solution being changed every 15 min. At this stage it is possible to observe lines of precipitation by staining the strip with Coomassie brilliant blue according to the technique of Pizzolato, Pizzolato & Agostoni (1972). In case the soy protein concentration in the product is very low one is able to perform the indirect immunofluorescence technique before staining: the strips are buffered in PBS, pH 7.2, for 15 min and then placed in a humidified box protected from light. After removing the excess liquid (with filter paper) from the surface of the strips, 200 μl of a 1:4 dilution of anti-rabbit γ-globulin fluorescent serum are distributed in the area of the strip between the deposits of the reagents.

After incubation at room temperature for 15 min the fluorescent antiserum which remains unbound is removed by washing the strip in PBS (2 washes of 15 min, continuously shaken), and results are recorded by examining the strips by transparency, laying them directly on the screen of the U.V. lamp.

If the reaction is positive, one or more arcs of neatly fluorescing precipitation are observed between the deposit of the positive sample and that of the soy antiserum.

As a control, all the tests are conducted by using extracts prepared from the same types of samples but where the soy is omitted.

Electrophoresis in polyacrylamide gel

Extraction of protein. The technique of Benincasa *et al*. (1978), is modified as follows: lipids are removed from every sample by acetone extraction followed by centrifugation at 18,000***g*** for 15 min. This treatment is repeated three times and then the residue undergoes hot extraction using a solution consisting of 96 parts tris-HCl (pH = 6.8), three parts sodium dodecylsulphate (SDS) and one part beta-mercaptoethanol. The extraction repeated four times, is performed at 100°C in a water bath for 10 min and then samples are centrifuged at 18,000***g*** for 15 min; the supernatant is saved from time to time in a single test tube and then analysed by electrophoresis.

Method. Electrophoresis is performed in a polyacrylamide gel according to the technique of Davis (1964), employing a discontinuous buffer system.

The polyacrylamide gel rods are prepared by mixing 1 ml of solution A (48 ml of 1N HCl, 36.6 g of tris and 0.23 ml of Temed, dissolved in distilled water to give a final volume of 100 ml); 2 ml of solution B (28.0 g of acrylamide and 0.735 g of bis-acrylamide dissolved in 100 ml of distilled water); 1 ml of distilled water and 4 ml of solution C (0.14 g of ammonium persulphate dissolved in 100 ml of distilled water). In such a way, a final concentration of acrylamide equal to 7% and a pH = 9 is obtained.

The polyacrylamide gel is then poured into glass tubes (12.5 cm × 5.0 mm diameter) to a height of 8 cm.

In order that polymerization of the gel occurs, leaving the upper surface level flat, distilled water is carefully layered on the acrylamide column; after polymerization has occurred (about 1 hr) the layer of water is substituted by an equal quantity of solution D (0.06% tris, 0.288% glycine and 0.01% SDS in distilled water with a pH = 8.4).

The gels are then loaded into the apparatus (Bio-Rad, model for tubes) which has a refrigeration of about 3°C. The anode and cathode partitions are then filled with solution D; after placing the samples to be examined (20 μl), 3mA for every gel are applied for 3 hr.

At the end of the migration, the gels are extracted from the tubes, fixed with 12% tricloroacetic acid in 25% isopropylic alcohol solution and then stained with Coomassie brilliant blue (fast stain) for 10 hr; 10% acetic acid in 25% methanol solution is used for destaining.

Results

Crossover electrophoresis, even if conducted without the use of fluorescent antiserum, allows the observation of specific precipitation arcs employing the extracts obtained from all samples (soy content varying from 10 to 1%) heated from 60 to 90°C for 60 min.

At higher temperatures, until 125°C for 25 min, such procedure reveals with certainty the soy protein, provided that its concentrations are equal to at least 5% (Fig. 1).

Crossover electrophoresis with indirect immunofluorescence shows more sensitivity in that it allows one to observe evident, specific precipitation arcs,

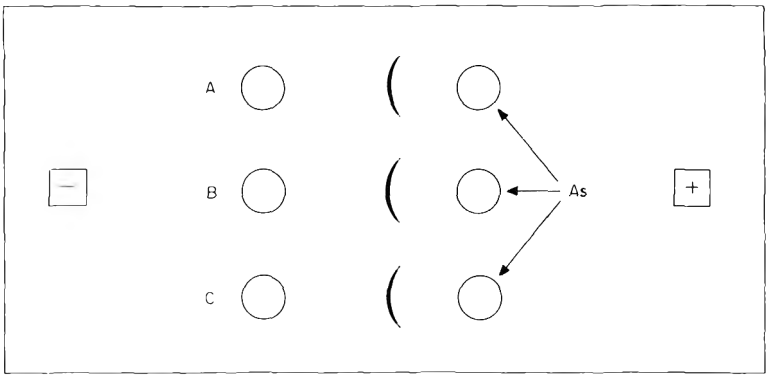

Figure 1. Crossover electrophoresis of meat samples containing different concentrations of soy protein and heated at various temperatures. A, 1% (90°C for 60 min); B, 10% (125°C for 25 min); C, 5% (125°C for 25 min); As, soy protein antiserum.

even when the soy content in the samples exposed to 125°C for 25 min is equal to 2.5%. Moreover, this procedure has revealed specific reactions, though the observation of them is difficult, even with soy concentrations equal to 1%.

Electrophoresis in polyacrylamide gel, even with all the difficulty connected with preparation of the extracts and execution of the procedure, still reveals in all of the samples examined the migration band characteristic of soy protein.

Discussion

The presence of even small quantities of soy protein in meat products heated to the level of commercial sterility (125°C for 25 min) can still be revealed through the aid of a new serological procedure: crossover electrophoresis with indirect immunofluorescence.

In effect, while the animal proteins undergo at this temperature a relevant denaturation with a consequent total loss of the original immunological reactivity, the more resistant vegetable proteins, although undergoing some configurational changes are able to present primitive antigenic sites which are still active.

The confirmation of this observation is the results of the present work, which demonstrate how it is possible to reveal, in our experimental conditions, a precipitation reaction between a specific antiserum and soy protein even when this is present in meat products exposed to high temperatures at levels corresponding to an addition of $\geq 2.5\%$. Naturally the reliability of the method has to be confirmed on extensive tests as suggested by the work of Beljaars & Olsman (1977).

We have observed that this method is more rapid (~3 hr to read the results) and sensitive compared to other immunological procedures such as radial and bidimensional immunodiffusion or electroimmunodiffusion techniques which reveal soy protein at these concentrations only using antisera prepared with heated soy protein.

In essence, the already high sensitivity of the crossover electrophoresis is further increased by the use of anti-rabbit γ-globulin antibodies (the animal species in which anti-soy serum is produced) marked with fluorescein, which eventually bind themselves to the antigen-antibody complex allowing visualization of the fluorescent precipitation arcs even when these are not visible by the classic staining techniques.

Furthermore, the precipitate marked with fluorescein can be further stained with Coomassie brilliant blue providing a two-fold finality of recording, and at the same time, making the reaction more evident.

The assays which have been conducted using electrophoresis in polyacrylamide gel, while on one hand confirming the extreme sensitivity of this method (detection of concentrations of soy $\geq 1\%$), have on the other hand confirmed how difficult and time-consuming this method is, both in the preparation of the extracts and execution of the procedure.

Moreover, it is likely that, as already observed with other immunological methods (Llewellyn & Sawyer, 1977), the use of monospecific antisera produced by inoculating soy protein previously denatured by heat will render the crossover electrophoresis even more sensitive than all the actual analytical procedures.

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Effects of water blanching on pea seeds
I. Fresh weight changes & solute loss

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Summary

Laboratory scale experiments were carried out to elucidate the main mechanisms by which weight changes and solute loss occur in immature pea seeds during blanching in water. Studies involved specially grown Dark Skinned Perfection peas, and commercial samples of carrot root. Net weight loss was shown to arise primarily from the contraction of the tissues on loss of cell turgor. With time, this net loss increased due to the continued diffusion of solutes out of the tissue. The presence of the testa surrounding the pea cotyledons significantly reduced the possible solute and overall weight losses from whole peas. Damage inflicted by bruising the pea and slitting the seed coat resulted in increased solute loss at a commercial blanch temperature of 97°C.

Introduction

The water relations of plant cells have been studied intensively for more than 70 years, and in particular much information is available concerning the process of water movement into and out of storage tissue such as potato tuber and carrot root. It is well known, for example, that fresh carrot root tissue when immersed in water will usually absorb it by osmosis and solutes may at the same time diffuse out of the tissue. At room temperature, water uptake may continue for some hours; at higher temperatures such as those used in blanching, the cell membranes may quickly be damaged, osmosis ceases, turgidity is lost and sap from the cell vacuoles can diffuse out freely.

The effects of blanching on the losses of water and solutes from vegetable tissues have been studied by many workers (Lee, 1958), but the mechanisms involved, particularly in complex plant organs, have not been clearly identified

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(Selman, 1978). Weier & Stocking (1949) concluded that five main internal factors probably controlled water retention by plant tissue during cooking: (i) the concentration of osmotically active materials within the cytoplasm and cell vacuoles, (ii) the permeability of the protoplasm, (iii) the amount of colloidally active material within the cytoplasm, vacuoles and cell walls, (iv) the elasticity of the cell walls, (v) the presence of intercellular spaces. It was considered that the state of turgor and cell wall elasticity would be the major factors influencing water loss from pea seed tissue following destruction of the cytoplasmic membranes. Loss of solutes would almost certainly be influenced by the initial concentration in the cells (Selman, 1977). Many solutes are lost from peas during commercial blanching, such as water soluble nitrogen compounds, vitamins and minerals, however sucrose contributes more than half the total loss of solids (Bosund, 1962). Other factors which must be involved include the maximum temperature, the time taken to reach that temperature and the concentration of solutes in the blanch water.

Although the osmotic basis of water movement in living cells is generally accepted (Steward, 1959), it should be noted that the early analogy between a plant cell and an inorganic cell bounded by a semi-permeable membrane has long been superceded, for solutes must enter and leave the living cell and cells capable of growth must have walls which are both elastic and plastic. It is perhaps pertinent to note that much of the early physiological work was carried out on mature tissues, incapable of growth, such as potato tuber.

These studies were made with young pea seeds (freezing maturity) in relation to the problems of blanching. The young pea seed is completely enveloped by a thin seed coat or testa, except for the micropyle, the site at which the pollen tube entered the ovule and through which the radicle will emerge (Hayward, 1938). Within the coat are two fleshy cotyledons which are actively growing, respiring and laying down food reserves including aleurone grains, starch grains and some lipid material, derived from amino acids, sugars and other compounds supplied by the parent plant. A small but growing embryo is attached to the cotyledons. The water relations of this complex organ were expected to differ somewhat from those of the more uniform masses of parenchyma tissue which comprise the potato tuber or carrot root.

Materials and methods

Plant material

Young pea seeds of the cultivar Dark Skinned Perfection were harvested at tenderometer reading 100–105 from plants grown on a specially prepared site in Surrey in 1974–5. The environmental conditions in 1975 resulted in Dark Skinned Perfection peas that were atypical to those harvested in 1974. The

variation between samples in 1975 was also greater than that in the previous year. Results obtained in 1975 cannot therefore be related directly to results obtained in 1974 (Selman, 1977). Commercial samples of mature carrot roots were also used for comparative purposes. There were forty pea seeds (*c.* 20 g fresh weight) per sample, the peas selected having a diameter between 8.7 and 10.3 mm, and a tenderometer reading between 100 and 105. In some experiments the seed coats were removed from twenty peas to provide forty separate cotyledons per sample. Carrot root tissue was cut into blocks (1 × 1 × 0.5 cm) comprising only secondary xylem tissue, and each sample contained 10 blocks (*c.* 5 g fresh weight).

The exposed surfaces of tissue samples were quickly and gently wiped twice with absorbent paper to remove drops of free liquid and the sample weighed in a closed dish. Sample weights after blanching or exposure to chloroform were determined in the same way. Dry matter contents were determined after drying duplicate samples to constant weight in a circulated air oven at 103–105°C. Pea cotyledons contained 20.7 ± 0.4% dry solids, and the carrot root 12.8 ± 1.6% dry solids.

Sap was expressed from the tissues by placing the sample between two rigid perspex plates (12 × 8 × 0.5 cm) and applying a maximum pressure of 4.1 MN/m^2 using a small hand operated hydraulic press. A measure of the solute content of the sap was obtained using an Abbe refractometer calibrated with sucrose solution at 20°C. Pea cotyledons and carrot root contained 19.8 and 7.8% soluble solids respectively (expressed as sucrose).

Control of turgor

By adjusting the initial water content of the tissues, varying degrees of turgor were obtained and the effects on water and solute loss studied after damaging the cell membranes by either immersion in warm water (blanching) or exposure to chloroform vapour at room temperature. The water content of fresh tissue was increased by immersion in distilled water for varying times at 20°C, and decreased by allowing water to evaporate from similar tissue placed in a circulated air oven at 30°C for various times.

Exposure of tissues to high concentrations of chloroform vapour results in a rapid increase in permeability of the membranes, followed by cell death due to irreversible disorganization of the lipid layer of the cytoplasmic membranes (Steward, 1959). Use was made of this to study water and solute losses divorced from the effects of heat and surrounding water associated with normal blanching procedures. Chloroform was placed in the lower compartment of a large desiccator to a depth of 2 cm, and in the upper chamber fresh tissues of known weight were placed in beakers. Serial samples were removed and weighed using the standard procedure.

Blanching

In this context blanching refers to the immersion of plant sample in water at temperatures from 20 to 97°C for periods not exceeding 25 min. 10 g samples of pea seeds (carrot roots) were blanched in 80 ml distilled water (pH 6.5–7.0) in a 250 ml beaker, agitating the water with a magnetic stirrer at 78 rpm. The tissues were kept immersed by a disc of aluminium mesh. To minimize temperature changes at 40°C and above when the tissues were added, the beaker was placed over a bunsen burner for 10 sec before returning it to an electric hot plate held to within ±0.5°C of the required temperature. The beaker was covered during blanching to minimize evaporation.

After the required blanch time, the peas (or other tissues) were sieved out and the blanch water collected in an ice cooled volumetric flask and subsequently made up to 100 ml with distilled water. The peas were blotted and their blanched weight determined. No post blanch cooling of the tissues was done in this work. A 50 ml aliquot of blanch water was dried to constant weight at 103–105°C to determine its solute content.

Experiments and results

Temperature of blanching

(a) *Carrot root*. An experiment was first made to study the changes in fresh weight and solute losses which occurred when blocks of fresh carrot root tissue were immersed in water for 10 min over the range 20–90°C. The results are summarized in Fig. 1 and illustrate the changes expected in a relatively homogeneous storage parenchyma. At the lower temperatures water is absorbed due to the diffusion pressure deficit of the cells, and at higher temperatures with increasing permeability leading to death of cells, water is lost. Solutes were lost under all conditions but the amount increased substantially in the temperature range 50–60°C. A small solute loss would be expected to occur at temperatures near ambient from the surface cells ruptured during the cutting of the blocks.

(b) *Whole peas*. A similar experiment was made to study the changes in fresh weight and solute losses which occurred when samples of whole peas were immersed in water for 10 min over the range 25–85°C. The results are summarized graphically in Fig. 2.

(c) *Pea cotyledons*. To study the effect of the testa on fresh weight changes and solute losses, samples of pea cotyledons were immersed in water for 10 min over the range 25–80°C. The results are summarized in Fig. 3. In this case losses showed a marked increase above 45°C and increased approximately linearly with temperature.

Consideration of Figs. 1 and 2 showed that the changes in whole peas induced by blanching were similar but not identical to those in a mature storage tissue; in

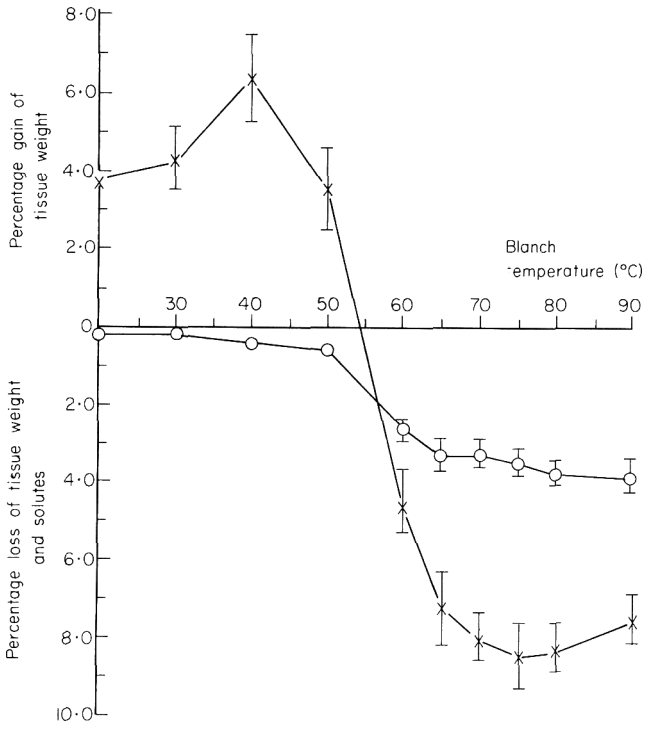
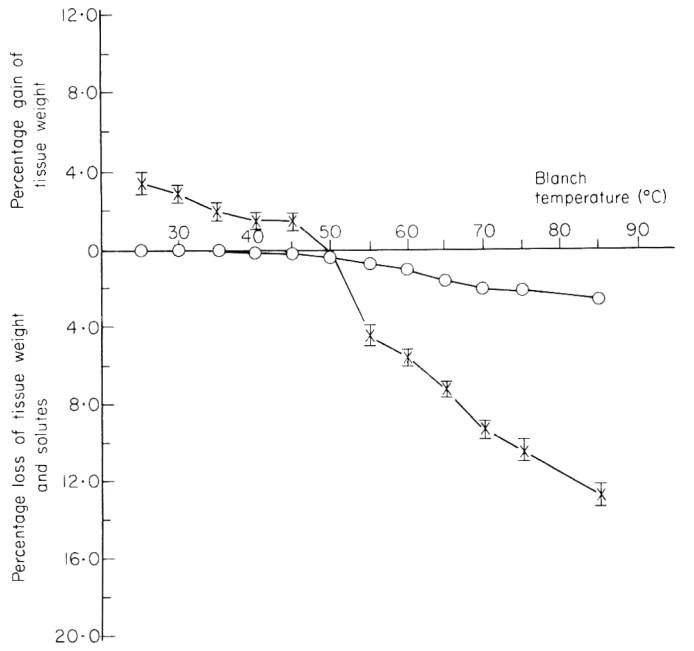


Figure 1. Percentage tissue weight changes and solute loss from carrot root samples blanched for 10 min at the given temperature. (Each point derived from three separate experiments and the losses in each experiment being determined in duplicate, i.e. means of three duplicated replicates, 1974). ○, solutes; ×, tissue.

particular solutes were lost more readily at the lower temperatures from the storage tissue than from the peas but at higher temperatures it was the whole peas which lost more solute. Comparison of Figs. 2 and 3 clearly showed the effect of the testa, which, when present, effectively reduced the movement of water both into and out of the seeds and also reduced the magnitude of the solute loss. The major difference due to the testa was that loss of tissue weight and solutes from whole peas increased regularly after the critical temperature of 45°C, whereas in carrot root and cotyledons, the losses reached a maximum at about 70°C. This temperature was then used in further studies.

Time of blanching

(a) *Carrot root*. An experiment was made to study the changes in fresh weight and solute losses which occurred when blocks of carrot root tissue were immersed in water at 70°C over the time interval 0.25–10 min. The results are summarized in Fig. 4.



Figu e 2. Percentage tissue weight changes and solute loss from whole pea samples blan· hed for 10 min at the given temperature (means of two duplicated replicates, 197⊂). ○, solutes; ×, tissue.

(b) *Wł ole peas*. Similar experiments were carried out using whole peas at 70, 85 and ⊊7°C. Results are summarized in Figs. 5, 6 and 7 respectively.

Comp arison of Figs. 4 and 5 indicate that the changes in carrots were again similar t⊕ those in whole peas, but solute loss occurred more rapidly in carrot root. Frcm Figs. 6 and 7 it is seen that blanching for 10 min at 85°C resulted in the same fresh weight loss as 2 min at 97°C but there was less solute loss at 97°C.

The effect of turgor on losses during blanching

From the pattern of results obtained it seemed likely that the bulk of the fresh weight lcss occurred as turgor was lost and the cells contracted. An experiment was therefore carried out to demonstrate loss of fresh weight when the cytoplasmic membranes were disorganized. Samples of carrot root and pea cotyledons we e exposed to chloroform vapour at room temperature and serial samples were removed and weighed in the standard manner. The results are shown in Fig. 8. It is seen that the maximum tissue weight loss is very similar to the maxi mum tissue weight loss induced by blanching for 10 min at 70°C shown in Figs. 1, 3 and 4. The relative slowness of the loss with time is attributed to the

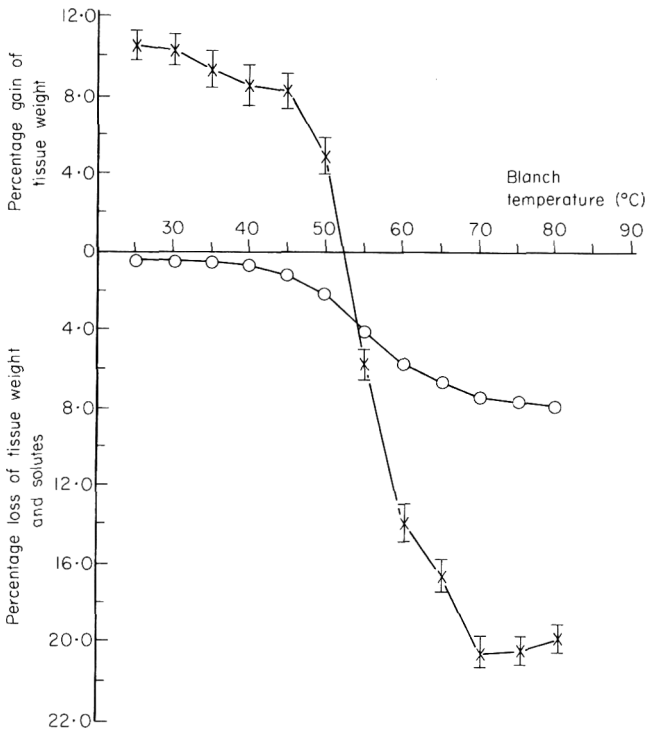


Figure 3. Percentage tissue weight changes and solute loss from pea cotyledon samples blanched for 10 min at the given temperature. (means of two duplicated replicates, 1974). ○, solutes; ×, tissue.

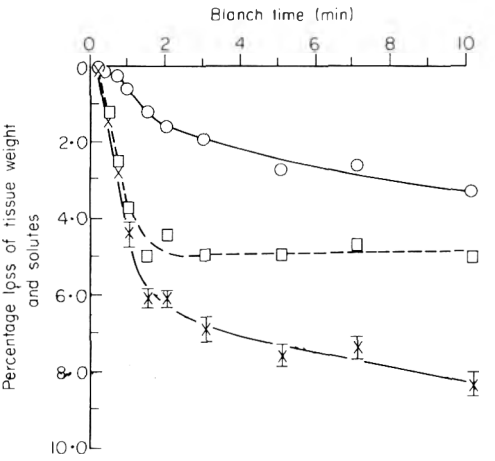


Figure 4. Percentage loss of tissue weight, solutes and water (by difference), from carrot root samples after the given blanch time at 70°C (means of two duplicated replicates, 1974). ○, solutes; □, water; ×, tissue.

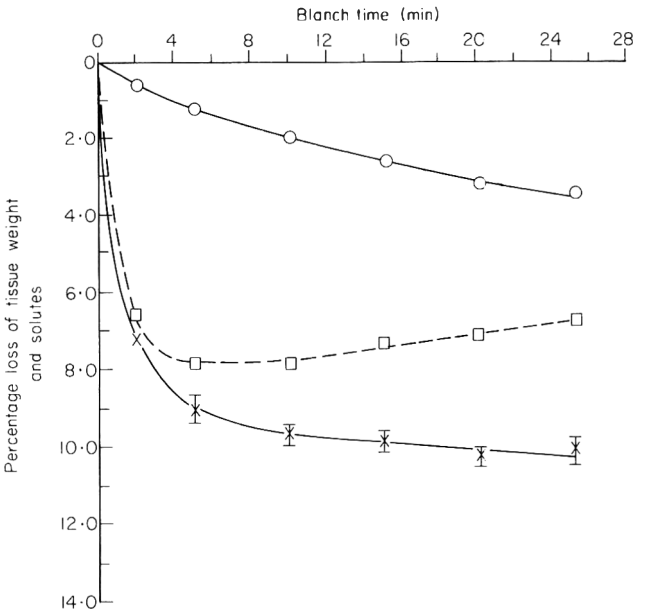


Figure 5. Percentage loss of tissue weight, solutes and water (by difference), from whole pea samples after the given blanch time at 70°C (means of two duplicated replicates, 1974). ○, solutes; □, water; ×, tissue.

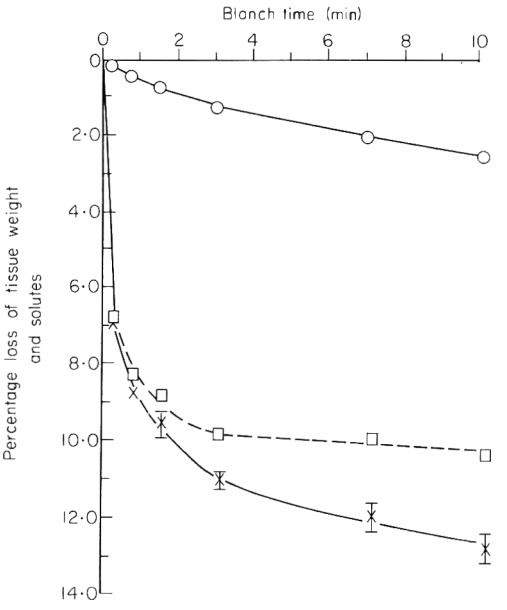


Figure 6. Percentage loss of tissue weight, solutes and water (by difference), from whole pea samples after the given blanch time at 85°C. (means of two duplicated replicates, 1974). ○, solutes; □, water; ×, tissue.

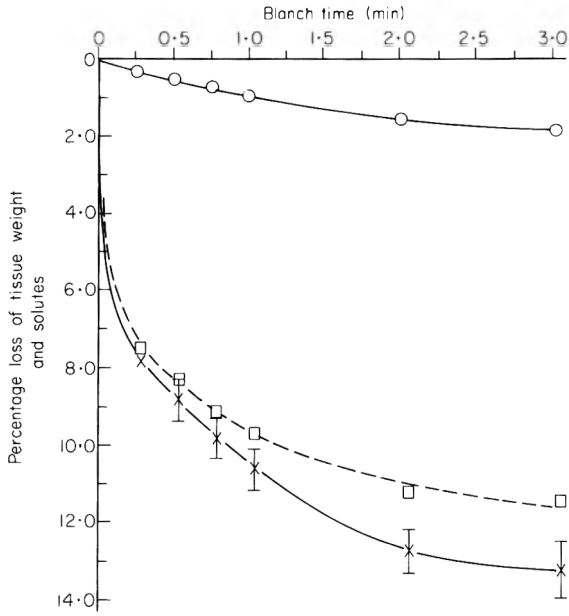


Figure 7. Percentage loss of tissue weight, solutes and water (by difference), from whole pea samples after the given blanch time at 97°C. (means of two duplicated replicates, 1974). ○, solutes; □, water; ×, tissue.

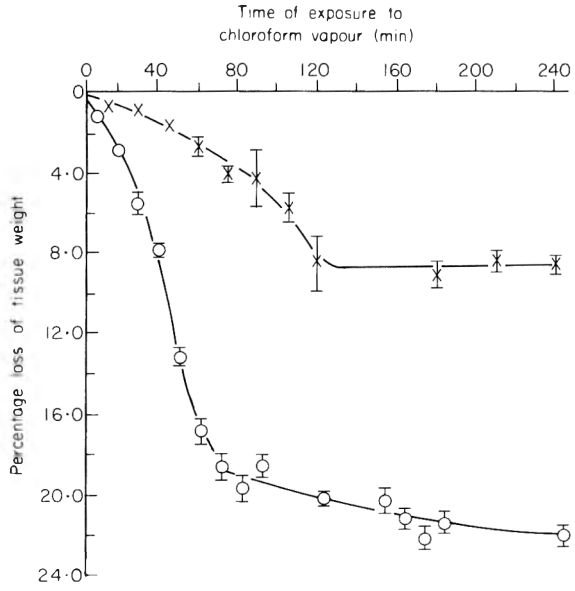


Figure 8. Percentage loss of tissue weight from samples of pea cotyledons and carrot root after the given time of exposure to chloroform vapour (means of two duplicated replicates, 1974). ×, carrot; ○, pea cotyledons.

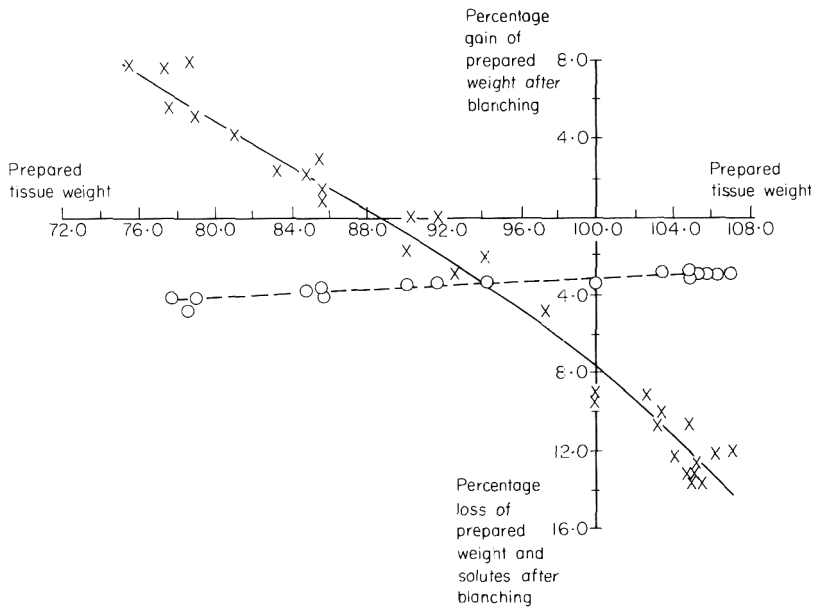


Figure 9. Percentage change of prepared tissue weight and percentage loss of solutes from carrot root samples blanched for 10 min at 70°C (points from four experiments, 1975). ○, solutes; ×, tissue.

time required for chloroform to diffuse into the tissue. Further experiments were carried out to study the effect of turgor on losses during blanching.

(a) *Carrot root*. Samples of carrot tissue were prepared of differing initial water contents (see methods). Fresh weight changes and solute losses were recorded after blanching for 10 min at 70°C and the results summarized in Fig. 9. In this material solute losses were positively correlated with the initial water content of the tissue. Increasing the original water content before blanching resulted in fresh weight losses greater than the amounts originally absorbed. To achieve an increase (or no change) in fresh weight after blanching entailed initial losses in the tissue greater than would have been obtained if the tissues had been blanched at their original water content.

In a second experiment, carrot tissue covering a range of prepared water content was exposed to chloroform vapour for 180 min at room temperature (see 'Materials and methods'). The resultant fresh weight losses are shown in Fig. 10. Thus material of the highest initial water content lost the greatest weight as in the previous experiment. This seemed to indicate the importance of the initial water content (or degree of turgor) in relation to water loss following disorganization of the cell membranes.

(b) *Whole peas*. Water contents of whole pea samples were adjusted as described above and the material blanched for 10 min at 70°C. The changes are summarized in Fig. 11.

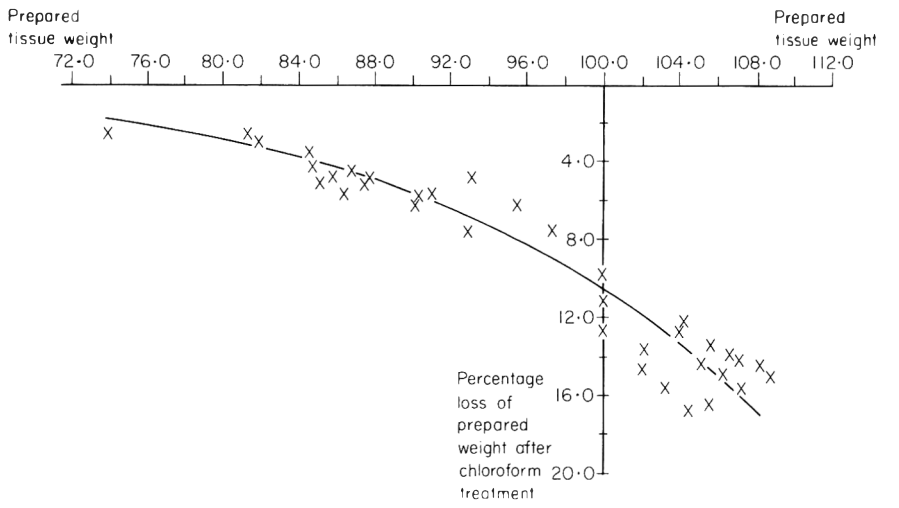


Figure 10. Percentage loss of prepared tissue weight from carrot root samples exposed for 180 min to chloroform vapour (points from three experiments, 1975).

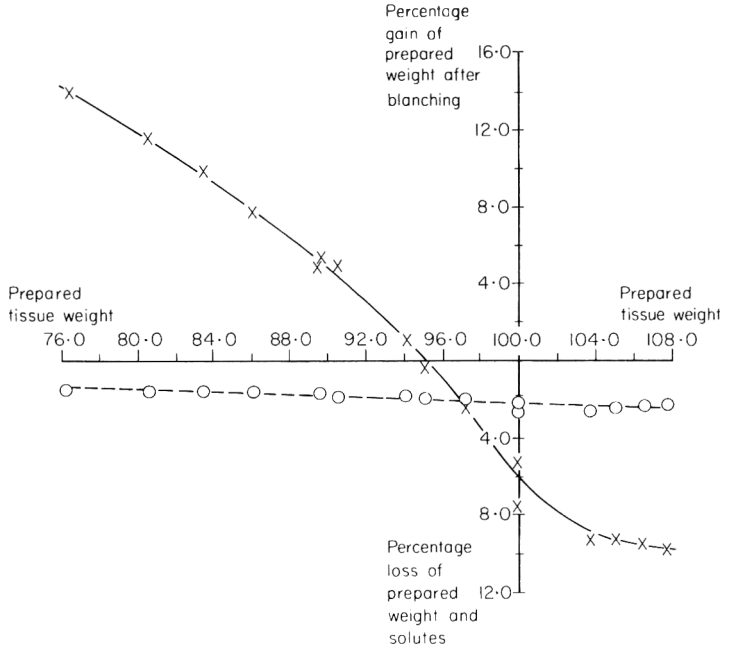


Figure 11. Percentage change of prepared tissue weight and percentage loss of solutes from whole pea samples blanched for 10 min at 70°C (means of two duplicated replicates, 1975). ○, solutes; ×, tissue.

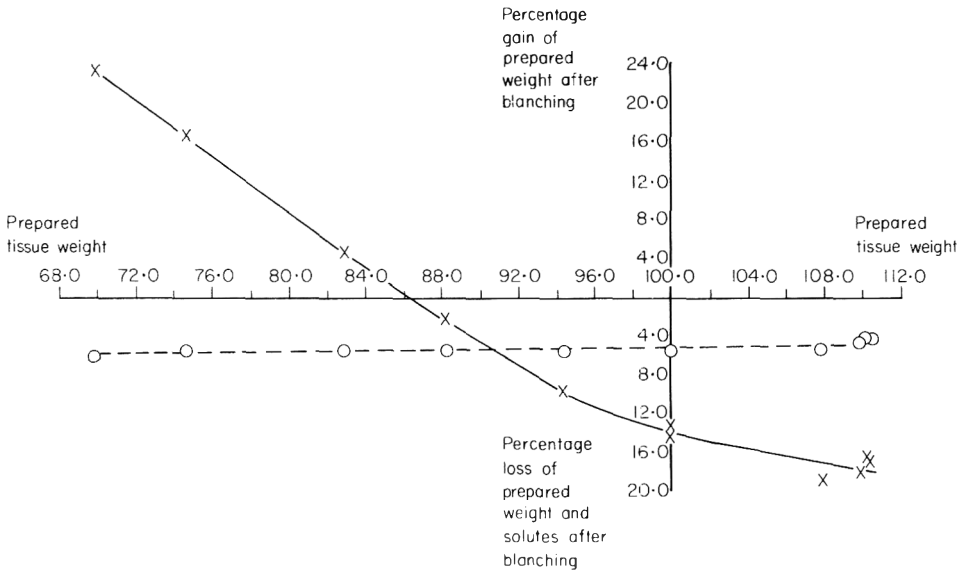


Figure 12. Percentage change of prepared tissue weight and percentage loss of solutes from pea cotyledon samples blanched for 10 min at 70°C (means of two duplicated replicates, 1975). ○, solutes; ×, tissue.

(c) *Pea cotyledons*. A similar experiment was made with samples of pea cotyledons which were blanched for 10 min at 70°C. The data are summarized in Fig. 12.

Thus with whole peas, prepared weight remained constant before and after blanching samples which had been initially allowed to lose about 5% of their original fresh weight. Seeds allowed to take up water amounting to more than 2% of their original fresh weight, lost less than the amount originally absorbed. Similar results were obtained with cotyledons except that fresh weight loss from the 'unprepared' material was about 14% as compared to 6% when the seed coat was present.

Solute losses from whole peas were slightly greater from the more turgid material, but in cotyledons were unaffected by turgidity. This suggested that the permeability of the seed coat to solutes is increased when it is stretched (or more hydrated), or that the micropyle opens wider, or both. The importance of the micropyle was next studied.

Significance of the micropyle

Observations made during blanching showed that both air and solution could be seen issuing from the micropyle. An experiment was made to determine the significance of the micropyle as a means for solute and water loss during blanching. Polyurethane varnish was applied in two coats to seal the micropyle

Table 1. Percentage weight and solute losses from whole peas and whole peas with sealed micropyles blanched for four different times at 70 and 97°C (means of two duplicated replicates, 1975)

Temperature (°C)	Time (min)	Percentage weight loss (range ± 0.7%)		Percentage solute loss (range ± 0.2%)	
		Unsealed	Sealed	Unsealed	Sealed
70	1	3.4	2.4	0.5	0.3
	3	5.7	2.2	1.3	0.7
	5	8.0	2.6	1.4	0.9
	12	9.4	2.0	2.5	1.6
97	0.5	2.4	2.2	0.3	0.3
	1	3.5	3.1	0.6	0.6
	2	4.6	5.2	1.2	1.2
	3	7.2	7.4	1.6	1.5

and surrounding area and the peas were then stored in a large desiccator containing water, to allow the polyurethane to harden overnight, whilst at the same time inhibiting dehydration of the peas. Unsealed peas to be used in the comparative blanching tests were stored under the same conditions. The fresh weight and solute losses from sealed and unsealed peas were studied after blanching for various times at 70°C and 97°C. The results are summarized in Table 1.

At 70°C both the fresh weight and solute losses from the unsealed peas were greater than from the sealed, suggesting than the micropyle played an important role. However at 97°C, both solute and fresh weight losses from sealed and unsealed peas did not differ appreciably. A firm conclusion from this experiment could not be drawn as it was observed that rapidly expanding intercellular air had unseated the polyurethane plug at 97°C. Nevertheless it seems likely from observations and the results at 70°C that the micropyle does serve as a passageway for water and solute loss.

Effect of damage

As the fresh weight and solute losses had been shown to be retarded by the presence of the testa, an experiment was made to study the effect of various types of damage to the pea on subsequent losses during blanching. The proportion of peas damaged during commercial harvesting may be of the order of 70% (Selman, 1977). Three grades of damage were chosen; slit, bruised and slit plus bruised. Standard pea samples were taken and the testa of each pea cut with a scalpel to produce a 5 mm slit. Bruised peas were produced by rolling a flat surface over each pea until the cotyledons were movable within the testa, effecting extreme bruising in a manner similar to that used by Eriksson & Von

Table 2. Effect of damage on solute loss from whole peas blanched at 97°C (means of two duplicated replicates, 1975)

Blanch time	Percentage solute loss (range ± 0.2%)			
(min)	Whole	Slit	Bruised	Bruised plus slit
0.5	0.3	0.4	0.4	0.9
1	0.5	0.8	0.8	1.4
2	1.0	1.3	1.7	2.0
3	1.8	1.6	2.0	2.6

Sydow (1964). The slit plus bruised grade was prepared by introducing a 5 mm slit to the bruised pea. The solute losses from damaged and undamaged peas were studied after blanching for various times at 97°C. The results are shown in Table 2.

Solute loss from slit and bruised peas was similar to that from whole undamaged peas. However slit plus bruised peas lost significantly more solutes ($P = 5\%$). The effect of damage on nutrient loss and subsequent factory effluent in commercial operations could thus be significant.

Conclusions

It is apparent that weight loss from vegetable tissue during water blanching occurs by two main mechanisms. On loss of turgor the cells contract and express some of the cell contents. Simultaneously the damaged cell membranes allow free diffusion of solutes out of the cells. Due to continued diffusion of solutes out of the tissue, the net tissue weight loss consists of a greater amount of the tissue solutes than that represented by the expressed cell sap.

In the case of peas, the presence of a testa around the cotyledons significantly retarded the loss of cell contents, and results suggested that at least initially, cell contents passed out through the micropyle. Damage to the pea in the form of a slit in the testa did not significantly increase the losses of solutes and this suggested that some cell contents do escape through the testa. Bubbles of air were seen to form on the surface of the testa, but it was not proven whether they originated from rapidly expanding intercellular air that had created openings in the testa, or from air dissolved in the blanch water. However a combination of bruising and slitting, as is most likely to occur during mechanical vining, did result in significantly increased solute loss. This is not surprising as bruising would cause substantial cell damage and hence assist in the release of cell contents during blanching.

Loss of solutes appears to be influenced largely by the initial solute concentration of the cell sap. Whereas the overall tissue weight loss appears to be governed largely by the initial cell volume, inherent cell turgor pressure and the

elasticity of the cell walls. Although full analysis of tissue volume changes was not carried out, the relative net weight and solute losses observed might be expected from consideration of the relative structures and functions of pea and carrot root tissue.

Preblanch water uptake by carrot tissue resulted in correspondingly increased net weight losses during blanching. However in the case of pea cotyledons and whole peas, water uptake amounting to over 2% of the initial weight, did not produce correspondingly larger losses during blanching. It is not clear whether this was due to the effect of heat on the cell wall elasticity in the cotyledons, but certainly the partial gelatinization of the pea starch observed at 70°C may to some extent have contributed to the retention of water in the tissues. The greater part of the net weight loss may therefore have occurred in the temperature range 55–70°C. Artificially increasing the water content prior to blanching could affect the yield and probably the eating quality of peas, but simply raising the yield is not necessarily in the interests of the consumer.

It is appreciated that there are other methods of blanching peas, but there have been difficulties for example with off flavours resulting after steam blanching. However if such problems could be overcome it seems possible that the presence of the testa encapsulating the cotyledons might aid nutrient retention even further during a 'dry' blanching operation.

Finally it must be emphasized that these studies were directed towards the effects of the blanching operation alone and therefore a post blanch cooling operation was not included. Simple tests indicated that some water uptake occurred when blanched whole peas were cooled in water. This demonstrated that these results cannot be directly compared to those of other workers who have included a cooling stage as an integral part of their defined blanching operation.

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Development and acceptability testing of a modified salt/fish product prepared from shrimp by-catch

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Summary

Methods for preparing salt/fish cakes from the minced flesh of fish characteristic of the shrimp by-catch are described. Heat treatment of the salt/fish mix at 70°C for 2 hr prior to low temperature dehydration promoted a rapid initial exudation from the material, a factor which considerably reduced total drying time. Acceptability testing in Mexican communities indicated a significant preference ($P<0.01$) for the preheated cakes when incorporated into local dishes. It is suggested that such processing is especially suitable for application to shrimp by-catch in developing countries.

Introduction

The abundance of potential resources of fish and other marine organisms capable of contributing to human food supplies has often been emphasized (FAO, 1975; Disney & Poulter, 1977). These fisheries resources exist in the main in tropical and subtropical waters adjacent to developing countries, where the demand for food of animal origin is most pressing. Whilst many of the underutilized species are not presently landed, in some circumstances large quantities of fish are caught incidentally and subsequently discarded at sea. A prime example of this is the by-catch of shrimp trawls, which represents a global annual loss of several million tons of marine food (Meinke, 1974; FAO, 1975; Allsopp, 1977). This wastage occurs above all in the developing regions of the world.

Although a number of problems are implicit with regard to the utilization of shrimp by-catch, these including economic and logistical drawbacks, information regarding possible processing methodology is scant. The nature of the by-catch, which mainly consists of an enormous variety of small demersal fish

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species (Young, 1978), would appear to preclude conventional distribution in iced or frozen form. Moreover, in tropical developing areas, it seems more appropriate to investigate the development of low-cost food products with extended shelf-lives at ambient temperatures.

It has previously been demonstrated that rapid mixture of fish flesh with sufficient salt to effect a loss of water holding capacity of the muscle proteins is a promising method of processing underutilized fish species (Del Valle & Nickerson, 1968; Del Valle & Gonzalez-Inigo, 1968, Andersen & Mendelsohn, 1972; Bligh, 1977; Poulter & Disney, 1977). The process affords an effective and simple means of preservation. Indeed, resultant dried, salted products may be stored without refrigeration and have been shown to be favourably received when incorporated into the diets of some communities in developing areas (Del Valle *et al.*, 1973).

The current article describes the application of rapid salting to fish minces prepared from shrimp by-catch. In particular, the influence of possible modifications to the salting and drying process is examined. Further attention has been given to the organoleptic acceptability of the developed products, with special reference to a rural area of Mexico.

Materials and methods

Raw materials

Samples of shrimp by-catch were recovered fresh from commercial vessels fishing in the Gulf of California and stored in frozen form prior to processing. A batch weighing 34 kg and comprising a representative mixture of fish species was used for the study. The formulated composition was as follows: Pacific flagfin mojarra (*Eucinostomus* spp.) 9.2 kg; Pacific sand perch (*Diplectrum pacificum*) 8.4 kg; grunt (*Orthopristis cantharinus*) 6.0 kg; gafftopsail pompano (*Trachinotus rhodopus*) 4.2 kg; bairdiella (*Bairdiella icistia*) 3.0 kg; red goatfish (*Pseudopeneus grandisquamis*) 1.8 kg; gulf croaker (*Micropogon megalops*) 1.0 kg; sharpnose lizardfish (*Synodus scituliceps*) 0.4 kg.

Fabrication of salt/fish cakes

Fish were headed and eviscerated manually and subsequently washed in a water bath. Elimination of the bones and skin was effected using a Paoli model 19 automatic meat and bone separator. The distance between the cylinder and the pressure plate of the separator was adjusted to 0.008 cm. The deboned flesh was intimately mixed with fine salt in a Hobart mixer. A salt concentration of 20% (as percentage of fish flesh weight) was employed throughout the study

since this amount has previously been shown to promote satisfactory cake formation (Young, 1978). The salt/fish mix was left to stand for about 30 min and then manually pressed into cakes using a simple hamburger press. About 130 g of mix was used for each cake.

The formed wet cakes were divided into two batches. One batch was preheated in an oven (Proctor and Schwartz, Philadelphia) at a temperature of 70°C for 2 hr. The other batch was not preheated. Each of these batches was further divided into two, half the cakes being dried to constant weight at 40°C in the oven and the rest being dried by exposure to the sun. The ambient temperature during solar drying varied between 33–36°C and the relative humidity between 81–89%.

Determination of drying rates

The degree of exudation from the cakes during dehydration was measured as an index of drying rate. Five individual cakes were selected at random from each batch and weighed on a Mettler P1200 balance at intervals throughout the drying period. Initially, weighing was carried out every 30 min and later at intervals of 2–8 hr.

Microbiological analysis

The microbiological quality of the products was assessed using procedures recommended by the International Committee on Microbiological Specifications for Foods (Thatcher & Clarke, 1975).

Chemical analysis

Crude protein (N × 6.25) was determined by the microkjeldahl method (Joslyn, 1970). A macrodigest was performed using 1–2 g sample, the resultant hydrolysate being diluted to 100 ml. Ten millilitre aliquots of this solution were used for distillation. Crude fat was measured by the soxhlet method using petroleum ether and NaCl by the ammonium thiocyanate and silver nitrate method (Pearson, 1970). Moisture content was determined using an Ohaus moisture determination balance at a power of 2.5 W and a drying time of 12 min.

Cooking procedure

Cakes were desalted by soaking in fresh water for 30 min. Approximately 2 l of water was used for up to four cakes. The soak water was replaced with fresh

water which was heated to boiling. The cakes were then simmered for 15–20 min.

The recipe used for the organoleptic evaluation was based on that described by Del Valle *et al.* (1973). In order to assess the acceptability of cakes prepared under the different processing conditions, the following sample treatments were compared:

Cake A: preheated at 70°C/2 hr, oven-dried at 40°C.
Cake B: non-preheated, air-dried at 40°C.
Cake C: non-preheated, sun-dried at 33–36°C.

Organoleptic evaluation

Assessors were drawn from ITESM staff, with an emphasis on the lower paid, from employees of INP including fishermen and from residents of Barrio Antena, a lower working-class area of Guaymas but some higher income staff were included and no assessor was noticeably malnourished.

Tasting took place at four separate sessions in the place where the assessors normally ate and immediately before their mid-day meal. In each case a group of 10–20 assessors were asked to consume a meal comprising the three samples under test, identified only by random numbers.

Assessors indicated which sample they liked most (and why) and which they liked least (and why) on very simple record sheets; panellists also made comments on acceptability and suggestions for methods of preparation. Plate waste was noted. From the preferences expressed a table of rank frequencies was constructed and the significance of the differences between rank totals determined either from tables (Kramer, 1963) or analysis (Kendall, 1948).

In addition to sessions at which preferences for the various samples were formally recorded there were a number of occasions on which samples were served to other tasters and their comments and reactions noted.

Results

Drying rates

The weight losses of oven-dried and sun-dried cakes are demonstrated in Figs. 1 and 2 respectively. In each case, the products could be dried to a final weight of 35–40% of their original weight. In the absence of preliminary heat treatment, the drying time for oven-dried cakes was 50 hr whereas that for sun-dried cakes was 65 hr under the atmospheric conditions indicated in Fig. 2.

Preheated cakes exhibited a very rapid initial drip loss, weight reductions of 45% of their wet weight being achieved during the first 2 hr at 70°C. When dehydration of the cakes was continued at the lower temperature, final dried

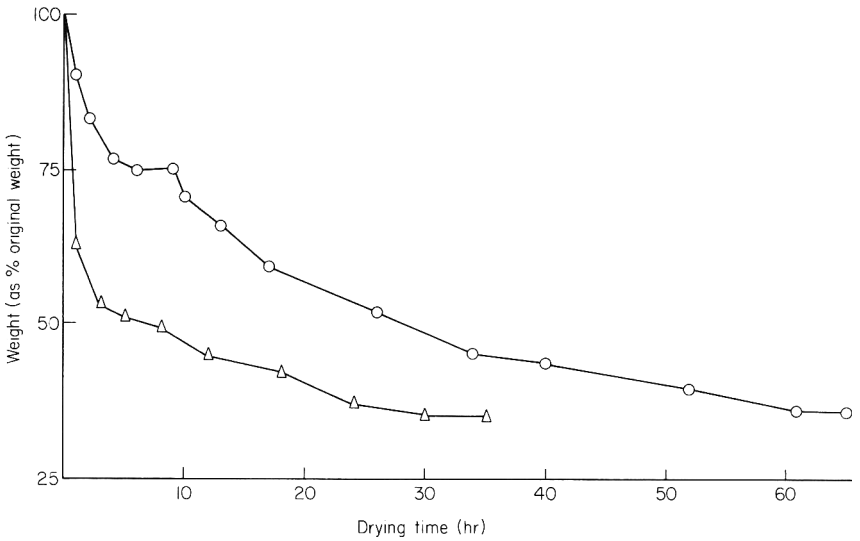

Figure 1. Weight losses of salt/fish cakes during dehydration. Δ, Cakes preheated at 70°C for initial 2 hr, drying continued at 40°C in oven; ○, cakes dried at 40°C in oven throughout.

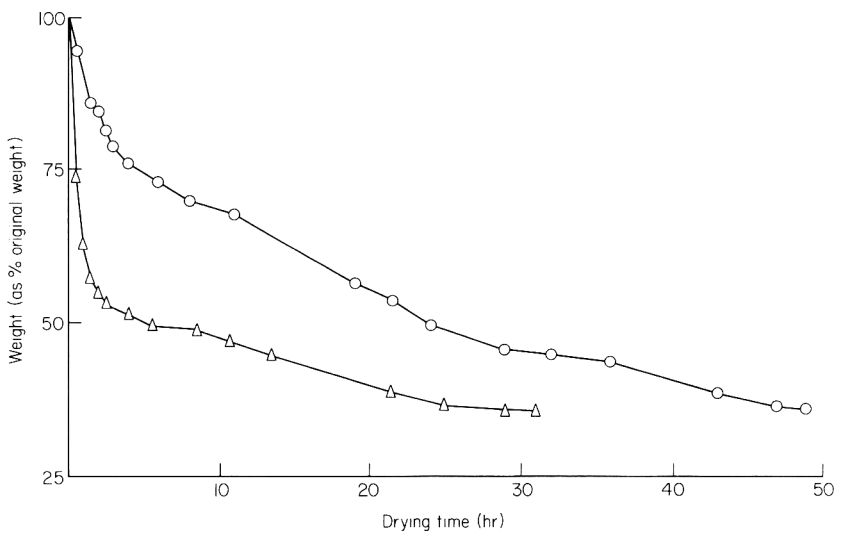

Figure 2. Weight losses of salt/fish cakes during dehydration. Δ, Cakes preheated at 70°C for initial 2 hr (in oven), drying continued in sun; ○, cakes dried in sun throughout. Sun-drying temperature 33–36°C, R.H. 81–89%.

weights were obtained after a total drying period of 30 hr. It was noted that, after preheating the salt/cakes, the total drying time was the same irrespective of whether oven-drying or sun-drying was employed.

Product characteristics

Samples of the three types of salt/fish cake used for acceptability testing are illustrated in the dried form in Fig. 3. The type A cakes (preheated) were regular in shape, smooth-surfaced and almost white in colour. Type B cakes (non-preheated, oven-dried) were less regular and compact, having a granular surface appearance and an even light brown colour. Type C cakes (non-preheated, sun-dried) appeared very coarse and granular with a grey-brown colour. White spots of crystallized salt were also present on the surface of the latter type of cake. The odour of dry cake A was mild and fresh, whereas dry cakes B and C possessed a stronger fish odour – albeit, not an unpleasant one. On handling, cakes B and C tended to crumble whereas cake A retained its original form.

During reconstitution and cooking in water, cakes B and C tended to disintegrate. However, the more compact cake A remained intact during this treatment but could be mashed equally easily in the cooked form. The final cooked material was textured and similar in appearance to cooked, minced meat. In all the samples, it was found that the salt was completely removed by the soaking and cooking method described.

Chemical composition and microbiological quality

Table 1 shows the proximate analyses and total viable counts obtained for the three types of dried salt/fish cake under test. All samples were very similar in composition although the preheated cake A appeared to have marginally lower

Table 1. Proximate analyses and total viable counts for dried salt/fish cakes. Sample A: preheated, oven-dried; sample B: non-preheated, oven-dried; sample C: non-preheated, sun-dried.

	Sample		
	A	B	C
Crude protein (%)	40.8	41.7	44.3
Crude fat (%)	8.6	8.7	7.2
Moisture (%)	7.6	7.2	7.9
Salt (%)	34.8	38.2	37.8
Total viable count (organisms/g)	1.8×10^2	4.3×10^2	6.5×10^2

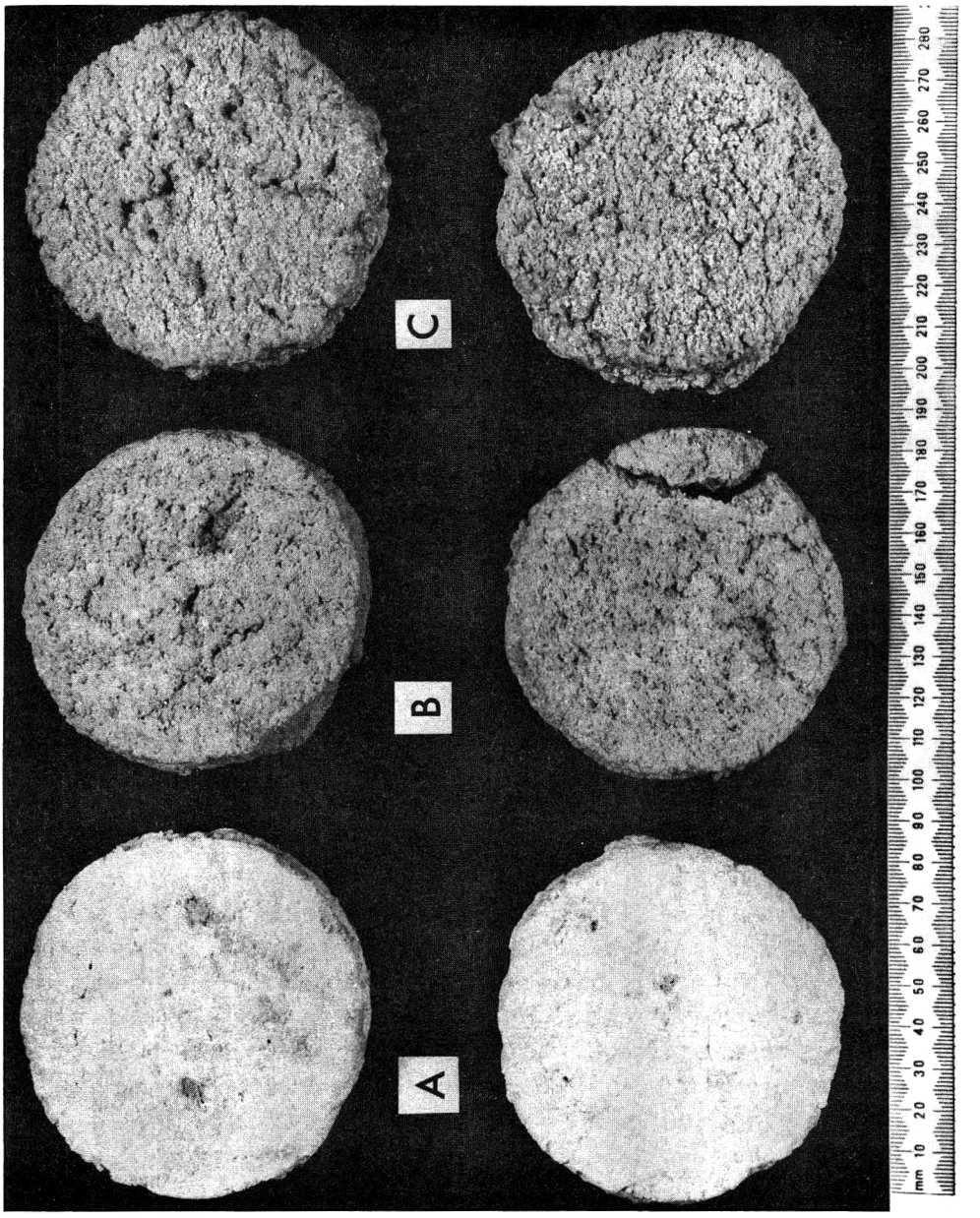

Figure 3. Samples of dried salt/fish cakes prepared from shrimp by-catch under different processing conditions. Sample A: preheated at 70°C for 2 hr, oven-dried at 40°C; sample B: oven-dried at 40°C; sample C: sun-dried at 33–36°C.

Table 2. Rank frequencies and totals determined from organoleptic testing of cooked salt/fish cakes. Sample A: preheated, oven-dried; sample B: non-preheated, oven-dried; sample C: non-preheated, sun-dried.

	Rank frequencies		
Rank	Sample A	Sample B	Sample C
1	32	12	16
1.5	1	1	2
2	18	23	14
2.5	1	4	5
3	10	22	25
Rank totals	102*	135.5	134.5

*Difference significant at 1% level.

protein and salt contents, probably due to greater losses of these components in the drip exuded during drying.

As may be expected in such heavily salted and dried products, total viable counts were low in all cases. Further analysis for the presence of pathogens indicated complete absence of coliforms, salmonella and shigella in the cakes.

Organoleptic acceptability

In general, the samples seemed acceptable. Comments from the panellists indicating outright rejection were low, one only for sample A, four for B and five for C from a total of sixty-two assessors. Most of the other comment, favourable and otherwise, was concerned with the recipes used rather than the basic material. Plate waste was low from assessors of both high and low income groups.

Tasters not taking part in the formal panels also indicated, with some exceptions, a general acceptance of the products. Not all of these tasters knew the origin of the products and comments sometimes suggested confusion with textured vegetable protein products.

Despite the general acceptability, the results from the panels indicated a significant preference ($P<0.01$) for the preheated sample A. Comments suggested that the flavour and texture of sample A were preferred, these being somewhat milder and less granular respectively than those of samples B and C.

Discussion

With the advent of devices capable of separating fish flesh from the skin and bone, a wider range of possibilities is now available for the utilization of

presently wasted fish. Quick-salting of fish minces has previously been suggested as an appropriate means of preservation in developing areas. In the studies of Del Valle and co-workers (1968), dewatering of the mince was effected by chemical separation (viz. lowering the water holding capacity of the muscle proteins by admixture with a high proportion of salt) and physical separation (viz. mechanical application of a high pressure). Drying could then be completed in an oven or in the sun. The process has been simplified, by omitting the pressure stage, and applied to oily fish by Poulter & Disney (1977). The present study has demonstrated that the inclusion of a heat treatment at 70°C for 2 hr, prior to low temperature drying, promotes an appreciable initial exudation from salt/fish cakes. This allows dehydration to be completed in much shorter times whether oven-drying or solar drying is employed.

Modification of the fish muscle proteins by combined salting and heat treatment, rather than salting alone, appears to confer further benefits on the final product. Thus, the cakes are more compact and smooth in appearance with improved mechanical properties during handling and reconstitution. The results of the acceptability tests demonstrated that some improvement was also transferred to the cooked product. The clear preference for dishes prepared from preheated salt/fish cakes appeared to be due to differences in flavour and texture. Several years ago, precooking of meat prior to drying was shown to be beneficial in that case-hardening during drying was obviated and reconstitution of the material was improved (Dunker, Hankins & Bennett, 1945; Sharp, 1953). However, the principle has not previously been applied to the preparation of salt/fish cakes.

The rapid drip loss caused by the heating process may encourage more appreciable losses of flavour components, an effect which could have been advantageous in this case. Certainly, soluble metabolites produced by microbial or enzymatic breakdown in the fish flesh and also blood pigments are likely to be more effectively eliminated. Preheating should also limit further enzymatic breakdown during dehydration and storage of the cakes, a factor which could conceivably assist the deterioration of salt/fish cakes prepared in the absence of preliminary heating.

It would seem that dried salt cakes formed from the flesh of shrimp by-catch fish show considerable promise as a component of local diets in developing countries. The variant process, involving preheating, appears to further enhance product acceptability. Shrimp by-catch could be a particularly suitable material for this type of processing. Cakes prepared from oily fish may meet consumer resistance outside those areas where dried, salted oily fish are traditionally consumed while cakes made from shrimp by-catch, using the procedure described, could have much wider acceptability. Indeed, the nature of the reconstituted material invites comparison with textured soya protein, although the result of a much simpler process. The good microbiological quality and high protein content of this type of product, noted elsewhere (Del Valle *et al.*, 1976; Poulter & Disney, 1977; Young, 1978), were confirmed during the present investigation. Moreover, recent studies show that the high biological value of

the fish protein is retained in the salted, dried products even after prolonged storage at ambient temperatures (R. H. Young & J. Velez, unpublished data).

Although it may be possible to develop a variety of food products from the fish characteristic of the shrimp by-catch, salt/fish cakes would appear to deserve special attention in lesser developed regions in view of their ease of preparation and conservation at tropical temperatures. These factors should facilitate distribution and sale of the products in rural areas, often distant from the coasts.

Acknowledgments

This work forms part of a joint research project between the Tropical Products Institute and the Instituto Nacional de Pesca as part of the technical cooperation programme between the Ministry of Overseas Development, London and the Government of Mexico. The authors are indebted to the Director and staff of the School of Marine and Food Sciences of the Instituto Tecnologico y de Estudios Superiores de Monterrey for the provision of the facilities required for the study. We also gratefully acknowledge the assistance provided by Mr. R. Peralta.

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Silver proteinate staining of neutral polysaccharides in apple cell walls: implications relative to fruit firmness

W. P. MOHR

Summary

The method of selective tissue extractions followed by silver proteinate staining to visualize neutral polysaccharides in cell walls at the ultrastructural level was shown to be potentially useful in future studies on apple fruit firmness and storage ability.

Introduction

The polysaccharide composition of cell walls of apple fruits has been related to firmness values characteristic of varieties at harvest and during storage (Wiley & Stembridge, 1961; Tavakoli & Wiley, 1968), a variety's characteristic rate of softening on cooking (Kertesz, Eucare & Fox, 1959), and the apple parenchyma cell's life history on and off the plant (e.g., Nelmes & Preston, 1968; Knee, 1973; Bartley, 1976). Nelmes & Preston's work included some transmission electron microscopy of methacrylate-embedded, heavy metal-shadowed, apple cell walls. Results with this technique, which visualizes the cellulose component, suggested that wall thickness, wall density, and lamination features of the cellulose microfibrils do, in fact, change during the cell's life history. Fuller (1976) observed structural symptoms of cell organelle and cell wall breakdown as related to differing initial content of calcium in the fruit. These are among the very few contributions reported on the cell wall ultrastructure of stored apples.

Refinement of polysaccharide localization methods at the electron microscope level (Thiery, 1967) in conjunction with selective extraction procedures (Jensen, 1960, as modified by Jewell & Saxton, 1970) offers further possibilities for cell wall studies. Of several modifications for visualizing the 1,2-glycol groups, the periodic acid-thiocarbohydrazide-silver proteinate (PA-TCH-AgPr, or 'silver proteinate') method was used in the present study because it has given higher specificity, more contrast, and better resolution than

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other methods presently available (Jewell & Saxton, 1970; Freundlich & Robards, 1974). The work reported is an attempt to apply the method to stored apple parenchyma and to relate the results to known firmness properties of the fruit.

Materials and methods

Small cubes of parenchyma tissue were cut from a region of the mesophyll near the equatorial middle of the apple fruit, about 10 mm in from the epidermis. The cubes were fixed in 2% glutaraldehyde (in 0.02 M sodium phosphate, pH 7.0). Some of the cubes were extracted with 0.5% ammonium oxalate and/or 4% sodium hydroxide according to procedures reported by Jensen (1960) and modified for electron microscopy by Jewell & Saxton (1970) to remove selectively pectic and hemicellulose materials. Extracted and non-extracted tissue cubes were dehydrated through graded ethanol, then infiltrated and embedded in styrene/methacrylate (Mohr & Cocking, 1968) and Spurr's epoxy resin (Spurr, 1969).

Sections (silver/gold) were cut on a Reichert ultramicrotome and picked up on 400 mesh uncoated gold grids. The presence of neutral polysaccharides was demonstrated in extracted and non-extracted sections by the PA-TCH-AgPr method (Thiery, 1967) as modified and adapted for plant tissue by Jewell & Saxton (1970). Dimedone-treated control sections were included to ensure that any positive reaction was due to aldehyde groups. Of several variations tried, best results were obtained using an immersion rather than flotation staining technique, and by making up the silver proteinate (from Taab Laboratories, Reading) in triple distilled water shortly before use. Grids were examined in a Philips 300 electron microscope operated at 60 kV.

Using this method several varieties of apples grown in 1975, 1976, and 1977 in the Smithfield Farm orchards were studied throughout the period of normal fruit development on the tree and subsequent senescence off the tree in 0°C storage. This report is limited to the differences in wall structure found between two stored fruit types of particular commercial interest – soft-textured 'short-keepers' and firm-textured 'long-keepers'.

Results and discussion

The finely particulate reaction obtained with silver proteinate staining clearly identified reaction sites in the wall. Selective extractions followed by staining indicated that some pectic and cellulosic components were reactive in addition to hemicelluloses, usually regarded as the main wall component stained by this method. Freundlich & Robards (1974) attribute the positive reaction given by cellulose in certain tissues to the presence of a reactive, less-crystalline (amorphous, para-crystalline) phase surrounding the normally unreactive crys-

talline microfibrils. Paton (1974) concluded from chemical studies that the cellulose of apples is more amorphous than crystalline.

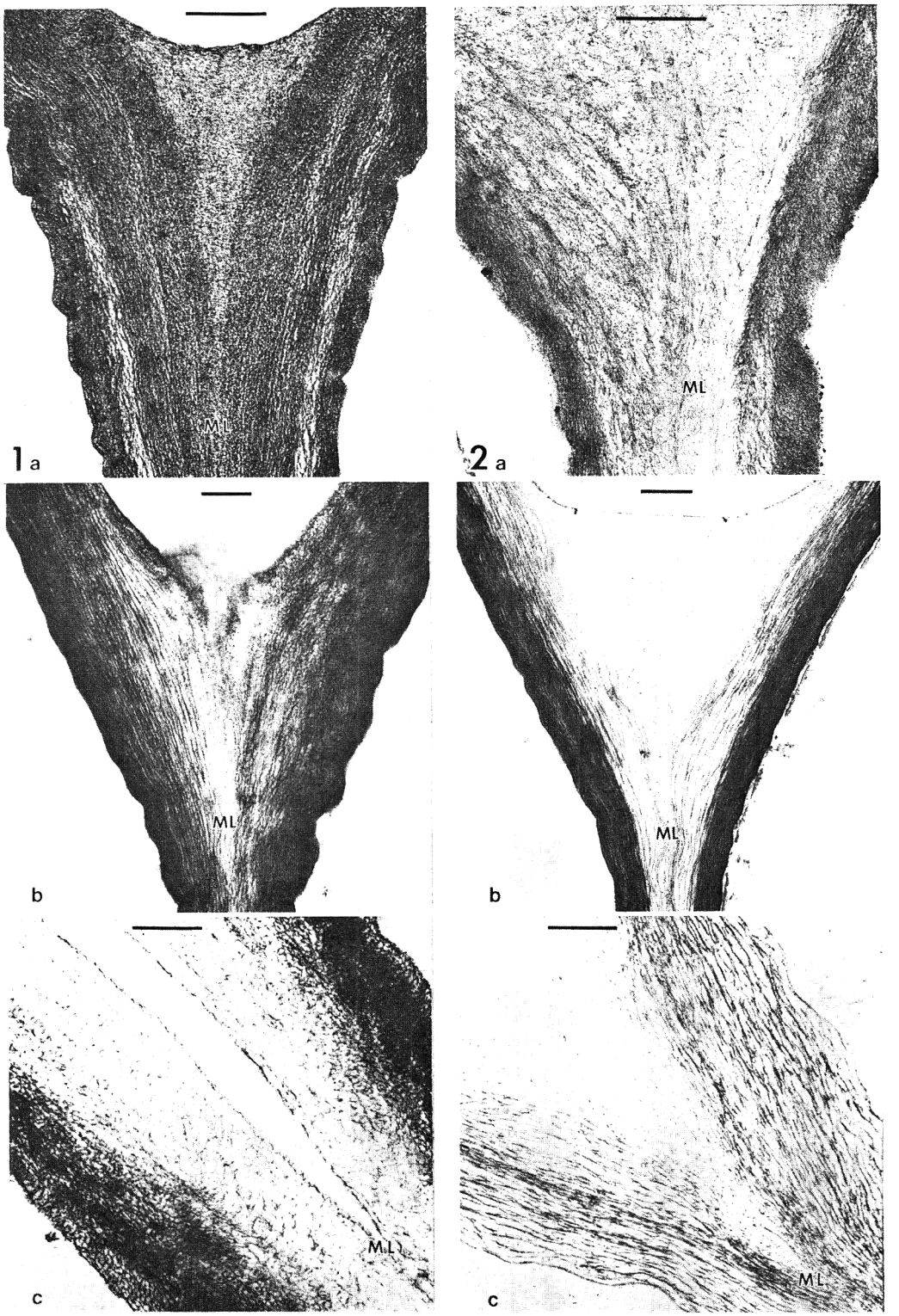
Cultivars with similar storage firmness and texture properties gave similar staining patterns. These patterns did not change significantly until the fruit began to soften noticeably. To avoid repetition, only micrographs of cultivars representing two general fruit types that differed appreciably in storage properties are reported.

During 0°C storage, before the fruit had begun to soften noticeably, long-keeping, firm, cultivars differed from short-keeping, 'soon-to-be-soft' cultivars in certain wall staining characteristics. Non-extracted, PA-TCH-AgPr-stained, cell walls of firm type fruit always stained densely (Fig. 1a. Shown here is cv. T-416 which remained very firm in storage for at least 10 months). Similarly prepared walls of soft type fruit often, but not always, stained less densely (Fig. 2a. Shown here is cv. Cortland which remained firm for only 2–3 months). A lamellate wall was usually evident in both. In tissue from which pectic substances had been removed, the staining reaction in the wall middle lamella region was considerably reduced – more so in the soft type (Fig. 2b) than in the firm type (Fig. 1b). In tissue from which both pectic and hemicellulose substances had been extracted, there was a further reduction in staining intensity, the soft type (Fig. 2c) again staining less densely than the firm type (Fig. 1c). Such differences in staining pattern and intensity must reflect compositional differences of the kind reported in analytical studies of apple fruit cell walls (Tavakoli & Wiley, 1968). They suggest that the cell walls of long-keeping, very firm-type apples contain greater amounts of at least some hemicellulosic, cellulosic, and pectic fractions than the cell walls of short-keeping, soft-type apples.

Another distinguishing feature of non-extracted walls of the soft-fruited type early in its storage life, before there was any noticeable fruit softening, is shown in Fig. 3. Some cell corners were conspicuous by their very light PA-TCH-AgPr staining reaction. This is thought to indicate very low content of wall components in these regions, although it can be argued that such regions could be the beginning of wall separation in 'soon-to-be-soft' tissue. These relatively unstained cell corners amidst otherwise densely-stained walls were not seen in any of the non-extracted firm-fruited material, even out of relatively late storage.

Once the fruit had begun to soften, typical features at the cellular level in non-extracted, stained, material were wall separation (Fig. 4a) and the enlargement of intercellular spaces and consequent rounding of cells (Fig. 4b). This supports the generally held view that these anatomical changes are the cause of, or at least intimately linked with, fruit softening and the development of a mealy texture.

In summary, silver proteinate staining at the ultrastructural level appears to be potentially useful in applied studies with apple cell walls. Future work should include its use in conjunction with tissue extraction procedures not yet investigated sufficiently, e.g., purified enzyme systems to remove specific fractions of the major wall components.



Figures 1 and 2. Typical wall structure near cell corners of very firm, long-keeping, apples (Fig. 1) and less firm, short-keeping, apples (Fig. 2) during 0°C storage, before the fruit had begun to soften noticeably: (a) not extracted; (b) extracted to remove pectic substances; (c) extracted to remove pectic and hemicellulose substances. Note the denser staining reaction in the long-keeping type than in the short-keeping type, particularly with extracted tissues.

ML, middle lamella. Scale bar = 1.0 μm.

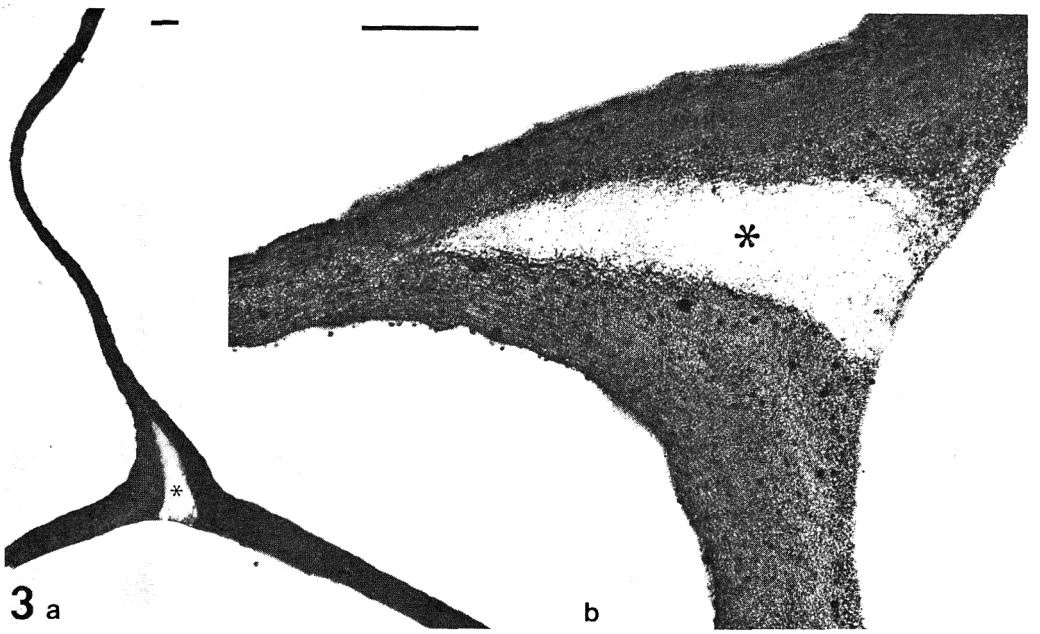


Figure 3. Wall structure at cell corners sometimes seen in short-keeping cultivars held in 0°C storage, before the fruit had begun to soften noticeably. Note the densely stained walls except for the very lightly stained middle lamella region (*) at a cell corner. This was not observed in long-keeping cultivars.

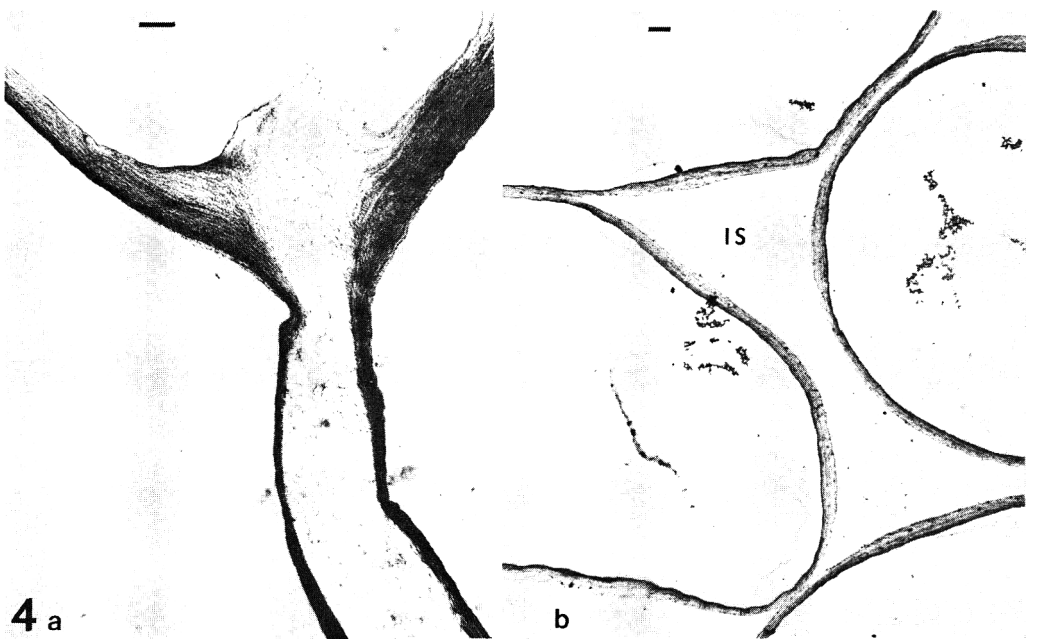


Figure 4. Separated wall (Fig. 4a) and resulting enlarged intercellular spaces and rounding of cells (Fig. 4b) typical of softening fruit.

Tissues shown in Figures 3 and 4 were not extracted before fixation. IS, intercellular space. Scale bar = 1.0 μm.

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Equations for fitting water sorption isotherms of foods. III. Evaluation of various three-parameter models

R. BOQUET,*‡ J. CHIRIFE† AND H. A. IGLESIAS†

Summary

The objective of the present study is to evaluate the capacity of four published three-parameter equations in describing water sorption isotherms of various types of foods. Thirty-nine food isotherms obtained from literature and relating to fruits, meats, milk products, proteins, starchy foods and vegetables were used to evaluate the fitting abilities of the various equations. The equations studied were: B.E.T. modified – Brunauer (1945), Chen (1971), Young & Nelson (1967) and Hailwood & Horrobin (1946).

Introduction

In part I of this work, Chirife & Iglesias (1978) have compiled and discussed twenty-three isotherm equations which have been reported in the literature for fitting water sorption isotherms of foods. Chirife & Iglesias (1978) suggested the need for an 'overall' evaluation of this large number of equations in order to have a precise and quantitative definition on their fitting abilities as applied to different foods. Accordingly, in part II of this work, Boquet, Chirife & Iglesias (1978) evaluated the capacity of eight published two-parameter equations in describing thirty-nine food isotherms obtained from the literature and corresponding to almost all type of foods. Their results allowed Boquet, Chirife & Iglesias (1978) to propose the best equations to fit the experimental sorption data for each group of foods.

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It is the purpose of the present work to study the fitting abilities of three-parameter equations as applied to the same isotherms used by Boquet, Chirife & Iglesias (1978). For this purpose a statistical analysis is made on the goodness of fit of the above isotherm equations as applied to the experimental data.

Results and discussion

Following the reasonings of Boquet, Chirife & Iglesias (1978) a fixed range of 0.10 to 0.80 water activity (a_w) will be utilized in the evaluation. Also and for the purposes of comparison, the same thirty-nine isotherms used by Boquet, Chirife & Iglesias (1978) will be utilized here. Those isotherms corresponded to room or near room temperature and were grouped in the following form: (a) fruits, (b) meats, (c) milk products, (d) proteins, (e) starchy foods, and (f) vegetables. The list of products utilized, specifications and source of data are listed elsewhere (Boquet, Chirife & Iglesias, 1978).

The three-parameter equations that will be evaluated have been discussed in Part I (Chirife and Iglesias, 1978) and are the following: (in all cases a_w refers to water activity and M to moisture content, dry basis)

B.E.T. modified equation

$$M = \left[\frac{M_m C a_w}{1 - a_w}\right]\left[\frac{1 - (n+1)a_w^n - n a_w^{(n+1)}}{1 - (C-1)a_w - C a_w^{(n+1)}}\right] \quad (1)$$

where M_m, C and n are parameters

Chen equation

$$a_w = \exp\ (k + a \cdot e^{bM}) \quad (2)$$

where k, a and b are parameters.

This equation can be written in the following form,

$$M = \frac{1}{b} \cdot \ln\left(\frac{1}{a}(\ln a_w - k)\right) \quad (3)$$

Hailwood and Horrobin equation

$$\frac{a_w}{M} = A + B a_w - C a_w^2 \quad (4)$$

where A, B and C are constants.

This equation was written as,

$$M = \left(\frac{A}{a_w} + B - C a_w \right)^{-1} \tag{5}$$

Young and Nelson equations

$$M_{\text{sorption}} = A\ (\theta + \alpha) + \beta \varphi \tag{6}$$

$$M_{\text{desorption}} = A\ (\theta + \alpha) + \beta\, \theta\, a_{w\max} \tag{7}$$

$a_{w\max}$ is the water activity from which the desorption commenced originally. The θ, φ and α functions are defined as,

$$\theta = \frac{a_w}{a_w + (1 - a_w)\, E}$$

$$\varphi = a_w\, \theta$$

$$\alpha = - \frac{E\, a_w}{E - (E - 1)\, a_w} + \frac{E^2}{(E - 1)} \ln \left\{ \frac{E - (E - 1) a_w}{E} \right\}$$

$$- \ (E + 1) \ln\ (1 - a_w)$$

where A, β and E are parameters.

Analysis of data

The general three-parametric form of equations (1), (3), (5) and (6) or (7) is:

$$M = f\ (a_w, k_1, k_2, k_3)$$

Defining,

$$S^2 = \sum_{i=1}^{n} \left(M_i - f\ (a_{wi}, k_1, k_2, k_3) \right)^2 \tag{8}$$

In order to find the maximum likelihood parameter, $\hat{k}_1$, k_2 and $\hat{k}_3$ we seek the minimum of S^2.

The goodness of fit of the various equations as applied to the experimental sorption data was evaluated through a mean relative percentage deviation in modulus (P) defined as (Boquet, Chirife & Iglesias 1978)

$$P = \frac{100}{n} \sum_{i=1}^{n} \frac{|M_i - f(a_{wi}, k_1, k_2, k_3)|}{M_i} \quad (9)$$

The results are shown in Tables 1–6 which indicate the goodness of fit (P) of the various isotherm equations as applied to the different food isotherms. In view of the large number of equations and experimental isotherms analyzed, fitting parameters are not reported here. All the results are summarized in Table 7 which shows the average of the P values for each group of foods.

Conclusions

The following conclusions can be drawn about the fitting abilities of the four isotherm equations studied, as applied to the various groups of foods in the range of a_w 0.10–0.80.

Fruits. The best equation for correlating the data is that of Young & Nelson (1967) followed closely by that of Hailwood & Horrobin (1946). Other equations do not perform satisfactorily.

Meats. The Hailwood & Horrobin (1946) equation gives the best fit for this group of foods; other equations fit the data less accurately.

Milk products. Hailwood & Horrobin (1946) and BET modified (Brunauer, 1945) equations (in that order) give by far the best description of the experimental data. The other equations fail to describe the experimental data accurately.

Proteins. Hailwood & Horrobin (1946), Chen (1971) and Young & Nelson (1967) equations give a very good fit of experimental data of this group of foods.

Table 1. Goodness of fit, expressed as mean relative percentual deviation (P) of the various equations as applied to the experimental water sorption isotherms in fruits

Experimental isotherm no.*	B.E.T. modified eqn (1)	Chen eqn (2)	Hailwood & Horrobin eqn (4)	Young & Nelson eqn (6) or (7)
1	6.2	18.4	3.6	3.2
2	13.3	11.6	3.5	4.3
3	5.7	15.5	4.2	7.0
4	14.0	29.1	4.3	1.6
5	20.3	42.2	6.5	4.2
6	6.2	18.4	3.6	3.2
7	16.0	43.8	5.2	3.1

*See Boquet, Chirife & Iglesias (1978)

Table 2. Goodness of fit, expressed as mean relative percentual deviation (P) of the various equations as applied to the experimental water sorption isotherms in meats

Experimental isotherm no.*	B.E.T. modified eqn (1)	Chen eqn (2)	Hailwood & Horrobin eqn (4)	Young & Nelson eqn (6) or (7)
8	6.3	2.5	1.8	0.6
9	4.6	3.0	4.0	0.8
10	4.8	2.0	1.3	3.1
11	1.2	5.6	2.6	4.9
12	2.4	2.5	0.9	3.4
13	2.4	8.1	3.2	3.7
14	1.4	4.0	1.0	2.9
15	2.2	5.9	1.4	13.1

*See Boquet, Chirife & Iglesias (1978)

Starchy foods. Young & Nelson (1967) and Hailwood & Horrobin (1946) equations (in that order) give the best fits for starchy foods followed very closely by Chen (1971) and B.E.T. modified (Brunauer, 1945) equations.

Vegetables. The Hailwood & Horrobin (1946) equation gives the best fit in this group of foods. The other equations have definite lower fitting abilities.

There are various conclusions which can be drawn from the results here obtained. The best and also most versatile equation is that of Hailwood & Horrobin (1946), which was developed in an attempt to interpret the water sorption isotherms of proteins. This equation is able to describe the sorption data of almost any food group, with a P value of only 2.0% or less; only for fruits and milk products the last value increased to 4.4% and 3.9% respectively. This versatile behaviour together with a remarkably good ability to fit experimental data for most types of foods, suggest that this equation may be considered as a sort of 'universal' isotherm equation (in the range of a_w here studied). Of course, and as it was previously discussed by Chirife & Iglesias (1978), the excellent fitting ability of this (or other) equation(s) is not in itself sufficient to

Table 3. Goodness of fit, expressed as mean relative percentual deviation (P) of the various equations as applied to the experimental water sorption isotherms in milk products

Experimental isotherm no.*	B.E.T. modified eqn (1)	Chen eqn (2)	Hailwood & Horrobin eqn (4)	Young & Nelson eqn (6) or (7)
16	5.9	11.7	5.2	20.9
17	1.0	6.5	1.7	3.7
18	5.6	11.6	4.9	18.3

*See Boquet, Chirife & Iglesias (1978)

Table 4. Goodness of fit, expressed as mean relative percentual deviation (P) of the various equations as applied to the experimental water sorption isotherms in proteins

Experimental isotherm no.*	B.E.T. modified eqn (1)	Chen eqn (2)	Hailwood & Horrobin eqn (4)	Young & Nelson eqn (6) or (7)
19	5.8	2.1	2.6	2.5
20	1.9	1.7	1.8	1.8
21	3.4	1.0	1.0	1.2
22	1.0	2.5	2.0	1.8
23	3.5	1.4	1.3	0.8
24	3.6	1.9	2.0	2.6

*See Boquet, Chirife & Iglesias (1978)

Table 5. Goodness of fit, expressed as mean relative percentual deviation (P) of the various equations as applied to the experimental water sorption isotherms in starchy foods

Experimental isotherm no.*	B.E.T. modified eqn (1)	Chen eqn (2)	Hailwood & Horrobin eqn (4)	Young & Nelson eqn (6) or (7)
25	0.3	1.7	2.1	2.3
26	4.8	3.4	3.0	2.5
27	2.0	2.6	2.6	2.0
28	1.4	0.9	1.4	1.7
29	1.4	1.1	0.7	0.5
30	3.0	2.2	1.4	1.8

*See Boquet, Chirife & Iglesias (1978)

Table 6. Goodness of fit, expressed as mean relative percentual deviation (P) of the various equations as applied to the experimental water sorption isotherms in vegetables

Experimental isotherm no.*	B.E.T. modified eqn (1)	Chen eqn (2)	Hailwood & Horrobin eqn (4)	Young & Nelson eqn (6) or (7)
31	5.9	6.9	1.9	2.3
32	1.7	7.2	1.9	10.8
33	3.4	7.3	3.4	4.9
34	7.3	8.1	2.1	6.8
35	4.1	8.2	4.2	6.8
36	2.1	5.5	2.6	4.7
37	1.9	3.3	1.2	3.4
38	2.1	1.1	0.5	2.2
39	2.2	2.5	0.5	3.7

*See Boquet, Chirife & Iglesias (1978)

Table 7. Average goodness of fit of the various equations for each group of foods, expressed as the average P value for the total number of experimental isotherms comprising each group

	B.E.T. modified eqn (1)	Chen eqn (2)	Hailwood & Horrobin eqn (4)	Young & Nelson eqn (6) or (7)
Fruits	11.7	25.6	4.4	3.8
Meats	3.2	4.2	2.0	4.1
Milk products	4.2	9.9	3.9	14.3
Proteins	3.2	1.8	1.8	1,8
Starchy foods	2.2	2.0	1.9	1.8
Vegetables	3.4	5.6	2.0	5,1

prove the validity of a particular sorption model. The most we can say is that it has an excellent 'mathematical flexibility'.

The other three-parameter equations here examined (B.E.T. modified, Chen and Young & Nelson) have, in an overall sense, inferior fitting abilities. None of them is able to fit the data for all food groups studied with comparable accuracy, although they can also perform very well for some of the groups studied.

It is noteworthy that some of the simpler two-parameter equations evaluated by Boquet, Chirife & Iglesias (1978) also give fits of comparable or even better accuracy than B.E.T. modified, Chen, or Young & Nelson equations, when considering some particular group of experimental isotherms. Thus, the use of a third parameter does not seem to be always worthwhile.

It is obviously important to have a relatively simple equation describing the sorption isotherm of foods with a limited number of parameters. If we balance simplicity and number of parameters against fitting ability, we conclude that the water sorption data of probably any food would be satisfactorily represented by one of only four isotherm equations, namely those of Hailwood & Horrobin (1946), Halsey, Oswin or Iglesias & Chirife (Chirife & Iglesias, 1978).

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Technical note: Determination of ascorbic acid in a blackcurrant cordial with dihaloiodate (I)

G. J. MOODY AND J. D. R. THOMAS

Of the methods available for ascorbic acid assay the one normally recommended is based on its reduction of the blue dye 2,6-dichlorophenolindophenol to give a colourless product (Nicholls, 1945; Pearson, 1962). Another procedure depends on the reduction of iodine generated by the action of standard iodate on excess iodide in 0.25 M sulphuric acid (Nicholls, 1945; Ballentine, 1941). Iodine monobromide prepared by mixing equimolar quantities of iodine and bromine in hydrobromic acid has also been employed to determine ascorbic acid among other compounds (Nazrullaev, Gengrinovich & Murtazaev, 1963).

A more convenient method of obtaining cationic iodine oxidant is to mix a standard iodide solution and either concentrated hydrobromic acid, or concentrated hydrochloric acid, with the appropriate amount of standard iodate solution (Moody & Thomas, 1963, 1964; Bark & Grime, 1974). Both the dihaloiodate(I) species thus obtained are stable over several weeks.

Experimental

Preparation of materials

Cationic iodine in hydrobromic acid. 0.100 M potassium iodide (25 cm^3) and hydrobromic acid (s.g. ~ 1.46; 25 cm^3) were treated with 0.0250 M potassium iodate (50 cm^3). A ten-fold dilution with distilled water gave a 3.75 mM solution of dibromoiodate(I).

Cationic iodine in hydrochloric acid. 0.100 M potassium iodide (25 cm^3) and hydrochloric acid (s.g. 1.16; 50 cm^3) were treated with 0.0250 M potassium iodate (50 cm^3). A ten-fold dilution with distilled water gave a 2.00 mM solution of dichloroiodate(I).

Stability of cationic iodine in dilute acid solution. Frequent standardization of the dihaloiodate(I) solutions with sodium thiosulphate showed both cationic iodine species to be stable for several weeks even in daylight.

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Titration procedure for ascorbic acid

Aliquots of freshly prepared ascorbic acid standards were titrated against the appropriate cationic iodine solution, using the starch preparation 'Thyodene' (Purkis, Williams Ltd) for end-point detection.

The neat cordial (25 cm^3) was diluted ten-fold with distilled water and aliquots (25 cm^3) treated with conc. hydrochloric acid (5 cm^3). After bubbling nitrogen for 5 min to remove sulphur dioxide the ascorbic acid was titrated as above.

Chromatography

Oxidation of the ascorbic acid by stoichiometric amounts of each cationic iodine species was confirmed by chromatography using Watman No. 1 paper. Thyodene was excluded from the solutions subjected to chromatography. After development with butan-1-ol/acetic acid/water (4:1:5) (upper phase), the chromatograms were treated with acetone/silver nitrate reagent, then 0.1 M sodium hydroxide/95% ethanol (1:1).

Results and discussion

Figure 1 indicates that both the cationic iodine species, IBr_2^- and ICl_2^- are consumed by the ascorbic acid (AH_2) according to

$$AH_2 + IX_2^- = \text{Dehydorascrobic acid} + I^- + 2HX \qquad (1)$$

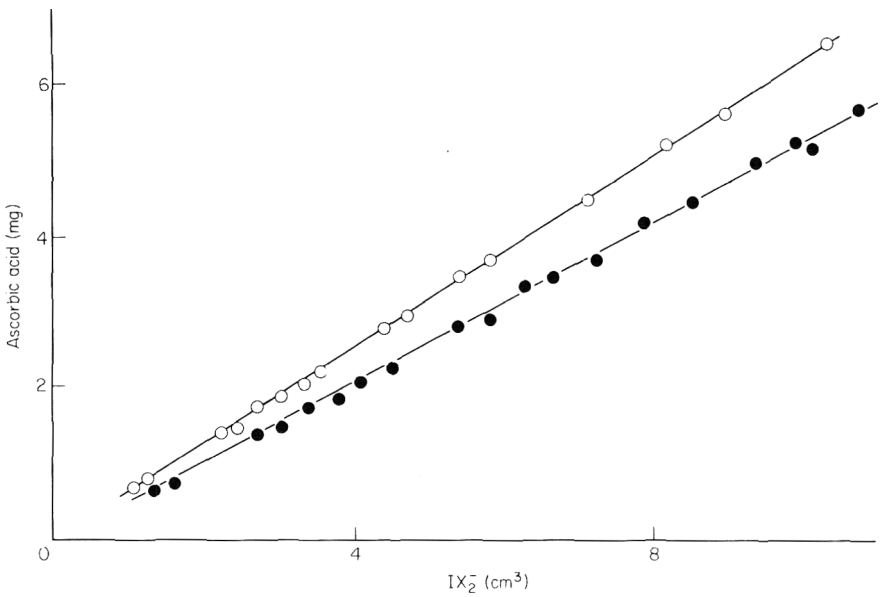

Figure 1. The consumption of dihaloiodate(I) species by standard ascorbic acid solutions: ○, IBr_2^-; ●, ICl_2^-.

Chromatography revealed no ascorbic acid after oxidation with stoichiometric amounts of either dihaloiodate(I) reagent. In all cases, a distinct spot at $R_F \sim 0.11$, corresponding to control applications of dehydroascorbic acid, was evident. No other products could be resolved.

The end-point of the titration is based on the iodine formed from the iodide produced by reaction 1 and the first excess of the dihaloiodate(I) reagent:

$$IX_2^- + I^- = I_2 + 2X^- \qquad (2)$$

The first trace of iodine gives a blue/purple coloration with Thyodene.

Sodium metabisulphite and D-glucose additives are present in this cordial. Control experiments with ascorbic acid showed sulphite, but not D-glucose, to be an interferent requiring additional cationic titrant (re eqn 2). Sulphite (15 mg) added to either ascorbic acid or actual diluted cordial was removed by adding conc. hydrochloric acid and briefly bubbling nitrogen prior to the titration.

The ascorbic acid content of the cordial was thus found to be 228 (s.d. 6) mg% and 229 (s.d. 7) mg% respectively using the ICl_2^- and IBr_2^- titrants for five replicate samples in each case. These values would have been about 6% higher if the sodium metabisulphite (ca 155 ppm quoted by the manufacturer) had not been removed.

A favourable feature of both the IBr_2^- and ICl_2^- reagents is their stability over several weeks, (Moody & Thomas, 1963, 1964), confirming the similar claim in favour of the $HIBr_2$ species prepared by the alternative method (Nazrullaev, Gengrinovich & Murtazaev, 1963). In addition, the reagents also provide a non-fading, sharp end-point, which is of special interest since the 2,6-dichlorophenolindophenol end-point involves a colour change which presents a problem when red coloured samples, e.g. fruit juices, are examined (Clegg & Morton, 1968) and standardization is required before each assay. Indeed in this work no end-point was possible when titrating the cordial with 2,6-dichlorophenolindophenol. Bark & Grime (1974) have also used dichloroiodate(I) for the thermometric assay of ascorbic acid but specialized equipment is needed and there is no significant difference in accuracy compared with the standard B.P. (1973) cerium(IV) titration technique.

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Technical note: Comparative aspects of pectin extracted from the peels of different varieties of mango

A. N. SRIRANGARAJAN AND A. J. SHRIKHANDE

Introduction

Mango is one of the important crops of India and occupies 60% of the area under fruit cultivation in the country. In the fruit processing industry seeds and peels are discarded as waste at present. The problems arising from the disposal of waste stress the needs of research to develop an acceptable commodity from waste. It was reported earlier that Alphonso mango peel contains about 13% of pectin, on a dry weight basis, suitable for jelly and jam preparations (Sriran-garajan & Shrikhande, 1976). The possible utilization of peels of two other commercial varieties of mangoes, Dassehri and Langra, for the extraction of pectin are also evaluated in this paper.

Materials and methods

Ripe mangoes were obtained from a local market. Peels of fruits were removed by hand and washed thoroughly in cold water and dried in a cabinet dryer at a temperature of 65°C to a final moisture content of 7–8%. Dried peels can be used for the extraction of pectin as and when desired and this also helps a plant of low capacity to work for a longer period. The pectin extraction was done in accordance with the procedure adopted by Kertesz (1951). Before the extrac-tion of pectin, dried peels are washed to remove the adhering pulp and other fibrous material. Extraction of pectin was performed at 98–100°C by using acidified water by maintaining the pH in the range of 3.0 to 3.2. The mixture was heated for 60 min. The extract was filtered with cheese cloth and the residue was re-extracted twice and the solutions were combined. The pectin was precipitated by adding ethanol equivalent to twice the volume of pectin extract and the precipitate was then dried at 37°C and powdered.

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Table 1. Comparative characteristics of pectins obtained from different sources

Composition	Variety Dassehri	Langra	Alphonso*	Apple Pomace†	Oranges§
Yield (% dwb)	18.0–19.0	15.0–16.0	13.0	15.0–18.0	13.0
Methoxyl content (%)	8.99	8.07	8.20	8.40	8.20
Anhydrouronic acid (%)	58.40	54.60	61.12	59.40	83.50
Degree of esterification	85.93	83.38	76.00	—	—
Molecular weight	147,900	161,700	105,000	—	—
Setting time (min)	10.00	10.00	10.00	—	—
Jelly grade	155	175	200	200	150

* Srirangarajan & Shrikhande (1976).
† Srirangarajan (1976).
§ Pruthi, Parekh & Lal (1961).

Anhydrouronic acid and methoxyl contents were determined by the standard method (Owens *et al.*, 1952). The esterification value was calculated on the basis of anhydrouronic acid and methoxyl contents.

The molecular weight of extracted pectin was determined by the method of Smit & Bryant (1967). Jelly was prepared for the determination of the grade as described in our earlier work by using an Instron Universal Testing Machine (Srirangarajan & Shrikhande, 1977). A jelly was prepared for comparison from apple pectin (200 grade) obtained from Messrs Obi pectin AG, Bischofszwll, Switzerland.

Results and discussion

The chemical composition and characteristics of the pectin extracted from Dassehri and Langra peels are given in Table 1. The quality of a pectin is known to depend upon the content of anhydrouronic acid and methoxyl group and also degree of esterification. Jelly prepared from peel pectin sets within 10 min indicating this to be a rapid set pectin. The jelly grade and yield of pectin from Dassehri and Langra mangoes are quite comparable to that from other sources. Jelly deformation curves indicating the breaking strength are shown in Fig. 1, which shows good quality pectins. The peels of these two varieties of mangoes would provide a cheap source for the production of pectin.

The possible utilization of mango peel waste as a good source of pectin for commercial exploitation has been presented in this paper. Mango peel is available in large quantities in the mango processing industry, which could be of a very useful source of raw material for processing for extraction of pectin.

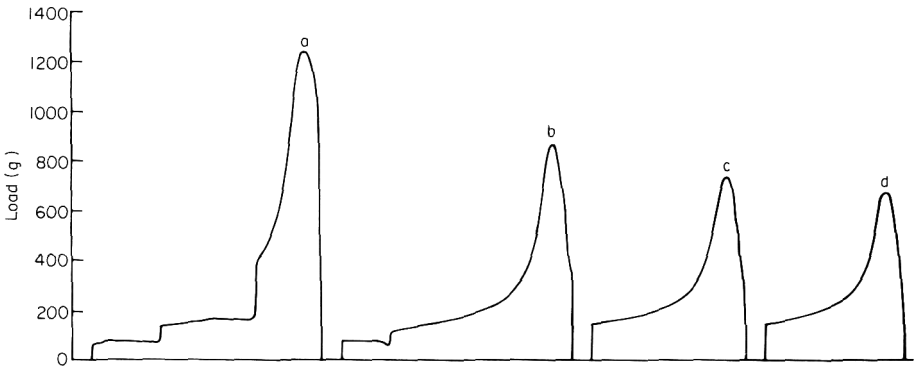

Figure 1. The deformation curves on Instron Universal Testing Machine for mango peel and apple pectins. a, Alphonso; b, Apple pectin (200 grade); c, Langra; d, Dassehri. The concentration of pectins used was 0·5% in each case.

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Book reviews

Nutritional and Safety Aspects of Good Processing. Ed. by S. R. Tannenbaum.
New York and Basel: Marcel Dekker, Inc., 1979. Pp. x + 488. Sw. Fr. 100.

The contents of this book cover an area which is currently receiving a great deal of attention. It is useful to find nutritional and safety aspects of food processing covered in one volume. The book is in the series of 'Food Science Monographs', under the general editorship of Owen R. Fennema. Several of these books, such as Volume 4, *Principles of Food Science* have already become classics in their field. The presentation on the back cover indicates the book as being crucial to the work of professionals in the fields of nutrition, food sciences, toxicology, dietetics, home economics and agricultural sciences. However, as each chapter is written by a specialist, it is doubtful if professionals in the latter three categories would normally have had sufficient training to fully appreciate many of them.

Human nutrient requirements, in particular protein, are discussed by V.R. Young & N.S. Scrimshaw. The accent is on the limitations of methodology for their estimation, interpretation in terms of recommended daily allowances (RDA) and problems of interpretation of RDA.

An overview of the effect of different processing methods on vitamin loss is given, with useful references on current methods of vitamin assay (M.C. Archer & S.R. Tannenbaum). Chapter 4 is a comprehensive review of lipids, including some 363 references. However, none of these is later than 1975. The characteristics and reaction mechanisms of the major lipid groups is given, together with sections on the effect of specific processing methods such as frying and oil refining.

Minerals receive some attention, in particular losses and gains in processing, availability in foods and safety. Proteins and amino acids are well reviewed by J. C. Cheftel. This section deals with interactions of proteins with other food components during processing as well as with other proteins and the formation of new, possibly toxic, amino acids. There is an interesting section on protein reactions with aldehydes (animal feeds), nitrites, sulphites and chlorinated molecules. The chapter is especially useful for discussion and diagrams of reaction mechanisms.

The remainder of the book deals with technology of fortification (short chapter), prediction of nutrient losses and optimization of processing conditions (a detailed chapter dealing with reaction kinetics, and the use of model systems), antinutritional and toxic substances (naturally occurring and accidental contaminants), agricultural chemicals, preservatives added to foods, and a brief discussion of immunological aspects of foods and food safety.

Of these last chapters, that on agricultural chemicals by D. J. Sissons & G. M.

Telling deserves further mention. This includes a discussion of degradative pathways of pesticides, their solubility and persistence. The distribution of the major classes of pesticides in the diet, legislative aspects and methods of analysis all receive attention, as well as distribution of pesticides in the major food classes and the food chain. With the knowledge we now have, processing conditions can be optimized to achieve maximum removal of pesticides but problems of residue reintroduction into the food chain may arise when waste materials from the food industry are recycled as animal feeds.

Overall, a very useful book for the nutritionist, food scientist and toxicologist to possess.

Ann F. Walker

Report on the Study Group on Vegetable Proteins in Foodstuffs for Human Consumption in Particular in Meat Products.
Brussels Commission of the European Community, 1978. Pp. v + 145. £13.50.

According to the introduction, this document is the work of an EEC study group established in 1974 to consider 'the scientific, technological and health aspects of the commercialisation of foodstuffs containing vegetable protein, in particular meat products'. Like the 1974 U.K. Food Standards Commitee *Report on Novel Protein Foods*, referred to in the text, it is designed to provide the legislative body, in this case the Commission, with adequate information on which to base its deliberations when preparing a draft directive. Unlike the FSC Report, however, it concentrates on the use of novel proteins in meat products, including possible vegetable protein sources other than soya, but specifically excluding microbial protein.

Despite this, the report is a somewhat weightier document than the FSC Report, because of its different format. I particularly liked the idea of presenting the important facts for consideration as appendices in the form of original papers. The use of this type of presentation certainly made the report more readable for me and, I suspect, will increase its exposure. The appendices would certainly form an ideal monograph for undergraduate reading on the subject of novel proteins, although there is a significant, but understandable, lack of detail on current technology. The papers on toxic substances and the *Review of Current Legislation* are particularly well prepared. From the Commission's point of view it must be considered an excellent presentation of the facts on novel proteins and their use, or potential use, in meat products, as presently available. It does, however, leave an enormous gap in the consideration of both new sources of protein and novel uses for the already available vegetable proteins although future deliberations on these topics are recommended in the conclusions.

The *Conclusions and Recommendations* are broadly in line with those proposed by the FSC, although the recommended crude protein content of novel proteins is 48% on a dry weight basis, rather than the 50% of the FSC Report,

and the addition of methionine is not considered necessary by this study group. The remaining recommendations on nutritional additives, levels of novel proteins in meat products and labelling are virtually identical to those of the FSC Report.

Altogether this report is informative and fulfils its purpose admirably.

C. Ovenden

Streptococci. Ed. by F. A. Skinner and L. B. Quesnel. Society of Applied Bacteriology Symposium Series No. 7.
London, Academic Press, 1978. Pp. xiii + 415. £18.00.

A symposium entitled *Streptococci* is an ambitious project and cannot cover all that is known about a large and diverse genus. A glance through the contributors to the Symposium held by the Society of Applied Bacteriology in Manchester in July 1977 should convince anyone that the book will contain useful information. A better title might have been 'The Streptococci and Their Relationship to Man and Animals'.

This is not a book for the taxonomist, the microbial physiologist or biochemist but for the ecologist and the technologist. Dr Jones starts by giving a wide ranging survey of the composition of the genus and explains the problems of classification. These are many, and most workers deal only with a few species from one ecological niche. A few papers mention some aspects of the physiological properties of streptococci in certain habitats. Dr Whittenbury's paper entitled 'Biochemical characteristics' is misleading for, as he explains, he discusses only the aerobic nature of some streptococci.

Most authors assume that the reader is familiar with the various species and apart from the oral streptococci no assistance is given in identification although media for isolation for a variety of habitats are well covered in the final chapter.

The streptococci and their relationship benign and pathogenic to animal hosts is discussed in several chapters. These deal both with the colonisation of the host and the distribution of the organisms. While many streptococci are potential pathogens, few are virulent, so that many species thrive outside as well as inside a healthy host, they can therefore readily contaminate food and water, the occurrence and significance of these unwanted streptococci is discussed.

Streptococci are, however, essential in the dairy industry and this aspect is covered in three papers. In the first the role of starters in cheesemaking has some useful biochemical information, while the second on the handling of starters is essentially practical. The third paper deals with NISIN.

This book will be of interest to the food technologist for it shows that organisms morphologically the same possess a wide variety of properties and it should help him to understand something of the ecology of the different species.

E. J. Garrie

Sugar: Science and Technology. Ed. by G. G. Birch and K. J. Parker. London: Applied Science Publications, 1979. Pp. xii + 475. £32.00.

This book is a collection of papers given at a symposium at the National College of Food Technology, Weybridge in April 1978. The progressive coverage of the subject is well organized and of excellent content. These lectures present a comprehensive picture of the sugar industry and make up a very useful reference book on the subject.

The papers may be grouped roughly into five sections. In section I the history of the sucrose industry and the current practices for refining cane and beet sugar are described. Two related papers on the storage of beet and the quality control of sucrose products are placed later in the text. Section II covers the potential uses of sucrose as an industrial energy source or a basis for a chemical industry as well as the more normal use in foodstuffs. Returning to basic food technology W. Nicol presents a useful survey of the physical properties of sugar solutions covering parameter of solubility, boiling point, osmotic pressure, relative humidity etc.

The other commercial sugars, glucose syrups, fructose and xylitol are described in the third section. A paper is also included on the more novel hydrogenated glucose syrups.

All sugars have a sweet taste and in section four a collection of papers cover this subject from several different aspects. Papers are given by the sensory analyst, the food technologist and the structural organic chemist.

The final section concentrates on the biochemical and medical aspects of consuming sugars. There is a general survey of medical aspects and more detailed papers on coronary thrombosis and dental caries.

This text should prove very useful to practicing food scientists and technologists and to students learning about the subject.

R. C. E. Guy

Books received

Nutrition and National Policy. Ed. by B. Winikoff. Cambridge, Mass, MIT Press.
1979. Pp. xi + 580. £15.75.

This is a collection of case studies in which the nutrition policies of governments of eleven developing countries and those of the United States are outlined and analysed.

Introduction to Nutrition (4th ed). By H. Andrews Guthrie.
St Louis: C. V. Mosby Company. 1979. Pp. vii + 693. £9.75.

A textbook for first year students.

World Review of Nutrition and Dietetics, Vol 33. Some special aspects of nutrition. Ed. by G. H. Bourne.
Basel, S. Karger. 1979. Pp. x + 234. Sw.Fr.158.

This volume contains monographs on the following subjects: nutrition during adolescence, some aspects of the role of cyanogenic glucosides in nutrition, carbohydrate sweeteners, structural requirements for taste, chronic marginal vitamin C deficiency, milk from semi-domesticated ruminants.

The Processing of Banana Products for Food Use. By P. C. Crowther.
London: Tropical Products Institute. 1979. Pp. iv + 18. £0.85.

A short report with an extensive bibliography.

Sugar Analysis: ICUMSA Methods. Ed. by F. Schneider.
Peterborough: ICUMSA. 1979. Pp. xiv + 265. £12.00.

Official and tentative methods recommended by the International Commission for Uniform Methods of Sugar Analysis.

The Next Seventy Years. Population, Food and Resources. By B. Gilland. Tunbridge Wells: Abacus Press.
1979. Pp. viii + 133. £9.50.

Analysis of the demographic situation and outlook, nutritional requirements and agricultural potential of the world in the next seventy years.

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Volume 3. Effect of Nutrient Deficiences in Man. 1979. 388 pages. £45.00

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Volume 1. Diets, Culture Media and Food Supplements. 1978. 672 pages. £54.00

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Volume 3. Effect of Nutrient Deficiences in Man. 1979. 388 pages. £45.00

Volume 4. Diets, Culture Media, Food Supplements. 1978. 472 pages. £39.00

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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	
litre	l = 10^{-3} m^3		

NON SI UNITS

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

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

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