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Enthalpy and specific heat of meat and fish in the freezing range

F. L. LEVY

Summary

Freezing time, weight loss and power demand can be calculated by equations in which the enthalpy gradient, namely, the specific heat requires to be expressed as a temperature function. Since the temperature at which freezing starts varies with the material of the food and its water content, the author has established equations for the freezing point t_f of beef, pork, lamb and fish and its variation with their water contents. That temperature serves as the reference point $\vartheta = -t + t_f = 0$ of a unified temperature scale with enthalpy $H = 0$. The change of enthalpy in the freezing range can be divided into two sections. The first one, which covers the band from $\vartheta = 0$ to $\vartheta_g = 6$, is characterized by a steep enthalpy gradient which can be expressed by a linear change of the ratio ϑ/H with the temperature ϑ. The second section covers the range $\vartheta > \vartheta_g$ and is characterized by a small enthalpy gradient with the resulting functions $H = C\vartheta^n$ and $c = n(H/\vartheta)$. The author has established equations for the numerical values of n and C for various meats and fish depending on their water contents and has produced diagrams of the specific heat in the low temperature range.

Introduction

To chill and freeze food correctly (preservation of quality) and economically (conservation of energy), the thermophysical properties must be known over a wide range of temperatures. Data such as 'frozen' or 'before freezing' do not serve the purpose. Graphs of the variation of the equivalent specific heat of meat and fish in the low temperature band are informative, but do not cover the needs of the refrigerating engineer or, more widely, the process engineer who wants to calculate freezing time, weight loss and power demand. He must handle equations in which the variation of enthalpy is expressed as a temperature function such as stated previously (Levy, 1977).

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In the present paper an attempt has been made to present 'unified' equations of the variation of the equivalent specific heat (the correct adjective 'equivalent' will subsequently be dropped) of meat and fish in the low temperature range down to −40C. Analysis of the data from the literature (Riedel, 1956, 1957, 1976, 1978; Rjutov, 1950; Latyshev, 1975; Fleming 1969) offers a welcome opportunity to pay tribute to the lifework and achievement of Riedel.

The mathematical form of temperature functions which the refrigerating engineer needs is simpler than that which the biologist might derive for food-water-systems. When presenting in this paper to the process engineer equations and diagrams based on a 'unified' temperature scale in the freezing range, biologists may not find the answer to their prayers. However, it is hoped that their loss may become the application engineer's gain.

Unified equations

The variation of specific heat of meat and fish in the freezing range depends largely on the initial water contents ψ (measured in kg/kg) and on the percentage of water that has been frozen. The temperature t_f at which freezing starts depends on ψ. Percentages of frozen water become comparable only when referred to the respective freezing point. With the relationship

$$\vartheta = -t + t_f \tag{1}$$

a unified, positive temperature scale in the freezing range, starting from $\vartheta = 0$, shall be established and the variation of enthalpy with temperature shall be referred to that zero-point, thus

$$H = 0 \text{ (kcal/kg) at } \vartheta = 0 \text{ (K)} \tag{2}$$

Similar to the French 'frigorie', H will remain a positive quantity as ϑ increases (t decreases).

Down to a certain temperature t_g the enthalpy gradient increases steeply until the majority (some 80%) of the initial water content has become frozen. Beyond t_g the change of enthalpy with temperature becomes gentle, or 'smooth'. For meat and fish the temperature at which the relationship changes its character can be ascribed to the unified temperature

$$\vartheta_g = 6 \tag{3}$$

At temperatures $\vartheta \geq 6$ the variation of enthalpy H' (in that range the distinctive dash shall be used) with temperature can be described by the power law

$$H' = C\,\vartheta^n \tag{4}$$

and the equivalent specific heat becomes

$$c' = dH'/d\,\vartheta = nH'/\,\vartheta = nC/\vartheta^{1-n} \qquad \text{(kcal/kg K)} \tag{5}$$

In the range from $\vartheta = 0$ to 6 the ratio ϑ / H can be expressed by the linear equation

$$\vartheta/H = (1/c_0) + \vartheta / \dot{H} \tag{6}$$

from which

$$H = (1/c_0\,\vartheta + 1/\dot{H})^{-1} \tag{7}$$

In this range of 'steep' enthalpy gradient the specific heat $dH/d\vartheta$ becomes

$$c = (H/\vartheta)^2/c_0 = (1/c_0) / (1/c_0 + \vartheta / \dot{H})^2 \tag{8}$$

The quantity c_0 is the specific heat at the freezing point ($\vartheta = 0$) and $\dot{H}$ is that enthalpy limit which would be reached at $\vartheta = \infty$ if equation (7) were to be valid beyond ϑ_g.

As shown in the Appendix, investigation of the variation of t_f with ψ for various foods shows that t_f can be evaluated as a function of ψ by numerical equations which shall be presented hereinafter.

When plotting H' against ϑ between 6 and 40 in the logarithmic scale, a straight line can be drawn through measured values from which the integer n and the constant C can be evaluated. With these values the enthalpy H'_g and the specific heat c'_g at ϑ_g can be calculated (equations 4 and 5). At ϑ_g they must equal H_g and c_g (equations 7 and 8) from which

$$1/c_0 = n\ (\vartheta_g/H'_g) \tag{9}$$

and

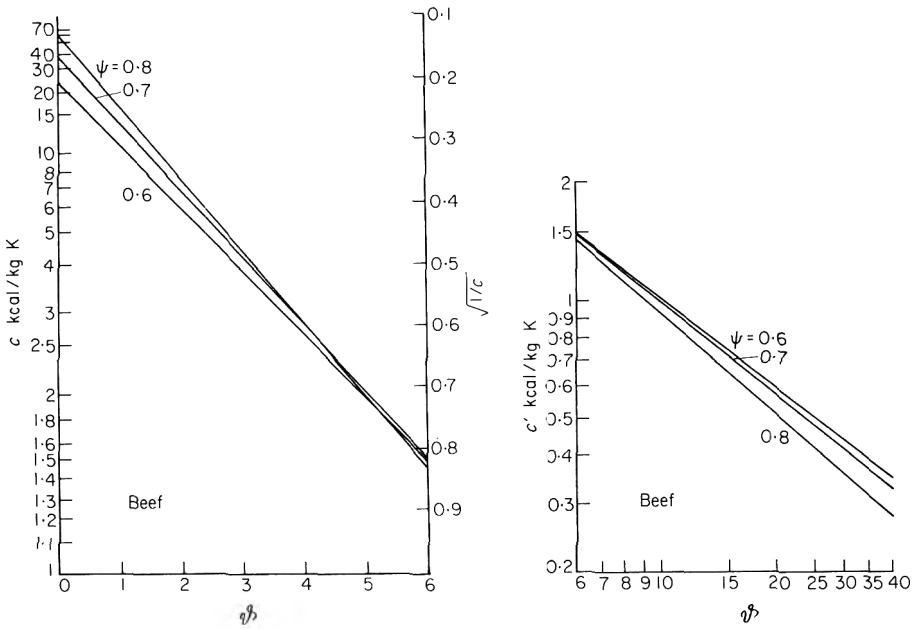
$$1/\dot{H} = 1/H'_g - 1/c_0\,\vartheta_g = (1-n)/H'_g \tag{10}$$

H and H', and c and c', can now be calculated as functions of ϑ in the respective temperature ranges for use in connection with the calculation of freezing time, weight loss and power demand. The following linear diagrams may be found useful since they can be rapidly drawn by using two values only, namely at $\vartheta = 0$ and ϑ_g, and in logarithmic co-ordinates ϑ_g and 40 (or any other pair of ϑ -values). Between 0 and 6 equation (6) yields a linear diagram for the ratio ϑ / H from which a plot of H against ϑ can be derived when dividing ϑ by (ϑ/H) (see Fig. 9). Similarly, equation (8) shows that a plot of $\sqrt{1/c}$ against $\vartheta = 0$ to 6 will yield a linear diagram in which the resulting c-values can be marked on the ordinate axis (Figs. 1, 3, 5, 7).

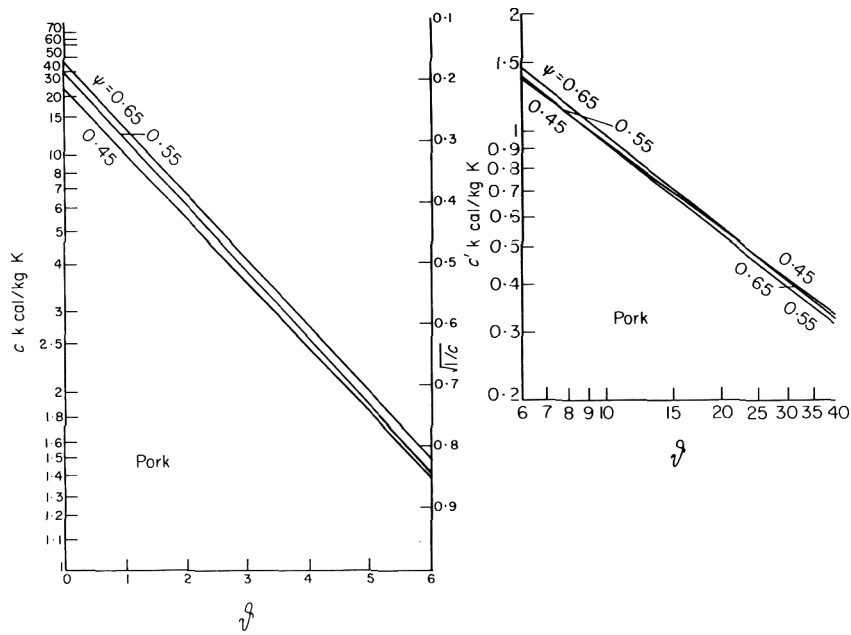
In the range of smooth enthalpy gradients ($\vartheta \geqq \vartheta_g$) both H' and c' will yield linear diagrams when drawn in a system of logarithmic coordinates (Figs. 2, 4, 6, 8).

Results

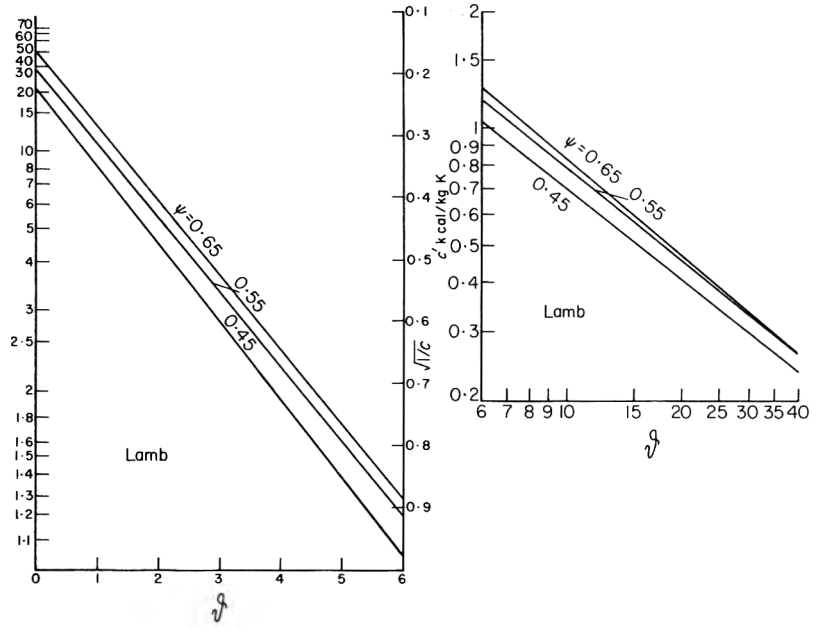
The thermophysical data of various meats and seafish will be presented in the following order:



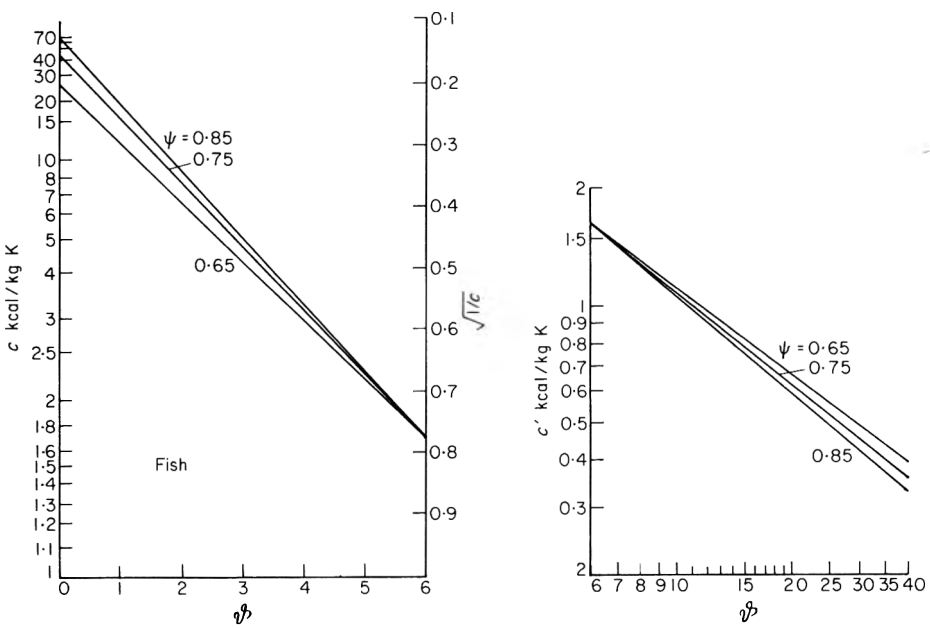
Figures 1 and 2. Beef ($\psi = 0.8, 0.7, 0.6$). Variation of specific heat between $\vartheta = 0$ and 6, and 6 and 40 K.



Figures 3 and 4. Pork ($\psi = 0.65, 0.55, 0.45$). Variation of specific heat between $\vartheta = 0$ and 6, and 6 and 40 K.



Figures 5 and 6. Lamb (ψ = 0.65, 0.55, 0.45). Variation of specific heat between ϑ = 0 and 6, and 6 and 40 K.



Figures 7 and 8. Fish (ψ = 0.85, 0.75, 0.65). Variation of specific heat between ϑ = 0 and 6, and 6 and 40 K.

Numerical equation of the freezing point t_f as a function of the water contents ψ.

Numerical equation of the integer n valid in the range of smooth enthalpy gradient (equation 4) as a function of the water contents ψ.

Numerical equation of the constant C valid in equation (4) as a function of the water contents ψ.

Particular values of interest for various parameters ψ.

Reference to illustrations.

Numerical equation of the specific heat c_+ at temperatures above t_f up to blood temperature as a function of the water contents ψ.

Beef

$t_f = -3(1/\psi - 1)$

$n = 0.066 + 0.360(1 - \psi) + 0.217(1 - \psi)^2$

$C = 11.31 - 43.7\,\psi + 106.33\,\psi^2$

ψ	0.80	0.75	0.70	0.65	0.60
t_f	−0.8	−1.0	−1.3	−1.6	−2.0
n	0.148	0.170	0.194	0.219	0.245
C	44.40	38.35	32.82	27.83	23.36
H'_g	57.9	52.0	46.5	41.2	36.3
H'_{40}	76.6	71.8	67.1	62.5	57.7
$1/c_0$	0.0153	0.0196	0.0250	0.0319	0.0405
$1/\dot{H}$	0.0147	0.0160	0.0173	0.0196	0.0208
c_0	65.2	51.0	39.9	31.4	24.7
c_g	1.43	1.47	1.50	1.50	1.48
c_{40}	0.28	0.31	0.33	0.34	0.35
c_+	0.85	0.83	0.81	0.78	0.75

Figs. 1 and 2.

At $\vartheta = 0$ the specific heat of beef with a higher contents of water is higher than that with lower ψ–value. When almost all water has become frozen at $\vartheta = 6$, the c-values are becoming almost equal. Below that temperature the influence of hardening fats becomes dominant and responsible for a reversal of the relative magnitude of c-values.

$c_+ = 0.35 + 0.825\,\psi\,(1 - 0.3\,\psi)$

Pork

Analysis of the data by Rjutov (1950), Latyshev (1975) and particularly those recently published by Riedel (1976) tends to confirm that, regardless of water contents, the freezing point of pork is practically constant, namely, $t_f = -0.9°C$

For n and C the following equations are valid.

$$n = 0.237 - 0.315(1 - \psi) + 0.604(1 - \psi)^2$$
$$C = -3.77 + 63.09\,\psi - 16.08\,\psi^2$$

ψ	0.65	0.60	0.55	0.50	0.45
t_f	−0.9	−0.9	−0.9	−0.9	−0.9
n	0.201	0.208	0.217	0.231	0.247
C	30.45	28.29	26.07	23.76	21.36
H'_g	43.63	41.08	38.45	35.95	33.26
H'_{40}	63.91	60.94	58.06	55.72	53.12
$1/c_0$	0.0276	0.0304	0.0339	0.0386	0.0446
$1/\dot{H}$	0.0183	0.0193	0.0204	0.0214	0.0226
c_0	36.18	32.92	29.53	25.94	22.44
c_g	1.46	1.42	1.39	1.38	1.37
c_{40}	0.32	0.32	0.31	0.32	0.33
c_+	0.86	0.87	0.88	0.90	0.91

Figs. 3 and 4.

$$c_+ = 1.031 - 0.293\,\psi\,(1 - 0.154\,\psi)$$

The contents of water ψ and fat χ can be linked by

$$[1 - (\psi + \chi)]^2 = 0.149\,\psi - 0.0455$$

from which

$$\chi = (1 - \psi) - 0.386\,\sqrt{(\psi - 0.305)}$$

Lamb

$$t_f = -0.75\,\sqrt{(1/\psi - 1)}$$
$$n = -0.031 + 0.817(1 - \psi) - 0.65(1 - \psi)^2$$
$$C = 25.70 - 69.9\,\psi + 122.7\,\psi^2$$

ψ	0.65	0.60	0.55	0.50	0.45
t_f	−0.6	−0.6	−0.7	−0.7	−0.8
n	0.175	0.192	0.205	0.215	0.222
C	32.11	27.93	24.38	21.43	19.10
H'_g	43.96	39.38	35.20	31.50	28.44
H'_{40}	61.23	56.73	51.93	47.38	43.34
$1/c_0$	0.0239	0.0293	0.0349	0.0410	0.0468
$1/\dot{H}$	0.0188	0.0205	0.0226	0.0249	0.0274
c_0	41.87	34.18	28.62	24.42	21.35
c_g	1.28	1.26	1.20	1.13	1.05
c_{40}	0.27	0.27	0.27	0.25	0.24
c_+	0.81	0.79	0.77	0.73	0.70

Figs. 5 and 6

$c_+ = 0.15 + 1.67\,\psi\,(1 - 0.60\,\psi)$

The contents of water and fat can be linked by

$\psi + \chi = 1 - 0.36\,\psi$

from which

$\chi = 1 - 1.36\,\psi$

Seafish

$t_f = -3(1/\psi - 1)$ (same as beef)

$n = 0.116 + 0.19(1 - \psi) + 0.6(1 - \psi)^2$

$C = 6.58 - 38.6\,\psi + 101\,\psi^2$

ψ	0.85	0.80	0.75	0.70	0.65
t_f	−0.5	−0.8	−1.0	−1.3	−1.6
n	0.158	0.178	0.201	0.227	0.258
C	46.74	40.34	34.44	29.05	24.16
H'_g	62.0	55.5	49.4	43.6	38.4
H'_{40}	83.7	77.8	72.3	67.1	61.1
$1/c_0$	0.0153	0.0192	0.0244	0.0312	0.0403
$1/\dot{H}$	0.0136	0.0148	0.0162	0.0177	0.0193
c_0	65.4	52.0	41.0	32.0	24.8
c_g	1.63	1.65	1.65	1.65	1.65
c_{40}	0.33	0.35	0.36	0.38	0.39
c_+	0.93	0.90	0.88	0.85	0.83

Figs. 7 and 8.

$c_+ = 0.5(1 + \psi)$

Enthalpy diagrams

An example of an enthalpy-temperature diagram between $\vartheta = 0$ and $\vartheta = 6$, derived from the linear equation (6), is shown in Fig. 9 for pork (full lines) and fish (broken lines) and the respective parameters ψ. The diagram is extended into the range of 'smooth' enthalpy gradients from $\vartheta = 6$ to $\vartheta = 10$ according to equation (4).

Conclusion

The very moment the water contained in foods starts freezing with a resulting discontinuity of the specific heat, calculations and diagrams can be simplified and become a more useful and meaningful tool in the hands of the process engineer when introducing the ϑ-scale at $-t+t_f = 0$ and starting the freezing range with enthalpy $H = 0$. Consequently, H can be expressed as a unified function of ϑ and ψ which has been analysed for various meats and fish.

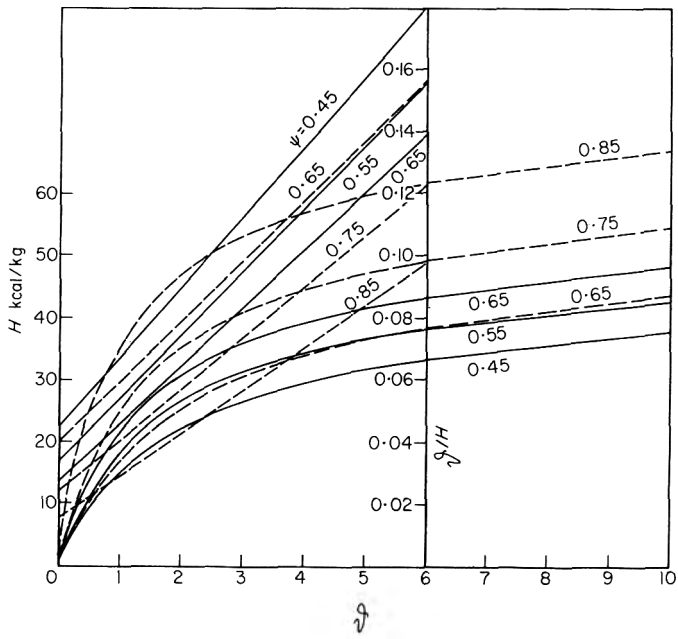


Figure 9. Pork (ψ = 0.65, 0.55, 0.45): ---, fish (ψ = 0.85, 0.75, 0.65). Linear function ϑ/H between ϑ = 0 and 6 and enthalpy H between ϑ = 0 and 10 K.

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Nomenclature

t	temperature
t_f	temperature at which freezing starts
$\vartheta = -t + t_f$	temperature difference between negative food temperature and its freezing point, i.e., temperature measured in the ϑ-scale
$\vartheta_g = -t_g + t_f$	temperature, in ϑ-scale, at which enthalpy gradient changes from 'steep' to 'smooth'
H	enthalpy in the freezing range at temperatures $< \vartheta_g$
$\dot{H}$	enthalpy limit at $\vartheta = \infty$ if the equation governing the enthalpy variation between $\vartheta = 0$ and ϑ_g were valid beyond ϑ_g
H'	enthalpy in the freezing range at temperatures $> \vartheta_g$
$H_g = H'_g$	enthalpy at ϑ_g

H'_{40}	enthalpy at $\vartheta = 40$
C	a constant
n	integer of exponential temperature variation of H' in the range $> \vartheta_g$
c_0	specific heat at $\vartheta = 0$
c	specific heat in the freezing range at temperatures $< \vartheta_g$
c'	specific heat in the freezing range at temperatures $> \vartheta_g$
$c_g = c'_g$	specific heat at ϑ_g
c'_{40}	specific heat at $\vartheta = 40$
c_+	specific heat between blood temperature and t_f
ψ	water contents of food
χ	fat contents of food

Appendix

The freezing point t_f

At temperatures above the freezing point, particularly in its vicinity, the enthalpy becomes a linear function of the temperature:- a plot of H against t is a straight line. As soon as a part of the water contents of the food starts freezing, its change from the liquid into the solid state requires the dissipation of a certain quantity of latent heat causing a sudden change of enthalpy with temperature at a rate much larger than above freezing. A plot of H against temperatures below freezing point intersects the straight line at the freezing temperature t_f. This procedure can be repeated for the same species of food, but for different parameters ψ. The resulting curve connecting freezing points for different water contents can be expressed by an equation for t_f as a function of ψ for various foodstuffs. The respective numerical equations are presented in the paper.

The graphical procedure is based on experimental H-values at different temperatures. Since the measurement of heat quantities supplied by electric energy is easier and more reliable than that of heat quantities extracted by cooling, the current method of establishing the variation of H with t in the freezing range consists in freezing the specimen to a low temperature, say $-40°C$, and recording its warming up as a function of the measured quantity of heat supply. Thus, the change of enthalpy variation that occurs at t_f marks, in fact, the melting point of the food which is assumed to be identically the same as its freezing point.

Due to the rapid change of H when approaching t_f from the frozen state, measurements in its proximity are rather difficult, and the accuracy of experimental values is bound to suffer.

In the following tables H-values have been calculated and have been compared with empirical values from the literature for beef, pork, lamb and fish. Discrepancies which in certain cases are noticeable at the approach to the freezing point may, possibly, be due to uncertainty of the accuracy of experimental values in that region.

Comparison of empirical and calculated H-values (kcal/kg) referred to $H=0$ at $\vartheta=0$ ($\vartheta=-t+t_f$)

Beef (Riedel, 1978)

ψ	0.8			0.6			0.5	
t_f	−0.8			−2			−3	
ϑ	emp.	calc.	ϑ	emp.	calc.	ϑ	emp.	calc.
4.2	54.9	54.2	3.0	28.3	29.2	2.0	15.4	17.1
9.2	61.4	61.2	8.0	38.9	38.9	7.0	27.9	28.7
14.2	65.0	61.3	13.0	43.6	43.8	12.0	33.8	33.8
19.2	68.4	68.7	18.0	47.7	47.4	17.0	37.5	37.5
29.2	73.7	73.1	28.0	52.9	52.8	27.0	43.0	43.1
39.2	78.4	76.4	38.0	57.4	57.0	37.0	47.4	47.4

Pork (Riedel 1976)

ψ	0.725			0.56			0.402	
t_f	−0.9			−0.9			−0.9	
ϑ	emp.	calc.	ϑ	emp.	calc.	ϑ	emp.	calc.
1.1	25.5	25.4	1.1	21.5	19.9	1.1	16.1	14.0
2.1	35.6	34.9	2.1	29.3	27.9	2.1	22.1	20.4
3.1	40.5	40.2	3.1	33.2	32.5	3.1	25.4	24.4
4.1	43.6	43.6	4.1	35.7	35.5	4.1	27.5	27.1
5.1	45.9	46.0	5.1	37.6	37.6	5.1	29.1	29.0
10.1	52.6	52.7	10.1	43.5	43.6	10.1	34.5	34.6
15.1	56.9	57.0	15.1	47.3	47.5	15.1	38.2	38.5
29.1	64.9	64.9	29.1	54.7	54.7	29.1	45.6	45.6
39.1	69.4	68.7	39.1	59.0	58.3	39.1	49.9	49.3

Lamb (Fleming 1969)

ψ	0.65			0.53			0.44	
t_f	−0.6			−0.7			−0.8	
ϑ	emp.	calc.	ϑ	emp.	calc.	ϑ	emp.	calc.
1.6	25.5	29.5	1.5	21.6	20.7	1.4	18.2	16.1
2.7	34.6	36.1	2.6	26.8	26.5	2.5	22.4	21.3
3.8	38.8	39.8	3.7	29.7	29.8	3.6	24.9	24.3
6.1	43.2	44.3	6.0	33.3	33.7	5.9	27.9	27.9
11.6	49.6	49.6	11.5	38.6	38.6	11.4	32.4	32.4
17.2	52.9	53.2	17.1	41.8	42.0	17.0	35.2	33.7
22.7	55.7	55.9	22.6	44.3	44.5	22.5	37.2	37.8
28.3	58.2	58.1	28.2	46.4	46.6	28.1	39.2	39.7
39.4	61.6	61.6	39.3	50.0	50.0	39.2	42.8	42.8

Fish (Riedel 1978)

ψ	0.8			0.6			0.5	
t_f	−0.8			−2			−3	
ϑ	emp.	calc.	ϑ	emp.	calc.	ϑ	emp.	calc.
4.2	52.0	51.6	3.0	23.9	25.7	2.0	12.2	13.9
9.2	59.9	60.0	8.0	36.0	36.0	7.0	25.3	25.3
14.2	64.4	64.7	13.0	41.9	41.4	12.0	31.5	30.7
19.2	67.7	68.3	18.0	45.9	45.5	17.0	35.4	34.8
29.2	72.7	73.5	28.0	51.5	51.6	27.0	41.2	41.2
39.2	77.6	77.5	38.0	56.4	56.4	37.0	46.1	46.1

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Uses of Pekilo, a microfungus biomass from *Paecilomyces varioti* in sausage and meat balls

JUHA KOIVURINTA, RAKEL KURKELA AND PEKKA KOIVISTOINEN

Summary

The possible inclusion of Pekilo ® biomass, which is a dried powder produced from the filamentous microfungus *Paecilomyces varioti* and containing about 50% protein, in meat based food systems was evaluated using two types of sausage and meat balls as test products. The textural parameters evaluated indicated that Pekilo can form, in combination with meat, structures which are needed in these type of food systems provided that the water and fat content of the system is optimized. A comparatively strong taste owing to Pekilo was detected in these products, but it was not unanimously considered a negative factor in organoleptic analysis. Torutein and Promine D were used as reference materials in part of the sausage tests.

Introduction

Little information is available on the biological or functional properties of microbial biomasses and their proteins and hardly anything has been published on their use in meat products. In this study the suitability of Pekilo biomass in sausage and meat balls was tested using Pekilo to substitute meat.

Pekilo biomass (in text Pekilo) is a dried whole cell microbiological material (SCP), containing about 50% protein (N×6.25) produced by the so called Pekilo process developed in Finland. The microbe used in this process is the filamentous microfungus *Paecilomyces varioti*. The Pekilo process consists of continuous aerobic, submerged cultivation of this microfungus aseptically in a carbohydrate containing sulphite spent liquor solution. The only existing industrial plant to date was started in 1975 in Jämsänkoski, Finland, by the United Paper Mills Ltd. (Romantschuk & Lehtomäki, 1978). The process was first patented in Finland (Patent No. 44366) and is now patented in six other countries. Since 1971 the biomass has been approved as livestock feed in Finland.

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Feeding studies (Laksesvela & Slagsvold, 1974; Alaviuhkola *et al.*, 1975; Farstad *et al.*, 1975) conducted with several animals such as hogs, cows, calves and poultry indicate that Pekilo biomass is nutritionally of high quality. It can therefore be expected to find extensive use as livestock feed.

Before direct application of Pekilo for human consumption a great deal of work is still needed in procuring all the information required in the guidelines 6 (1970) 7 (1970) and 12 (1972) of the Protein Advisory Group of the UN. To approach this situation it was decided to estimate first the technological potential of Pekilo biomass. The decisions on future experiments will be made on the basis of this information. The very good emulsifying property and also the gelling ability of Pekilo reported in a previous paper (Koivurinta & Koivistoinen, 1979a) are assumed to the beneficial for meat product textures. Ziemba (1966) has named three functions for a protein preparation in sausage: to hold water, to disperse fat, and to form a gel-like structure.

The aim of this study was to obtain information on the technological suitability of Pekilo in sausage and meat balls. Special attention was paid to the texture and organoleptic properties of the products.

Materials and methods

Pekilo biomass

Pekilo biomass is a powdered product produced from the filamentous microfungus (*Paecilomyces varioti*) grown aseptically in sulphite spent liquor (Forss, 1974; Romantschuk, 1974; Forss, Passinen & Sjöström, 1974).

Pekilo biomass was mechanically dewatered. washed and dried on a conveyor drier. Chemical characterization and other details are given in Tables 1–3.

Reference materials

Two different protein preparations were used as reference materials in part of the experiments: Torutein (Amoco Foods Co., U.S.A.) and Promine D (Central Soya, U.S.A.). The chemical compositions are given in Table 4.

Table 1. Chemical composition and bulk density of Pekilo

Type of dryer used	Dry matter	Percentage of dry matter		Ash	Bulk density (g/dm^3)
		Crude protein	Crude fat		
Conveyor	94.4	52.3	1.7	5.2	230

Table 2. Essential amino acids of Pekilo protein

Essential amino acids	g/16 gN*
Isoleucine	4.4
Leucine	7.0
Lysine	6.5
Phenylalanine	3.4
Methionine	0.8
Threonine	3.7
Valine	4.9
Tyrosine	3.1

*Determinations were made at Kemira Research Institure, Oulu, Finland

Table 3. Mineral content of Pekilo biomass

Minerals	mg/kg *
Ca	1900
K	10000
Mg	1100
Fe	135
Mn	105
Zn	195
Cu	15
Co	0
Pb	0.46
Se	0.14
Mo	<0.05
Br	<2
B	0.8
As	<0.05
Rb	4.5
Al	27
P	14800
S	4300
F	1.0
Si	110
Cd	0.06
Cr	3.7
Ni	1.8

*Determinations were made at Kemira Research Institute, Oulu, Finland

Table 4. Chemical composition of reference materials

	Dry matter	Crude protein	Fat	Crude fibre	Ash
Torutein	94	55.2	1.0	5	8.4
Promine D	95.2	92	0.5	0.25	4.0

Sausage tests

Sausages were made in the pilot plant of the Department of Meat Technology of the University of Helsinki using the following recipes:

	sausage A	sausage B
Beef meat, kg	2.2	1.0
Pork meat, kg	2.8	1.25
Pork skin/ice water mixture (1/1), kg	0.9	0.75
Potato starch, kg	0.5	0.3
Milk powder, kg	0.5	0.3
Water, kg	3.1	1.25
Additives, spices		

Pekilo was used in sausage A to substitute meat (0–30%) as a 67% (below 10% substitution) or 50% (above 10% substitution) suspension. The substitution levels used for Pekilo were 2, 4, 6, 10, 20 and 30%. The reference materials (substitution levels for Torutein and Promine D were 2,5 and 10%) were used as dry formulations. In sausage B substitution levels of 1,5 and 10% were used. The amount of meat substituted by the materials was calculated from the total amount meat (beef + pork) in the recipe, but in practice the beef fraction in the recipe was reduced by the amount of substitute. The effect of an additional amount of water and water-fat mixture (1:1) on the organoleptic quality of the product was tested separately in the Pekilo sausages A made at 5% substitution level. The sausages were prepared by the normal procedure used at the Department of Meat Technology. All ingredients except water and test materials were first chopped in a cutter. Water was added gradually in the form of ice to cool the mass during chopping. When the mass was uniform the test material was added together with the rest of the water and chopping was continued until the mass was uniform. The raw sausage was extruded into cellulose casing and cooked. A control sausage was prepared with each test series to eliminate possible differences in raw materials.

Meat ball tests

The recipe used in the meat balls tests was:

Mixture of Pekilo suspension and minced meat	300 g
Water	200 g
Ground toast	60 g
Salt and other spices	

The substitution levels used were 2, 4, 8, 12 and 20%. All minced meat was purchased at the same time, mixed well, divided into small portions and stored frozen. The water and fat contents of meat balls were kept constant by adding extra water and fat together with Pekilo. The ingredients were mixed in a cutter, formed manually into balls of approximately 20 g each and fried in oil at 130–140° C (Kultasula oil, Raision Tehtaat, Finland, a mixture of soybean oil and rapeseed oil).

Evaluation of products

The consistency of meat balls and sausages A was measured on three whole products with an Instron consistometer (Table Model M 100, Instron Ltd., England). The effect of additional amounts of water in the sausages A on the consistometer values was determined at the 5% substitution level.

Appearance (0–4 points), texture (0–4 points), aroma (0–2 points) and taste (0–8 points) of sausages were evaluated be a panel of five members, using a scoring method. The most important parameters of evaluation which should be considered before giving a score were listed.

Appearance, gumminess, hardness, colour, juiciness and chewability of the meat balls were evaluated by asking the panel members to indicate their opinion on a segment of a line. The score on the segment ranged from +2 to −2. Standard meat balls were known and analysed at the same time. The possible effects of Pekilo on the taste and aroma of meat balls as compared to the reference ones were evaluated by the same method, using a range of 0 to −4 for taste and 0 to −2 for aroma.

Results

Sausages

The organoleptically determined appearance and texture of all sausages containing test materials differed little from the control up to the 10% substitution level (Table 5). In Pekilo sausages A, which were tested also on higher substitution levels, only slight differences were observed up to the 30% substitution level. Off-odour and off-flavour of all the test materials was observed already at the lowest substitution level. Pekilo had a clear off-flavour which was observable already at the 2% level (sausage A), but it was considered acceptable by some of the panel members and unacceptable by others. Taste was not evaluated at the highest substitution levels (sausage A) because of the distinct off-flavour. The scores given for Promine D sausages for taste were somewhat higher than those given to Pekilo or Torutein sausages at 5 and 10% substitution level.

The aroma and taste of sausages B were considered even superior to the control at lowest substitution level. Even at 10% substitution level the scores were almost comparable to the control.

Table 5. The effect of test materials on the organoleptic properties of the sausages tested

Substitution	Appearance (0–4 p)	Texture (0–4 p)	Colour (0–2 p)	Aroma (0.2 p)	Taste (0–8 p)
Sausage A					
Control (for Pekilo)	3.2	2.8	1.9	1.9	4.4
Pekilo					
2%	3.2	2.7	1.7	1.0	3.8
4%	3.2	2.8	1.7	1.0	3.8
6%	3.0	2.6	1.9	0.9	3.8
10%	3.1	2.5	1.6	1.1	
20%	2.8	2.7	1.2	0.5	
30%	2.2	1.8	0.6	0.3	
Control (for other materials)	3.4	3.2	1.7	1.8	5.5
Torutein					
2%	3.2	3.2	1.7	1.4	4.6
5%	3.3	2.9	1.5	1.3	3.8
10%	3.4	2.6	1.7	1.2	2.8
Promine D					
2%	3.5	3.0	2.0	1.4	4.5
5%	3.5	3.1	1.6	1.5	3.7
10%	3.4	3.0	1.7	1.4	4.0
Sausage B					
Control	2.9	3.6	1.9	1.9	5.4
Pekilo					
1%	3.1	3.6	1.8	1.9	6.1
5%	2.7	3.3	1.9	1.7	5.3
10%	3.4	3.4	1.7	1.6	5.0

Better scores were generally given to sausages A (5%substitution) in which both water and fat were added than when only water was added (Fig. 1). The aroma of Pekilo sausages was considered quite unpleasant at all levels of water and mixture of water and fat addition and therefore no organoleptic evaluation on taste was performed. Optimum appearance and texture were obtained on 300 g of fat and water addition level.

The consistency of Pekilo sausages A, measured with an Instron consistometer (Fig. 2) was harder than that of the control up to the 30% substitution level where it was approximately equal to the control. Torutein and Promine D did not affect the consistency until at the 10% substitution level where the sausages were harder (Promine D 8.9,Torutein 7.4) than the control (6.6). When the amount of water was increased the Pekilo sausages became softer in consistency at the same rate as the control (Fig. 3).

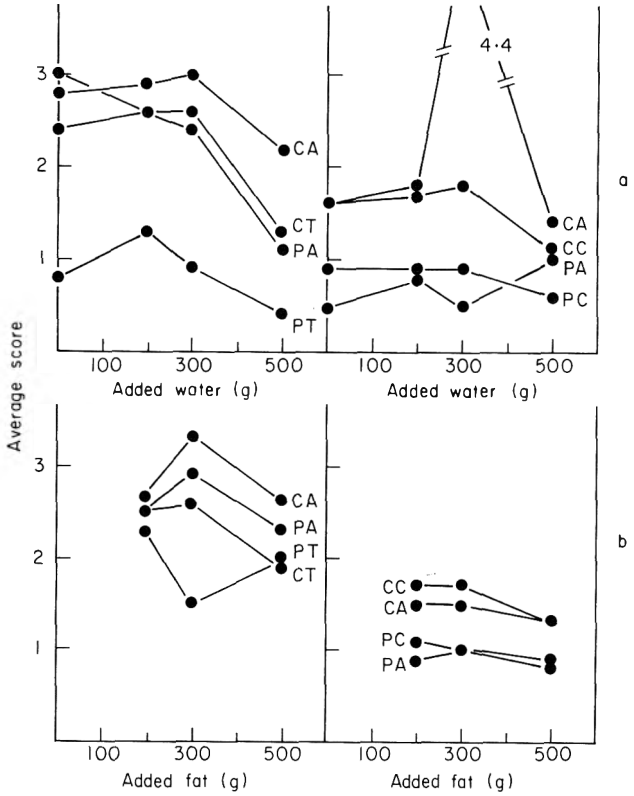

Figure 1. Effect of additional water (a) and water + fat (b) on sausages (type A) at 5% substitution level. Left: CA, control appearance; CT, control texture; PA, Pekilo appearance; PT, Pekilo texture. Right: CA, control aroma, CC, control colour; PA, Pekilo aroma; PC, Pekilo colour).

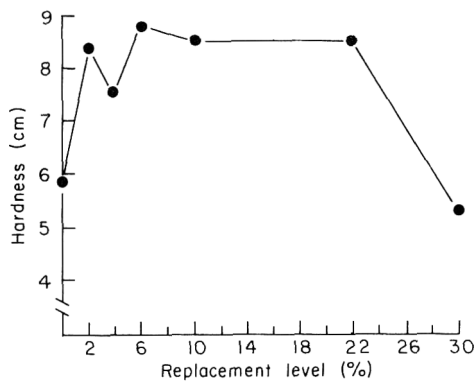

Figure 2. Hardness (consistometer) of Pekilo sausages at different substitution levels

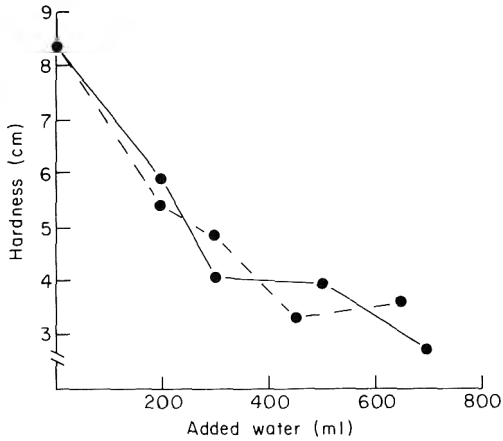

Figure 3. Effect of various amounts of water on the hardness (consistometer) of Pekilo sausages at 5% substitution level (——— Pekilo; - - - Control)

Meat balls

Meat balls in which, 2, 4, 8, 12 or 20% of the minced meat was substituted with Pekilo water suspension were generally considered inferior to the standard meat balls as judged by the organoleptic properties studied. With increasing substitution level the number of negative scores as compared with the control increased (Table 6). Only the juiciness of the Pekilo meat balls was considered better than that of the control. A relatively small change was observed in the appearance and colour of Pekilo meat balls up to the 8% substitution level. For taste the Pekilo meat balls were given distinctly lower scores than the control meat balls already at the 2% replacement level (Table 7).

The consistency of the Pekilo meat balls became softer when the substitution level increased except at the 12% level where the Instron values were almost equal to the control values (Table 8). A slight exception was also observed in the scores given for hardness in the organoleptic evaluation at the same substitution level.

Table 6. The differences in scores given to Pekilo meat balls at different substitution levels and to the control for appearance, colour, gumminess, hardness, chewability and juiciness

Substitution level	Appearance	Colour	Gumminess	Hardness	Chewability	Juiciness
2%	0.1	−1.1	−2.9	−2.8	−0.4	0.9
4%	−1.2	−0.4	−4.5	−7.8	−4.5	2.2
8%	−0.8	−0.4	−4.2	−7.5	−6.6	4.9
12%	−2.4	−2.2	−7.8	−5.2	−5.5	1.8
20%	−3.8	−4.7	−11.9	−10.7	−10.8	6.3

Table 7. The differences in scores given to Pekilo meat balls at different substitution levels and to the control for aroma and taste

Substitution level	Aroma	Taste
2%	−2.6	−6.5
4%	−2.8	−12.8
8%	−3.8	−14.5
12%	−5.6	−14.5
20%	−7.1	−22.0

Table 8. The effect of Pekilo on the consistency of meat balls at different substitution levels

Substitution level	Hardness	Standard deviation
0%	6.5	0.60
2%	6.5	0.90
4%	4.7	1.05
8%	4.2	0.45
12%	6.1	0.95
20%	3.9	0.62

Discussion

The sausage A used in these experiments has a very mild taste, no strong spices or onion are included in the recipe. Small changes in raw material composition are therefore expected to have a marked effect on the taste of this type of sausage. The mild taste peculiar to Pekilo powder was evident already at the 2% substitution level but the panel members were not in agreement as to whether this taste was negative or positive compared to the taste of the control sausage. The sensitivity of the kind of sausage used to taste variations caused considerable variability also in the scores given to the various control sausages.

In sausage B, which had a stronger taste, off-flavour was not registered. The results indicate that it is possible to eliminate the typical Pekilo flavour in sausages by selecting the right recipe with suitable spices.

Pekilo increased the hardness of sausages A up to 30% substitution level probably owing to the good water binding and gelling properties reported earlier (Koivurinta & Koivistoinen, 1979 a, b). Soy protein products have been reported to act similarly (Schweiger, 1974; Hermansson & Åkesson, 1975).

The water and fat contents in Pekilo sausage A had a strong effect on the structure of the product. The favourable effect of additional fat in the sausage is assumed to be due to the good fat binding and emulsifying properties of Pekilo and to the good heat stability of Pekilo emulsions (Koivurinta & Koivistoinen, 1979 b). The results obtained with meat balls followed the same pattern as the results for sausages. The off-taste of Pekilo was evident already at the 2% substitution level.

The functional properties of Pekilo biomass, such as water and fat binding, gelling and emulsifying properties reported earlier (Koivurinta & Koivistoinen, 1979 a, b) have been found to lead to structures almost comparable to those of meat in the food systems studied. Elimination of the off-taste that Pekilo has in these products by selecting the best suitable recipe, by modifying it to obtain a more acceptable taste or by removing possible taste components would most probably make its use in the type of food systems studied profitable.

Acknowledgments

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Stability of β-carotene in isolated systems

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Summary

The effects of water activity, antioxidants and fatty acid methyl esters on the stability of β-carotene in isolated model systems has been investigated. The rate of β-carotene degradation decreases with the increase in water activity. Both BHA and PG stabilized β-carotene at all water activity levels. Methyl stearate and oleate enhanced the stability of β-carotene. Methyl linoleate stabilized β-carotene during initial stages of slow oxidation after which it exhibited a strong prooxidant effect.

Introduction

Losses in carotenoids during processing and storage of foods are of commercial significance. Degradation of carotenoids not only affects the attractive colour of foodstuffs but also their nutritive value and flavour. The main cause of carotenoid degradation in foods is oxidation. In processed foods the mechanism of oxidation is complex and depends on many factors such as moisture, temperature, presence of pro- and anti-oxidants and lipids. Many workers have investigated the effect of water activity and lipids on the stability of carotenoids but the conclusions of various studies have been at variance. Baloch, Buckle & Edwards (1977) have reported that β-carotene is most stable at monolayer water activity. But Martinez & Labuza (1968) found that rate of degradation of astacene in freeze dried salmon decreased with increase in water activity even above the monolayer value. Kanner, Mendel & Budowski (1978) have also observed that stability of paprika carotenoids slightly increases with rise in water activity. Chen & Gutanis (1968) reported that degradation of carotenoids in ground chilli paprika follows a second order reaction kinetics and the reaction constants were 2–3 times higher at 4–5% moisture than at 8–9% moisture. Quackenbush (1963) on the other hand has found that stored corn having 3% moisture retained more carotenoids than that having 11% moisture.

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Knowles *et al.* (1968) also observed that xanthophylls in alfalfa meal are better retained at low moisture (3–4%) than at higher moisture (8–12%).

In the presence of lipids carotenoids have been reported to undergo coupled oxidation at rates dependant on the system. Bickoff *et al.* (1955) and Nazar *et al.* (1976) reported that addition of vegetable oils to alfalfa meal and carrot powder increases the stability of carotenoids. Budowski & Bondi (1960) found that the addition of unsaturated oils increased the rate of autoxidation of carotenoids. Lime (1969) has also reported that both methyl linoleate and methyl linolenate accelerated the rate of β-carotene destruction in dehydrated foods. Koloman & Gerhard (1974) on the other hand have concluded that in highly unsaturated systems, carotenoids are more stable because lipid itself accepts free radicals more readily than carotenoids. The present study was therefore undertaken to observe the effect of water activity, fatty acid esters and antioxidants on the stability of carotenoids.

Materials and methods

Reagents

All the solvents were of analytical reagent grade and used as such without further purification. Microcrystalline cellulose and β-carotene were from E.Merck. Methyl stearate, methyl oleate and methyl linoleate were obtained from V.P. Chest Institute, Delhi. Butylated hydroxyanisole (BHA) and propyl gallate (PG) were from May & Baker Ltd., Dagenham, and Ward Blenkinsop & Co., London respectively.

Impregnation of β-carotene on cellulose

β-carotene (85 mg) was dissolved in 50 ml of benzene and quantitatively transferred to a 1 litre RB flask containing 50 g microcrystalline cellulose. Weighed quantities of BHA (10 mg) or PG (10 mg) and methyl esters of fatty acids (775 mg) were dissolved in benzene and added to the cellulose powder along with β-carotene. The flask was swirled for uniform mixing and the solvent was evaporated under vacuum using a rotary vacuum evaporator. The coloured powder was ground in a glass mortar in order to break any lumps and redried under vacuum to remove the traces of benzene All operations were carried out under subdued light.

Storage tests

Impregnated cellulose powder was adjusted to different water activity by keeping 8 g of samples in glass petri dishes of 8.5 mm diameter over saturated salt solutions in desiccators according to the method of Rockland (1960). The desiccators were wrapped in black polyethylene and stored at room temperature (16–32°C). On alternative days desiccators were opened for 2 min for replenishing depleted oxygen in the storage atmosphere. The concentration of

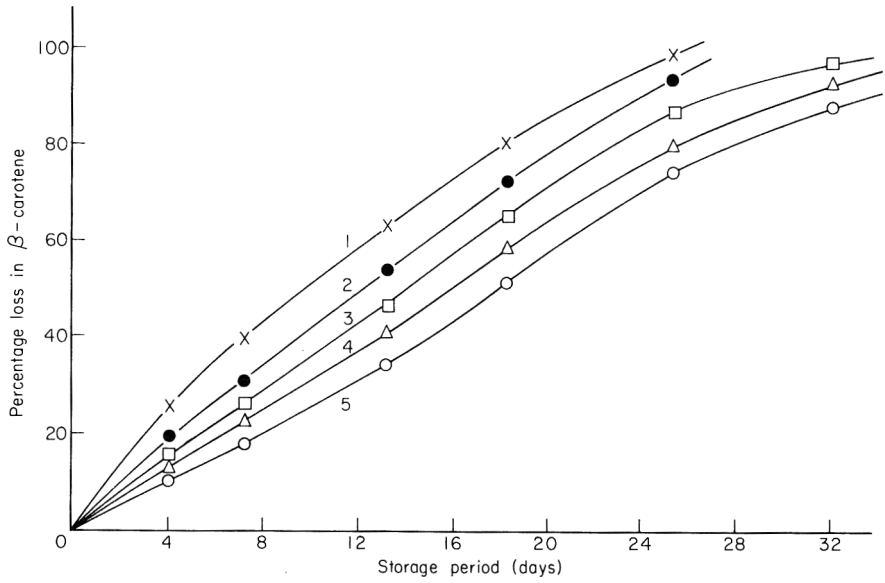

Figure 1. Percentage loss of β-carotene during storage at different water activities. 1, 0.0; 2, 0.22; 3, 0.33; 4, 0.43; 5, $0.73a_w$.

β-carotene in the samples was determined by extracting 250 mg samples with 50 ml hexane and measuring the absorbance at 449 nm using a Perkin-Elmer Model 124 Spectrophotometer. For determining peroxide value, 1 g samples were treated with 25 ml chloroform-acetic acid (2:3) mixture. The mixture was treated with 1 ml saturated potassium iodide solution and kept in the dark. After 15 min the contents were treated with 30 ml distilled water and titrated against 0.01 N sodium thiosulphate solution using 1% starch solution as indicator.

Results and discussion

Effect of water activity on the rate of β-carotene degradation is shown in Fig. 1. It may be seen that in isolated systems the rate of β-carotene degradation significantly decreases with rise in water activity. Baloch *et al.* (1977) had previously reported that β-carotene was most stable at monolayer water activity (0.32). However, in the present study, stability of β-carotene increased with increasing water activity even above the monolayer value. Kanner *et al.* (1978), Chou & Breene (1972), Chen & Gutanis (1968) and Martinez & Labuza (1968) have also found that the protective effect of water continuously increased from dry to multilayer region.

Both BHA and PG significantly lowered the rate of β-carotene degradation (Figs. 2–4). Relatively BHA was more effective in the dry systems whereas PG was more effective above the monolayer region. Higher effectiveness of PG above the monolayer region may be due to its higher hydrophilicity compared

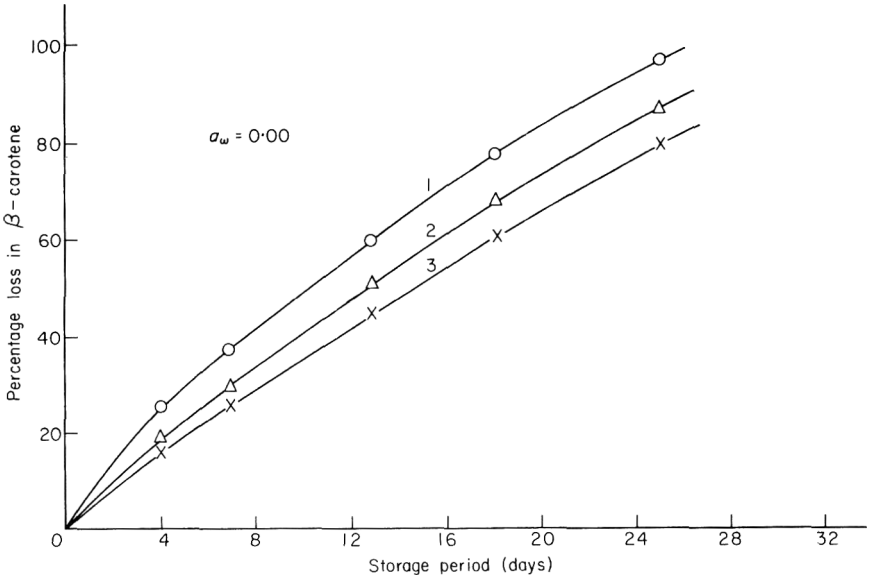

Figure 2. Effect of antioxidants on losses in β-carotene during storage at 0.0 a_w. 1, control; 2, β-carotene + Pb; 3, β-carotene + BHA.

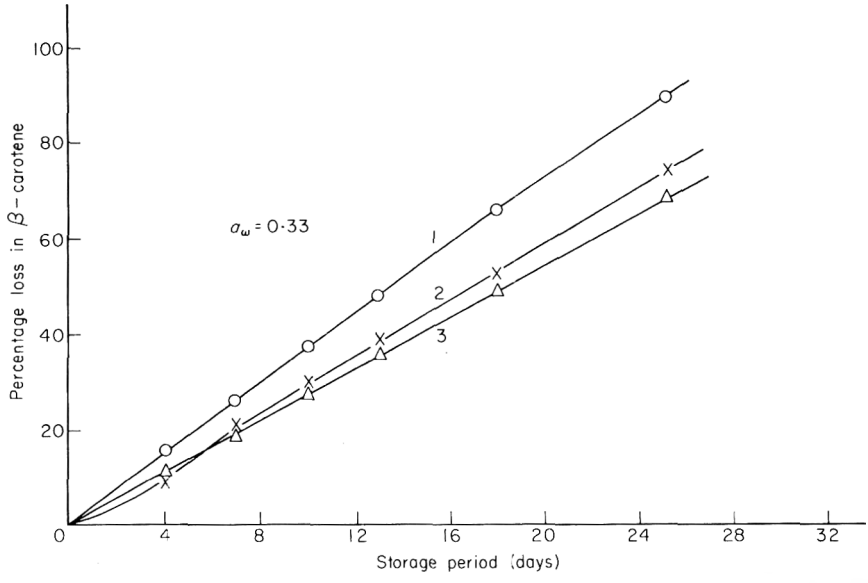

Figure 3. Effect of antioxidants on losses in β-carotene during storage at 0.33 a_w. 1, β-carotene; 2, β-carotene + Pb; 3, β-carotene + BHA.

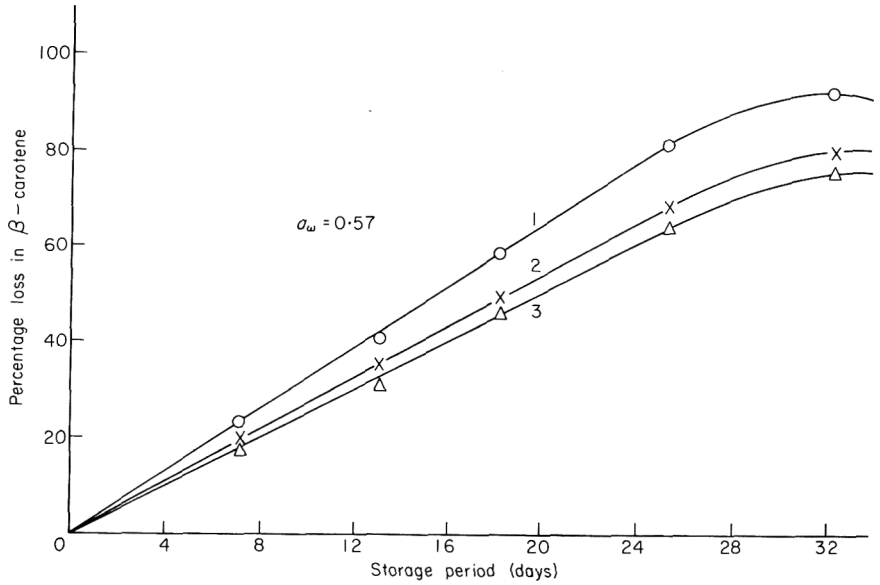

Figure 4. Effect of antioxidants on losses in β-carotene during storage at 0.57 a_w. 1, β-carotene; 2, β-carotene + BHA; 3, β-carotene + PG.

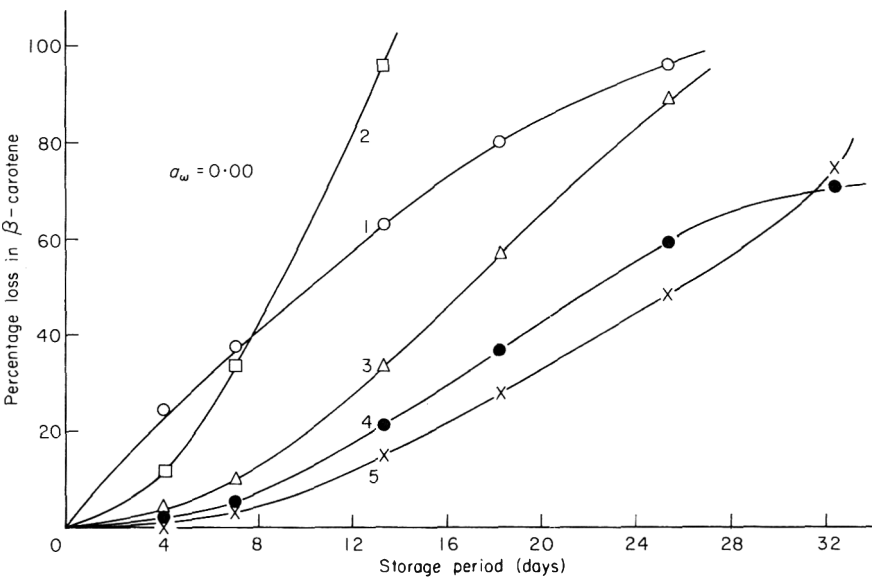

Figure 5. Effect of fatty acid methyl esters on the losses in β-carotene during storage at 0.0. 1, control; 2, β-carotene + methyl linoleate; 3, β-carotene + methyl oleate; 4, β-carotene + methyl linoleate + BHA; 5, β-carotene + methyl stearate.

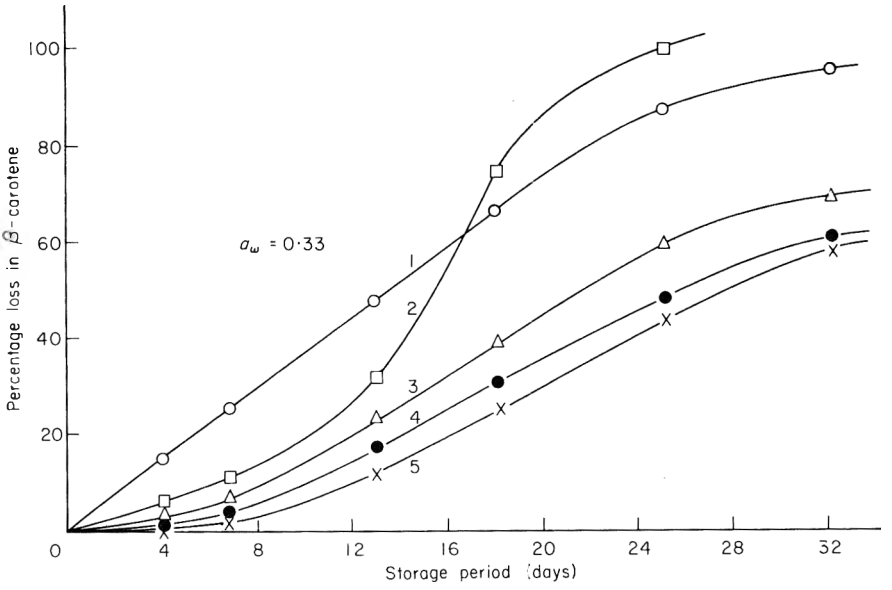

Figure 6. Effect of fatty acid methyl esters on the losses in β-carotene during storage at 0.33 a_w. 1, β-carotene; 2, β-carotene + methyl linoleate; 3, β-carotene + methyl oleate; 4, β-carotene + methyl linoleate + BHA; 5, β-carotene + methyl stearate.

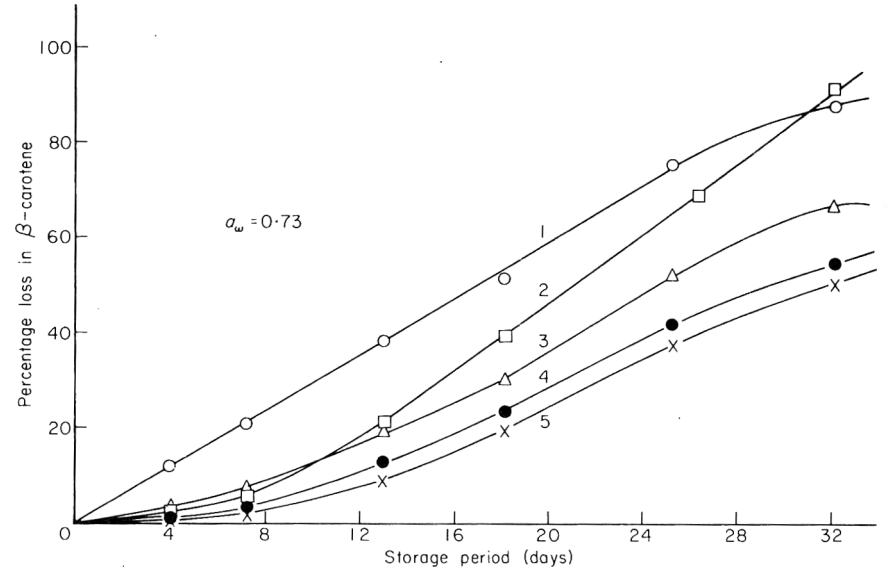

Figure 7. Effect of fatty acid methyl esters on the losses in β-carotene during storage at 0.73 a_w. 1, β-carotene; 2, β-carotene + methyl linoleate; 3, β-carotene + methyl oleate; 4, β-carotene + methyl linoleate + BHA; 5, β-carotene + methyl stearate.

Table 1. Peroxide value (meq O_2/Kg powder) of control and methyl oleate model systems

Storage period (days)	Peroxide value											
	Control						Methyl oleate					
	0.0	0.22	0.33	0.43	0.57	0.73	0.0	0.22	0.33	0.43	0.57	0.73
17	3.2	3.1	3.1	2.6	2.5	2.5	6.3	5.2	4.5	4.7	3.7	3.2
26	4.1	3.8	3.8	3.2	3.0	3.1	8.9	8.4	7.2	6.0	5.3	5.4
32	5.2	4.2	3.8	4.0	3.5	3.4	16.8	9.4	7.2	6.8	6.3	6.1

with BHA (Cornell *et al.*, 1969) and concomitant increased mobility in the system. Increased mobility of PG above the monolayer region is likely to increase the chances of its interaction with the free radicals in the autoxidative process and thereby result in its enhanced protective action. Any increase in the mobility of BHA due to rise in water activity is likely to be small because of its very pronounced hydrophobic nature (Cornell, Devilbers & Pallansch, 1969). Incorporation of fatty acid methylesters along with BHA should increase its mobility and thereby its protective action. In fact the protective action of BHA in the presence of methyl linoleate has been found to be significantly higher than that of BHA alone (Figs. 2–7). Shearer & Blain (1966) have also observed the activity of antioxidants in stabilizing β-carotene increased in the presence of methyl linoleate of linoleic acid.

Effect of methyl stearate, methyl oleate and methyl linoleate on the rate of β-carotene degradation is shown in Figs. 5–7. Incorporation of methyl stearate and oleate considerably enhanced the stability of β-carotene in cellulose model systems at all the water activities (0.00 to 0.73) and during the entire period of storage. Methyl linoleate on the other hand stabilized β-carotene during initial stages of storage only; the stabilizing effect being evident only up to 7, 16 and 30 days of storage at 0.00, 0.32 and 0.73 water activities respectively. During subsequent periods methyl linoleate significantly enhanced the rate of β-carotene oxidation and exhibited a marked prooxidant effect. Apparently

Table 2. Peroxide value (meq O_2/Kg powder) of methyl linoleate and methyl linoleate + BHA model systems

Storage period (days)	Peroxide value											
	Methyl linoleate						Methyl linoleate + BHA					
	0.0	0.22	0.33	0.43	0.57	0.73	0.0	0.22	0.33	0.43	0.57	0.73
7	8.4	4.8	4.8	4.8	4.9	4.5	6.5	2.9	2.9	2.9	2.9	2.5
14	106.9	55.9	12.0	12.0	7.9	7.0	10.2	10.3	6.3	6.3	6.4	5.4
18	104.9	110.3	19.8	—	10.5	8.1	17.7	14.2	8.2	8.1	7.6	4.9
27	77.6	77.2	96.3	86.7	35.8	12.8	20.2	—	9.7	8.9	8.7	5.8
33	67.9	68.0	96.5	88.1	92.8	17.5	—	—	—	—	—	—

both anti-oxygenic and pro-oxygenic factors are involved in determining the effect of lipids on the stability of carotenoids. The increased stability of β-carotene in the presence of methyl esters of fatty acids may be due to the dilution effect whereas pro-oxidant effect of highly unsaturated fatty acids like methyl linoleate may be due to their increased susceptibility towards antioxidation and concomitantly increased concentration of peroxides which catalyse the destruction of β-carotene. In the case of methyl stearate and loeate, the autoxidation proceeds too slowly (Table 1), the stabilization due to dilution effect manifests itself over the pro-oxidant effect resulting in an overall increased stability of β-carotene. But in the case of methyl linoleate the dilution effect is prominent only during the induction period when the concentration of peroxides is low (Table 2). After the induction period the pro-oxidant effect becomes more pronounced resulting in an overall enhanced rate of β-carotene degradation. Since the rate of autoxidation of fatty acid esters decreases with an increase in water activity, the enhanced stability of β-carotene due to dilution effect of methyl linoleate is more prominent at higher water activities (7 days at 0.00 a_w and 30 days at 0.73 a_w). In the case of methyl linoleate plus BHA, autoxidation proceeds too slowly, the stabilizing action due to dilution effect manifests itself during the entire period of storage. This is also supported by the relatively low peroxide value of the system containing linoleate and BHA (Table 2).

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Stability of carotenoids in dehydrated carrots

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Summary

The effects of water activity, salt, sodium metabisulphite and Embanox-6 on the stability of carotenoids in dehydrated carrots has been investigated. Carotenoid pigments have been found to be most stable at 0.43 a_w. Incorporation of salt, sodium metabisulphite and Embanox-6 significantly reduced the rate of carotenoid destruction and non-enzymic browning in dehydrated carrots.

Introduction

Several deteriorative reactions affect the colour, nutritive value, texture and flavour of dehydrated vegetables during storage. Degradation of carotenoids has been reported to play an important role in determining the overall storage life of dehydrated carrots. Tomkins *et al.* (1944), Tajiri, Matsumoto & Hara (1973) and Tomkins, Mapson & Wager (1946) reported that off-flavours became noticeable when about 20–45% of the total carotenoids had been destroyed. Falconer *et al.* (1964) also reported a significant correlation between the development of off-flavours and carotenoid destruction in freeze dried carrot powder stored at 18°C. Fishwick (1969) and Walter *et al.* (1970) have reported that voilet- and hay-like aroma prevalent in stored dehydrated vegetables arise from the autoxidation of carotenoids. A number of factors such as blanching, water activity and the presence of pro- and anti-oxidants may influence the stability of carotenoids in foods. In the present study the effects of water activity, blanching and additives like NaCl, bisulphite and Embanox-6 on the stability of carotenoids in dehydrated carrots has been studied.

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Materials and methods

Carrots

Good quality carrots were purchased from the local market, trimmed and hand peeled. The carrots were washed in running tap water, sliced (3–4 mm thick) and mixed thoroughly. Sliced carrots (3 kg) were taken in a muslin cloth and blanched in boiling water for six minutes to inactivate the peroxidase activity. In order to study the effect of salt, sodium metabisulphite and Embanox-6, the blanched carrots (3 kg) were dipped in 5% NaCl solution (3 l) both with and without sodium metabisulphite (0.1%) and Embanox-6 (0.1%) for 20 min.

Hot air drying

The blanched and treated carrots were dehydrated in a Kilburn hot air drier at 70°C to a final moisture level of 4–5%.

Accelerated freeze drying

Both blanched and unblanched carrots were frozen in a blast freezer and dehydrated by accelerated freeze drying technique in a 'Socaltra' freeze drying plant having a radiation heating system. During freeze drying, the product temperature was increased from −25°C at the beginning to 50°C at the end of the drying cycle (8 h). The moisture content in the dried product varied from 3–4%.

Packaging and storage

The dehydrated carrots (50 g) were packed in paper-aluminium foil-polyethylene laminated packs and stored at room temperature (16–32°C). For studying the effect of water activity on the rate of carotenoid destruction, 15 g samples of powdered freeze dried carrots were kept in glass petri dishes over saturated salt solutions in desiccators which were wrapped in black polyethylene and stored at room temperature.

Estimation of carotenoids

Total carotenoids in air dried carrots were determined according to Livingston, Knowles & Kohler (1971). Carotenoids from raw, blanched and freeze dried carrots were extracted with acetone-hexane mixture according to AOAC Procedure (1975). From the extract acetone was washed with five 100 ml portions of 5% NaCl solution. The volume of the hexane layer was made up to 100 ml and the absorbance was measured at 449 nm in a Perkin-Elmer model 124 spectrophotometer. Total carotenoids were expressed as β-carotene using $E^{1\%}_{1cm}$ 2500.

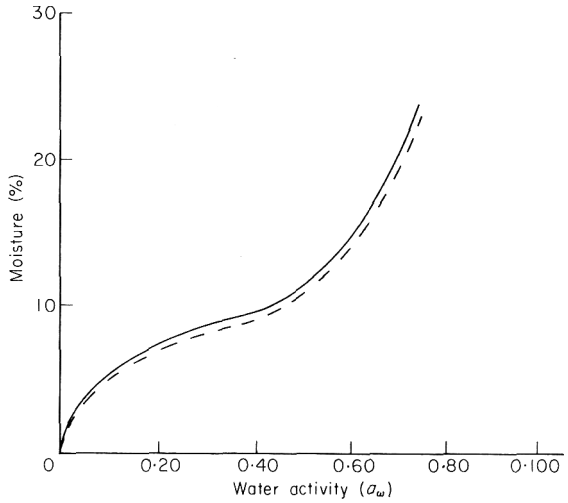

Figure 1. Sorption isotherm of freeze dried carrot powder. -----, Blanched; ———, unblanched.

Lipid peroxidation

TBA values were determined by the steam distillation method of Tarledgis *et al.* (1960). The peroxide values of dehydrated carrots were measured by extracting 5 g samples with 20 ml chloroform. The chloroform extract was treated with 30 ml acetic acid and 1 ml saturated KI solution and kept in the dark. After 15 min, the contents were treated with 50 ml water and titrated with standard sodium thiosulphate solution (0.01 N) using 1% starch solution as indicator.

Non-enzymic browning

Five g samples were shaken with 100 ml water in a wrist shaker for 2 hr. The solution was centrifuged at 5000*g* and the absorbance was measured at 420 nm.

Moisture

The moisture content in dehydrated carrots was estimated according to ISI method (I.S.4625–1968).

Results and discussion

Effect of water activity

Changes in carotenoids in freeze dried carrot powders stored at different water activities (a_w, 0.0 to 7.3) are shown in Table 1. It may be observed that carotenoids are relatively more stable in the range of 0.32 to 0.57 a_w; the maximum stability is near about 0.43 a_w. Both below and above this level the rate of carotenoid destruction increased significantly. The increase in the rate of carotenoid destruction was greater at lower a_w than at higher a_w. The moisture

Table 1. Effect of water activity on the stability of carotenoids in freeze dried carrots

Storage period (days)	Total carotenoids (μg/g) Unblanched 0.0	0.23	0.33	0.43	0.57	0.73	Blanched 0.0	0.23	0.33	0.43	0.57	0.73
0	1035	—	—	—	—	—	1240	—	—	—	—	—
6	801	830	861	896	867	866	832	936	973	981	948	935
10	751	790	822	833	832	834	774	884	934	940	924	929
15	709	741	775	781	777	718	615	806	822	849	837	813
21	608	689	746	747	710	633	42	750	767	821	813	667
	(5.8)	(2.1)	(1.8)	(1.1)	(1.2)	(2.8)	(20.7)	(2.8)	(1.8)	(1.1)	(1.6)	(3.4)
27	368	—	681	687	650	365	5	643	730	745	735	364
	(38.3)	(1.9)	(1.6)	(1.2)	(3.1)	(9.8)	(86.7)	(3.1)	(2.5)	(1.1)	(2.3)	(15.8)
31	58	567	603	646	602	300	—	561	615	678	675	218
	(79.1)	(1.6)	(1.6)	(1.2)	(4.8)	(14.2)	(60.5)	(8.5)	(2.8)	(1.1)	(3.8)	(26.2)
41	6	453	470	568	549	140	—	428	502	575	556	145
	(5.4)	(3.5)	(4.7)	(4.5)	(5.7)	(11.4)	(5.1)	(3.5)	(3.5)	(4.0)	(4.4)	(10.9)

Values in brackets indicate the peroxide value of the samples after the respective storage periods.

sorption isotherm of freeze dried carrots is shown in Fig. 1. At an a_w of 0.43 freeze dried carrots equiliberated to 8.8–10.0% moisture. Previously Tomioka *et al.* (1973) have also observed that carotenoids in freeze dried carrots were more stable at 10% than at 0% moisture. Tomkins *et al.* (1944), however, reported that proportion of β-carotene oxidized during storage was higher at 8.2% moisture than at 5.4% moisture.

In isolated systems the rate of carotenoid destruction has been found to decrease continuously with increasing a_w (Arya *et al.*, 1979; Kanner, Mendel & Budowski, 1978). The increase in the rate of carotenoid destruction at higher water activities (a_w, 0.73) in freeze dried carrots may be due to solubilization and concomitantly increased mobilization of catalysts present and exposure of new sites on solid matrix as a result of swelling of the matrix. An increase in the rate of lipid peroxidation at higher a_w in the presence of metal catalysts has been observed previously by Labuza & Chou (1974). In the present study also the

Table 2. Changes in total carotenoids, TBA value and peroxide value of freeze dried carrots stored at 5°C

Storage period (months)	Unblanched Carotenoids (μg/g)	TBA value	Peroxide value	Blanched Carotenoids (μg/g)	TBA value	Peroxide value
0	1035	0.12	1.8	1240	0.09	2.1
2	390	1.27	17.5	253	2.44	43.4
4	100	2.09	15.2	76	3.05	22.3

TBA value, mg of malonaldehyde per kg substance.

peroxide value of freeze dried carrots stored at an a_w of 0.73 was significantly higher than those stored at a_w of 0.32 to 0.57% (Table 1). Since in the presence of lipids carotenoids undergo coupled oxidations increased concentration of peroxides would be expected to enhance the rate of carotenoid destruction. In our previous studies in isolated systems, no metal catalysts were included and consequently increase in the carotenoid destruction and lipid peroxidation due to increased mobilization of catalysts may not be of significance. Accordingly the protective action of water increased continuously with increasing a_w.

Relatively the rate of carotenoid destruction was higher in blanched freeze dried carrots than in unblanched freeze dried carrots. This effect was particularly more pronounced at very low a_w. Tomkins *et al.* (1944) have reported that scalding did not significantly alter the rate of carotenoid destruction in dehydrated carrots. Feinberg *et al.* (1964), however, reported that blanching before dehydration enhances the stability of carotenoids. This effect is generally believed to be due to the inactivation of peroxidase and lipoxidase activity which catalyse the destruction of carotenoids and lipids during dehydration and storage. But in freeze dried carrots, especially at low moisture content, blanching seems to accelerate the rate of autoxidation of lipid and carotenoids (Table 2). This suggests that some substances which stabilize carotenoids are either degraded or leached out during blanching. It has been reported that about 10–30% of total solids are lost due to leaching during steam and hot water blanching operations (Baloch, Buckle & Edwards, 1977; Gooding, Tucker & MacDougall, 1960). Water soluble compounds like ascorbic acid, amino acids, bioflavonoids and other polyphenolic compounds present in carrots are expected to be leached out during processing. In unblanched freeze dried carrots these substances may act as anti-oxidants against autoxidation of carotenoids and lipids. A beneficial effect of these substances in stabilizing lipids and carotenoids has been reported previously (Lea, 1958; Pratt & Watts, 1964; Karel *et al.*, 1966; Tajiri, Matsumoto & Hara, 1973; Ben Aziz *et al.*, 1968; Kanner *et al.*, 1978). Alternatively it may also be argued that at low water activity, catalytic activity of peroxidase enzymes may become limited on account of inadequate substrate mobility because the rate of enzyme reactions in dried foods is limited by the rate at which the substrate diffuses to enzyme. Possibility of reactivation of peroxidase activity in blanched freeze dried carrots during storage is ruled out as no activity could be detected in stored samples. Whichever be the mechanism leaching of soluble solids during blanching is a potential food loss during the dehydration process and more efforts are necessary in understanding their role in deteriorative reactions vis-a-vis optimizing the conditions of blanching for maximizing the shelf life of dried carrots.

Effect of additives

Many workers have investigated the role of additives such as bisulphite, NaCl and antioxidants on the stability of carotenoids in dehydrated foods. Conclusions of these studies, however, have been at variance (Speck, Eschar & Solms,

Table 3. Effect of NaCl, $Na_2S_2O_5$ and Embanox-6 on total carotenoids, TBA value and non-enzymic browning in air dried carrots

Storage period (months)	Control			Salt treated			Salt + metabisulphite treated			Salt + metabisulphite + Embanox-6 treated		
	Caro-tenoids (μg/g)	TBA value	NEB	Caro-tenoids (μg/g)	TBA value	NEB	Caro-tenoids (μg/g)	TBA value	NEB	Caro-tenoids (μg/g)	TBA value	NEB
0	1120	0.12	0.08	1137	0.12	0.06	1114	0.10	0.05	1135	0.09	0.05
3	505	0.92	0.14	669	0.83	0.10	691	0.64	0.08	827	0.28	0.09
6	316	1.38	0.21	416	0.92	0.15	449	0.78	0.18	620	0.46	0.14
9	222	1.50	0.28	308	1.05	0.24	353	0.92	0.22	408	0.58	0.18

TBA value, mg of malonaldehyde per kg substance; NEB, non-enzymic browning reported as optical density at 420 nm.

1977; Ellis *et al.*, 1970; Nutting, Neumann & Wagner, 1970; Roberts & McWeeny, 1972; Masure *et al.*, 1950; Nazar *et al.*, 1976; Zhedek, 1968; Tomkins *et al.*, 1946; Braddock & Kesterson, 1974). In the present study the effects of NaCl, $Na_2S_2O_5$ and Embanox-6 on the stability of carotenoids and non-enzymic browning in dehydrated carrots have been investigated and the results are presented in Table 3. It has been observed that addition of NaCl, $Na_2S_2O_5$ and Embanox-6 helps in stabilizing of carotenoids and lipids in dehydrated carrots. Speck *et al.* (1977) have previously reported that salt treatment before air drying significantly improves colour, texture, flavour and carotenoid stability in dehydrated carrots. Ellis *et al.* (1970), however, observed that NaCl acted as pro-oxidant in dehydrated products but as an antioxidant in hydrated gels. In the present study soaking of blanched carrots in 5% NaCl prior to drying significantly reduced the rate of degradation of carotenoids. The salt uptake in the dehydrated carrots was 23% of the total solids as compared to 37% reported by Speck *et al.* (1977). Salt treatment also significantly reduced the rate of non-enzymic browning and improved reconstitution characteristics of the dehydrated carrots. As is evident (Table 3) NaCl, $Na_2S_2O_5$ and Embanox-6 had complementary effect in stabilizing β-carotene in dehydrated carrots. Beneficial action of SO_2 and antioxidants in stabilizing carotenoids in dehydrated vegetables has been reported by Roberts & McWeeny (1972), Nutting *et al.* (1970), Bakai & Zabara (1971); Guether & Henning (1974). Tomkins, Mapson & Wager (1946) and Feinberg *et al.* (1964), however, reported that sulphite treatment did not significantly lower the rate of carotenoid destruction. Nazar *et al.* (1976) have reported that incorporation of BHA along with fats stabilizes but incorporation of BHA with propylene glycol had no effect on carotenoid stability in dehydrated carrot powder. In the present study, however, incorporation of Embanox-6, (PG, BHA and citric acid in propyleneglycol) significantly enhanced the stability of carotenoids and lipids in dehydrated carrots. Incorporation of Embanox-6 along with salt and metabisulphite also tended to decrease the rate of non-enzymic browning.

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The rapid detection of polyphosphate in broiler chicken breast muscle

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Summary

A method for rapid detection of the presence of injected polyphosphate or a mixture of polyphosphate and sodium chloride solutions in broiler breast muscle has been developed. Detection is based on the ratio of sodium and phosphorus (as P_2O_5) concentrations found in a trichloroacetic acid (TCA) extract of the muscle. Comparison of acid digested and undigested TCA extracts showed that the presence of these added salts could be demonstrated equally well without acid digestion. The method has been satisfactorily applied to the analysis of deep-frozen broilers purchased from supermarkets.

Introduction

The ratio of sodium and phosphorus obtained by analysis of broiler breast muscle was used by Grey, Robinson & Jones (1977) to confirm the presence or absence of injected solutions containing polyphosphate or sodium chloride or a mixture of the two. However, the analytical methods involved the use of perchloric acid, both in the digestion of the muscle and in the colorimetric reagent for phosphorus determination, and the careful control of its concentration in the determination of sodium by atomic absorption spectrophotometry.

This paper describes a speedy, routine and reliable method of detection which involves the use of trichloroacetic acid, a deproteinising reagent widely used to extract phosphate intermediates of muscle metabolism (Cardini & Leloir, 1957).

Materials and methods

Reagents

Analytical reagents were purchased from B.D.H. Chemicals Ltd., Poole, Dorset and were all 'Analar' grade except for ammonium metavanadate. Glass distilled water was used for the preparation of aqueous solutions.

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0022–1162/79/1200–0587 $02.00

Injection solutions

These were used as previously described (Grey *et al.*, 1977). Concentrated polyphosphate solution (PURON 6040) was obtained from Albright and Wilson Ltd., Oldbury, Warley, Worcs. and diluted according to the manufacturer's directions. A volume of the diluted solution was mixed with an equal volume of 4% sodium chloride when a mixture was injected.

Solutions were injected into the breast muscle using a hand operated pneumatic injector unit (Autarky Machine Co. Ltd., East Grinstead, Sussex). Carcasses were weighed (a) before and (b) 3 min after injection to obtain the weight of injection solution retained in the muscle (see Grey, Robinson & Jones, 1978). The range of weights of injection solution retained varied from 1.0–70.0 g in birds ranging in eviscerated weight from 1.3–1.8 kg.

Livestock and processing

Ross I broiler chickens grown under commercial rearing conditions were part of the normal daily throughput of a local commercial processing plant. Carcasses were removed from the overhead conveyor system at the end of evisceration, placed in insulated containers and transported to the laboratory where they were subsequently injected, air-chilled at 0° for 1.5 hr, bagged and frozen to –20°C.

A number of carcasses which had been injected, immersion chilled and frozen in another commercial processing plant were also used for comparison. Their origin and treatment were as described by Grey *et al.* (1978). Control carcasses were always given exactly the same treatment but not injected.

Supermarket deep-frozen broilers

Twelve broilers representing three brand names were randomly selected from supermarkets, thawed as described below and the breast muscle analysed.

Sampling procedure

Carcasses were thawed overnight at 15°C and the breast muscles dissected out and weighed. They were then cut up into a 1 litre stainless stell homogenizer (Atomix, M.S.E. Ltd., Manor Royal, Crawley, Sussex) containing 100 ml of chilled 15% trichloroacetic acid (TCA) and homogenized for 30 sec. A further 100 ml of 15% TCA were added and homogenization continued for a further 30 sec or until a uniform paste was formed. The paste was then transferred to a Büchner funnel containing TCA-washed pulped Whatman 3 MM paper and suction applied. The homogenizer was rinsed with 50 ml of 15% TCA followed by 100 ml of 7.5% TCA; the washings each time were poured over the homogenate in the Büchner funnel. The clear TCA filtrate thus obtained was finally made up to 500 ml with glass distilled water and thoroughly

mixed. Appropriate dilutions of the filtrate were immediately analysed or samples taken for acid digestion.

Acid digestion of TCA extracts

Samples (20 ml) of extract were pipetted into 300 ml capacity Kjeldahl flasks and 20 ml of concentrated sulphuric acid (sp. gr. = 1.84) slowly added. After mixing, the solution was gradually heated to boiling. When all the water had been removed, the flasks were refluxed for a minimum of 10 min. They were then allowed to cool and 10 ml of concentrated nitric acid added and the heating recommenced. Digestion was complete when the evolution of nitrogen dioxide had ceased and the solution was virtually colourless. Occasionally some solutions remained coloured, in which case a further volume (2–5 ml) of nitric acid was added and the heating continued. Time for sulphuric acid digestion was approximately 30 min and digestion with nitric acid usually took a further 30 min. Solutions were finally cooled, diluted and allowed to cool further before quantitatively transferring to a 100 ml volumetric flask with glass distilled water.

The sodium and phosphorus (as P_2O_5) contents of the digest were then determined after appropriate dilutions had been made.

Estimation of phosphorus (as P_2O_5)

Undigested TCA extracts were accurately diluted 1 in 5 with glass distilled water and 1.0 ml aliquots of these and of the diluted digested extracts taken for analysis; 9.0 ml of glass distilled water were added followed by 10.0 ml of freshly prepared molybdovanadate reagent (Statutory Instruments, 1976) and mixed. The solutions were allowed to stand for 10 min at ambient temperature and the absorbance then measured at 430 nm using 10 mm cells in a Pye-Unicam SP500 series 2 spectrophotometer, against a reference solution containing 10.0 ml of reagent and 10.0 ml of a blank solution consisting of either the TCA solution used for extraction or an acid digest of this appropriately diluted. All estimations were carried out in duplicate.

Estimation of sodium

Acid digests and undigested TCA extracts were diluted to give sodium concentrations in the range 5 to 30 μg/ml. Sodium concentration was determined by atomic absorption spectrophotometry using a Pye-Unicam SP 90/A series 2 spectrophotometer in the emission mode. Conditions used were: wavelength 589 nm, slit width 0.1 nm, lamp current 6.0 mA and an oxidizing air/acetylene flame. Estimations were also made on a VARIAN AA6 spectrophotometer (Grey *et al.* 1977). Readings on both instruments were converted to concentration using calibration curves prepared from standard

Table 1. Ratio of sodium and phosphorus in TCA extracts of air-chilled breast muscle at various levels of injected polyphosphate

	Undigested			Digested		
Weight of solution retained (g)	Concentration of P_2O_5% of muscle wet weight	Concentration of sodium % of muscle wet weight	Ratio $\frac{P_2O_5}{Na}$	Concentration of P_2O_5% of muscle wet weight	Concentration of sodium % of muscle wet weight	Ratio $\frac{P_2O_5}{Na}$
Nil	0.28	0.032	8.8	0.34	0.030	11.3
Nil	0.33	0.040	8.3	0.37	0.036	10.3
Nil	0.31	0.036	8.6	0.38	0.032	11.9
10	0.37	0.074	5.0	0.43	0.070	6.1
20	0.44	0.097	4.5	0.49	0.089	5.5
40	0.41	0.109	3.8	0.47	0.102	4.6
50	0.46	0.166	2.8	0.58	0.153	3.8

sodium chloride solutions diluted with the extraction reagent (TCA) or with a diluted blank acid digest solution. No suppression of response was encountered when the acid digests were diluted at least tenfold.

Results

Effect of efficiency of extraction on $\frac{P_2O_5}{Na}$ *ratio*

The average concentrations of phosphorus (as P_2O_5) and sodium in breast muscle of uninjected broiler carcasses estimated by the perchloric acid method were 0.51 and 0.053% respectively (Grey *et al.*, 1977). TCA extraction and digestion resulted in approximately two thirds of these values (Tables 1 and 2). Variation in the weight of whole breast muscle (150–350 g) removed from various weights of carcasses and extracted in a final volume of 500 ml of TCA made no significant difference to the ratio. Similarly, when the volume of TCA extract was varied by increasing the number of extractions, the concentrations of P_2O_5 and sodium increased, but the ratio remained constant.

Effect of weight of polyphosphate injected on $\frac{P_2O_5}{Na}$ *ratio*

As the weight of injected polyphosphate solution retained in breast muscle increased from nil to 50 g, the ratio P_2O_5/Na decreased systematically (Table 1) in both digested and undigested TCA extracts. The consistent difference in ratio between the two extracts was mainly attributable to the higher P_2O_5 value obtained in the digested extracts.

Table 2. Sodium and phosphorus ratios in factory processed TCA -extracted broiler breast muscle.

	Undigested			Digested		
Treatment	Concentration of P_2O_5% of muscle wet weight	Concentration of sodium % of muscle wet weight	Ratio $\frac{P_2O_5}{Na}$	Concn. of P_2O_5% of muscle wet weight	Concentration of sodium % of muscle wet weight	Ratio $\frac{P_2O_5}{Na}$
Control	0.29	0.047	6.2	0.35	0.045	7.8
	0.31	0.047	6.6	0.40	0.045	8.9
	0.32	0.048	6.7	0.37	0.043	8.6
	0.35	0.042	8.3	0.39	0.039	10.0
5% * Polyphosphate injection	0.50	0.186	2.7	0.62	0.178	3.5
	0.50	0.170	2.9	0.58	0.155	3.7
	0.48	0.176	2.7	0.56	0.162	3.5
	0.55	0.207	2.7	0.65	0.204	3.2

* Average weight injected = 62 g.

Effect of commercial processing on $\frac{P_2O_5}{Na}$ *ratios.*

The ratios obtained from commercially processed spin-chilled broilers (Table 2) were lower than in the birds air-chilled after injection in the laboratory. This consistent difference was the result of higher concentrations of sodium found in the TCA extracts (*cf.* Table 1). Nevertheless the ratios in digested and undigested TCA extracts enable injected and uninjected carcasses to be differentiated.

Effect of injection of saline and mixed solutions of sodium chloride and polyphosphate on $\frac{P_2O_5}{Na}$ *ratios*

Injecting 4% sodium chloride and a 50:50 mixture of 4% sodium chloride and 5% polyphosphate produced similar, and low, P_2O_5/Na ratios (Table 3). There were only small differences between undigested and digested muscle extracts. Thus confirmation of salt alone being injected can be obtained from the low P_2O_5 and high sodium values and the presence of the mixture can be deduced from the low ratio and elevated P_2O_5 and sodium values.

Table 3. Effect of injection of saline and mixed solution of sodium chloride and polyphosphate on $\frac{P_2O_5}{Na}$ ratios.

Treatment	Weight of injection solution retained* (g)	Undigested Concentration. % wet weight of muscle P_2O_5	Undigested Concentration. % wet weight of muscle Sodium	Undigested Ratio $\frac{P_2O_5}{Na}$	Digested Concentration. % wet weight of muscle P_2O_5	Digested Concentration. % wet weight of muscle Sodium	Digested Ratio $\frac{P_2O_5}{Na}$
4% sodium chloride							
	42	0.30	0.147	2.0	0.33	0.149	2.2
	45	0.29	0.115	2.5	0.35	0.122	2.9
	48	0.26	0.150	1.7	0.41	0.161	2.5
	52	0.29	0.182	1.6	0.44	0.184	2.4
	66	0.29	0.210	1.4	0.36	0.203	1.8
2% sodium chloride + 2½% polyphosphate							
	59	0.44	0.228	1.9	0.61	0.220	2.8
	66	0.44	0.207	2.1	0.53	0.210	2.5
	68	0.48	0.234	2.1	0.50	0.238	2.1
	69	0.43	0.210	2.0	0.49	0.213	2.3
	70	0.42	0.220	1.9	0.50	0.223	2.2

* Carcase weighed 3 min after injection, air-chilled and frozen.

Application of method to broilers of unknown history

Broilers purchased from two of three local supermarkets each with a different brand name were found to contain no added polyphosphate whereas those from the third supermarket had concentrations and ratios of sodium and P_2O_5 which indicated that a mixture of salt and polyphosphate had been injected (Table 4).

Conclusions

Extraction of chicken breast muscle with TCA, although resulting in lower concentrations of P_2O_5 and sodium than found previously using the perchloric acid digest method (Grey *et al.*, 1977), nevertheless gave consistent ratios even when the acid digestion stage was omitted. It is possible, therefore, to recommend a procedure for the detection of added polyphosphate or mixtures of polyphosphate and salt in chicken muscle based on a simple TCA extraction without digestion. The new procedure is thus both quicker and safer than the original method. Also, the weight of muscle taken for analysis can be ignored, provided a representative sample is taken, because the ratio of P_2O_5 and sodium can be obtained from their concentrations per unit volume of extract. Thus, a complete analysis need now only take 1.5 hr.

Table 4. Analysis of TCA extracted breast muscle from commercially processed broilers

Supermarket number	Bird number	Undigested Concentration. % wet weight of muscle		Ratio	Digested Concentration. % wet weight of muscle		Ratio	Confirmation
		P_2O_5	Sodium	$\frac{P_2O_5}{Na}$	P_2O_5	Sodium	$\frac{P_2O_5}{Na}$	of added salt/phosphate
1	1	0.19	0.027	7.0	0.21	0.029	7.2	–
	2	0.29	0.038	7.6	0.30	0.038	7.9	–
	3	0.24	0.029	8.3	0.25	0.032	7.8	–
	4	0.30	0.047	6.4	0.32	0.053	6.0	–
2	1	0.42	0.289	1.4	0.62	0.287	2.2	+
	2	0.49	0.294	1.7	0.57	0.305	1.9	+
	3	0.44	0.250	1.8	0.48	0.229	2.1	+
	4	0.46	0.257	1.8	0.51	0.250	2.0	+
3	1	0.30	0.045	6.7	0.38	0.052	7.3	–
	2	0.29	0.056	5.2	0.33	0.066	5.0	–
	3	0.31	0.046	6.7	0.37	0.053	7.0	–
	4	0.22	0.031	7.1	0.27	0.038	7.1	–

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A relation between gluten protein amide content and baking performance of wheat flours

F. MacRITCHIE

Summary

Solubility –pH curves of gluten protein components from flours of varying baking performance showed small displacements, believed to be associated with differences in average isoelectric points. The apparent isoelectric points were higher for glutens from flours of good baking performance. Amide contents were also found to be higher for these glutens. A highly significant correlation was found between gluten protein amide content and loaf volume index for thirty seven flours covering a wide range of baking quality. The percentage of (glutamine + asparagine) in gluten protein was found to vary between 31.2 and 33.7% for the flours examined. Loaf volume index and gluten protein amide content were shown to be related to both cultivar and site.

Introduction

Previous work has shown that the gluten protein component is invariably responsible for differences in baking performance between wheat flours (Finney, 1943; MacRitchie, 1978). Several hypotheses have been proposed to explain gluten quality (e.g. gliadin/glutenin ratio, thiol/disulphide content) but no confirmation of these has come from experimental data (Sullivan, 1965; Bloksma, 1975; Graveland, Bosveld & Marseille, 1978). Amino acid analyses of gluten protein have shown small differences between flours but there has been no obvious relation between these differences and baking performance. Many flours appear quite similar in characteristics such as those measured in routine physical dough testing but behave differently in the baking test. The baking test is a very sensitive measurement for distinguishing between gluten protein components from different flours and, at present, no proven test exists which independently predicts baking potential with any degree of certainty. It may be concluded that compositional variations occur between the gluten protein from different flours but these variations must be quite small.

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One of the problems in attempting to relate protein composition to baking performance is to be able to make an objective assessment of the latter. The approach of Finney (1943), in which most of the important variables are optimized to allow a flour to perform to its full capacity, has been followed in the present work, with certain provisos. In general, the baking test has been devised so that performance of the flour is not limited by factors such as diastatic activity, water addition, oxidant level or mixing time. However, it was considered important not to include shortening in the formulation. Earlier work (MacRitchie, 1978) has shown that deficiencies in gluten protein often only appear in baking at lipid contents near the natural flour lipid content and that these deficiencies could, in many cases, be eliminated either by removal of all extractable lipid or by addition of extra lipid. The aim in a baking test, designed for research purposes, is to obtain true, relative rankings of flours by allowing the natural flour constituents to perform to their capacity but not to mask any of their deficiencies.

The two main ways in which a protein may change its properties are by changes in molecular weight distribution and by changes in chemical composition. A third way is by conformational changes arising from environmental factors. A preliminary study of the first possibility was made by initially separating gluten protein into fractions of relatively low and high molecular weight, utilizing the difference in solubility of the two fractions in dilute acid. These fractions were then combined in different ratios, reconstituted in flours at constant protein content and baking tests carried out. Although mixing characteristics varied, loaf volumes remained reasonably constant over a wide range of low to high molecular weight protein, only decreasing at extreme values of the ratio. This suggested that molecular weight distribution is probably not the most important factor causing differences in baking performance.

However, this work revealed that the amount of protein obtained in a single extraction with dilute acetic acid (0.002 M) varied appreciably from one gluten sample to another and that these amounts appeared to be correlated with the baking performance of the parent flours. Preliminary measurements suggested that the variations might arise from small differences in the average isoelectric points, the gluten protein from good baking flours having slightly higher values. One way in which this could occur is if the degree of amidation of the glutamic and aspartic acid residues should vary. A higher degree of amidation would lead to a higher isoelectric point since there would be a reduction in the number of carboxyl groups which contribute negative charges. This hypothesis was therefore tested by measuring amide contents of a number of flours covering a range of baking quality.

Materials and methods

Flours were obtained from the Bread Research Institute of Australia's programme of quality testing of Australian wheats from the 1977–78 season. The wheats were known cultivars and were milled on a Laboratory Buhler mill. A

selection of flours was made to cover as wide a range of baking quality as possible.

The baking test was that described previously (MacRitchie & Gras, 1973; MacRitchie, 1976; MacRitchie, 1978). In general, relative rankings of flours obtained in this test agreed closely with those from the Bread Research Institute of Australia 3 hr bulk fermentation baking test, although specific volumes were consistently about 10% lower, a result of the relatively smaller sample (35 g compared to 120 g of flour). The only exceptions were three flours which had unusually long mixing times and these performed considerably better in the no-time baking test, in which mixing time is optimized. Loaf volumes of flours were converted to loaf volume index (LVI) by the formula:

$$\text{LVI} = \frac{\text{loaf volume}}{\%\ \text{protein in flour} \times \text{flour weight}} \times 200$$

This enabled comparisons to be made between flours of different protein content and also between differently scaled baking tests. The factor of 200 was arbitrarily chosen in order to give values near 100 for flours of good baking performance. The standard deviation per mean LVI was 2.5 in the present work.

Glutens were hand-kneaded from doughs at a temperature of 15°C, freeze dried and protein determined by the Kjeldahl method using a factor of 5.7. Solubility measurements were made by stirring a sample of gluten, containing 2.0 g of protein, with 60 ml of solution in a Janke and Kunkel Ultraturrax mixer for 2 min followed by centrifugation. The concentration of protein in the supernatant was calculated from the absorbance of a diluted sample at 278 nm, using previously obtained calibrations for each buffer system. A check on the effect of stirring time on supernatant concentration showed that a plateau value was obtained after 1 min. Very slight increases were observed at longer times apparently due to small increases in the temperature of the solution during agitation.

The buffer systems used were acetic acid-sodium hydroxide (pH 3–6), potassium dihydrogen phosphate-sodium hydroxide (pH 6–8) and sodium bicarbonate-sodium hydroxide (pH > 8), all at an ionic strength of 0.05. Checks with other buffer systems (citric acid for low pH, sodium acetate for intermediate and borax for high pH) showed that relative solubilities of glutens were maintained in these solutions when compared at a fixed final pH value of the supernatant. This showed that the solubilities were a function of pH and that specific ion effects, if present, were of minor importance.

Amide determinations were made by the method of Eastoe, Long & Willan (1961), based on hydrolysis in boiling 2N hydrochloric acid. Constant values for amide contents of glutens were obtained after 45 min reflux and no changes could be detected at longer times, up to 7 hr. A reflux time of 1 hr was therefore adopted and no correction for decomposition of serine and threonine groups

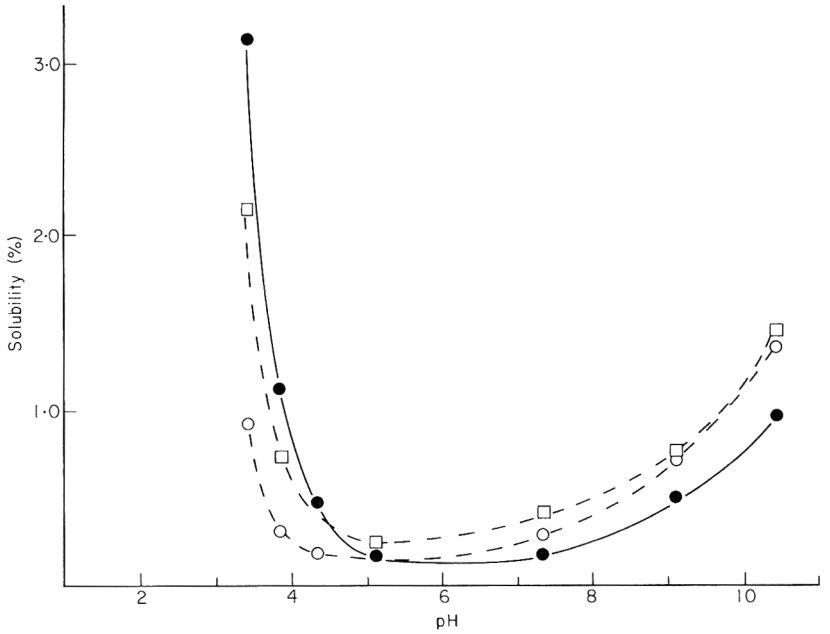

Figure 1. Solubility v. pH for gluten protein components from different flours. ●, Flour 1; □, flour 3; ○, flour 5. The curve for flour 2 was the same as for flour 1, that for flour 6 was almost identical to flour 5 and that for flour 4 fell between those of flours 3 and 5.

appeared necessary (Rees, 1946). The percentage of (glutamine + asparagine) in the gluten protein was calculated from the formula:

$$\%\ (\text{glutamine} + \text{asparagine}) = \frac{\text{moles ammonia} \times 128 \times 100}{\text{wt of protein in sample}}$$

The value of 128 represents a weighted average residue weight based on an acidic amino acid side chain composition of 93% glutamic and 7% aspartic acid (Wu & Dimler, 1963; Pomeranz *et al.*, 1970) and assumes that the proportion of amidated groups is the same in the two types of side chain. The standard deviation per mean (glutamine + asparagine) determination was 0.3%.

Results

Six flours were chosen for an initial study. Two of these had very good and four had extremely poor baking performance. The solubility-pH relations were measured for the gluten protein component from each flour and the results are shown in Fig. 1. Results for loaf volume index and (glutamine + asparagine) content of the gluten protein are given in Table 1.

A second study was carried out on flour samples obtained from a trial in which four Australian wheat cultivars were grown at four sites in the state of

Table 1. Loaf volume index and gluten protein amide content of six flours.

Flour	Loaf volume index	Glutamine and asparagine in gluten protein (%)
1	97.7	32.9
2	109.7	33.2
3	77.3	31.7
4	81.4	31.9
5	46.8	31.2
6	49.2	31.7

New South Wales. Data for loaf volume index, solubility in 0.002 M acetic acid and (glutamine + asparagine) content of gluten protein are summarized in Tables 2 and 3. Average values for cultivars are given in Table 2 and for sites in Table 3.

Figure 2 shows plots of loaf volume index versus (glutamine + asparagine) contents of gluten protein for all flours which have been examined to date, including those from Tables 1, 2 and 3.

Discussion

Protein solubility is usually a minimum at the isoelectric point and increases as the net charge on the molecule is raised by moving the pH to higher or lower values. The solubility-pH curves of Fig. 1 show relative displacements which indicate differences in the average isoelectric points of the gluten protein samples from the different flours. If this is caused by differences in the relative proportions of amide to free carboxyl groups in the acidic side chains, we would expect the protein samples with the higher solubilities at acid pH to have the greater amide contents. This is confirmed by the results of Table 1. The lowering of the isoionic point of a protein as the side-chain amide groups are converted to free carboxyl groups has been observed for gelatin (Eastoe *et al.*, 1961). Removal of all the amide groups by alkali treatment caused the isoionic point of gelatin to fall from above 9 to 4.8.

Table 2. Data for four cultivar-four site trial, expressed as averages for cultivars.

Cultivar	Loaf volume index	Solubility in 0.002 M acetic acid (g/100 ml)	Glutamine and asparagine in gluten protein (%)
Songlen	108.4	0.62	32.8
Kite	92.6	0.34	32.4
DKH3	85.8	0.27	32.3
DKH4	95.8	0.36	32.7

Table 3. Data for four cultivar-four site trial, expressed as averages for sites

Site	Protein (%)	Loaf volume index	Solubility in 0.002 M acetic acid (g/100 ml)	Glutamine and asparagine in gluten protein (%)
Baradine	14.2	85.4	0.18	32.5
Warren	12.4	92.4	0.16	32.1
Tamworth	12.9	97.9	0.21	32.2
North Star	13.7	106.9	1.04	33.4

A clear correlation between gluten protein primary amide content and loaf volume index is evident from Fig. 2. It is not certain whether it is the total side-chain amide content of the gluten protein or the degree of amidation which is the more critical factor. To ascertain the relative importance of the two will require measurements of the total acidic side chain content. Wheat flour is characterized by having both a high content of (glutamine + asparagine) and a high ratio of amidated to non amidated acidic side-chains. Since there is a good correlation between loaf volume index and solubility of gluten protein as well as side-chain amide (Fig. 1 and Tables 2 and 3), this indicates that the degree of amidation is important. The solubility in dilute acid is a measure of the degree of

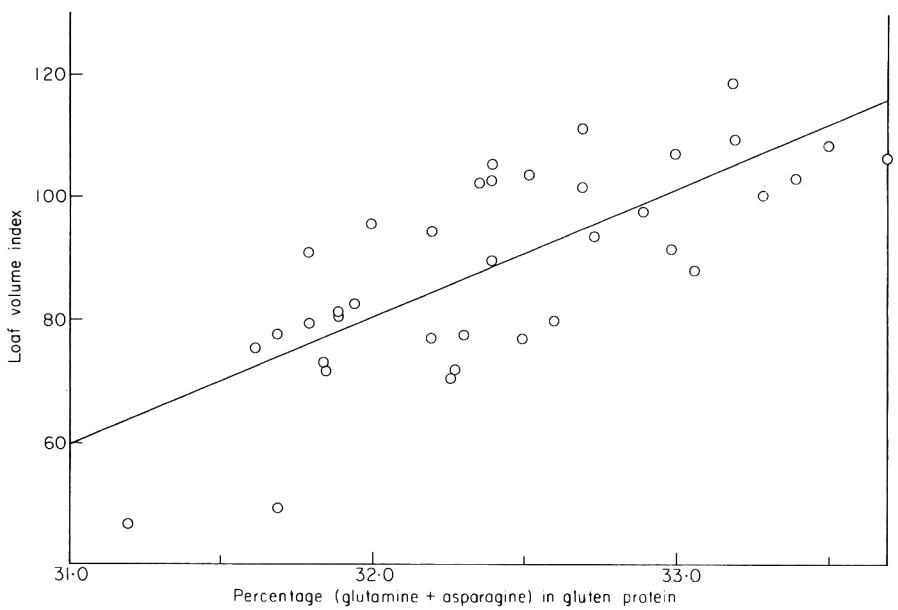

Figure 2. Loaf volume index, LVI, v. percentage (glutamine × asparagine), A, in gluten protein for flours covering a range of baking performance. The least squares regression equation and linear correlation coefficient were: LVI = 20.85A–587.3, r = 0.756. Correlation was significant at the 1% level.

amidation, a higher solubility indicating a higher isoelectric point and therefore a smaller number of free carboxyl groups providing negative charges.

Because the standard deviation for the amide determinations is large (0.3%) relative to the range of amide content encountered (2.5%), a certain amount of scatter of points is expected. Two additional factors could be expected to influence the scatter. Firstly, variable contamination of the glutens by albumins and globulins, which have much lower amide contents, could affect the determinations although this effect is thought to be minor. Secondly, variations in the relative amounts of different protein fractions of the gluten will be important because of their varying amide contents. For example, preliminary analyses in the present work gave (glutamine + asparagine) figures of 36.5% for gliadin and 30.9% for glutenin fractions.

The results of Table 3 have interesting implications. They show that the baking performance of all varieties is superior at one site (North Star) and that gluten protein solubility in dilute acid as well as amide content are both higher for flours at this site, even though total protein contents are not very different for the four sites. This is a result which warrants closer study.

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The production of soluble fish protein solution for use in fish sauce manufacture

I. The use of added enzymes

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Summary

The production of a fish hydrolysate, using plant proteases, which could be added to traditionally fermented fish sauce to increase the total volume without affecting the overall nutritional quality was investigated.

The effect of adding bromelain, papain or ficin, on the rate of hydrolysis and the extent of the conversion of insoluble fish protein, to soluble nitrogen was examined. The conditions employed were similar to those used in traditional fish sauce manufacture but both whole and minced Ikanbilis (*Stolephorus* sp.) were investigated. Measurement of the extent of hydrolysis after 1, 2, 4, 7, 14, 27, 28 and 35 days at 33°C showed that bromelain tended to give slightly better results with some 65% of the protein being hydrolysed. The effect of temperature enzyme, co-enzyme and salt concentrations for the hydrolysis by bromelain were investigated and the optimum conditions established at the pH normally found in fish sauce production. The hydrolysate produced in 18–21 days was comparable to traditional fish sauce in the distribution and concentrations of nitrogenous compounds and had very little aroma. The product could be added to the traditional sauce without affecting its quality.

Introduction

Fish sauces are manufactured, predominantly in S.E. Asia, by the mixing of certain species of fish (e.g. *Stolephorus* sp.) with salt in the ratio 3:2 (Van Veen, 1965). A supernatant liquor is soon produced, which, over a period of months, increases in volume and in its soluble protein content. This soluble nitrogen is mainly derived by the breakdown of the fish protein by the enzymes of the fish. (Saisithi *et al.*, 1966; Van Veen, 1965; Beddows, Ardeshir & Daud, 1979). The supernatant liquor is decanted and is often blended with other materials. For example, the fish sauce of Thailand (nam-pla) is mixed with 'meiki' which is a concentrate obtained from the bacterial production of mono-sodium glutamate

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from starch; this increases the volume of the sauce whilst keeping the protein concentration within the legal requirements. (Sukhumavasi, 1977, personal communication). In Malaysia the liquor is blended with tamarinde which imparts some colour and additional flavour; the fish sauce is known as 'budu' (Wan Johari bin Daud, 1977, personal communication).

To the consumer aroma is a prime indicator of the quality of a fish sauce. Part of the aroma, trimethylamine and ammonia, appears readily, early in the fermentation whilst other constituents, volatile fatty acids, appear mainly to be formed prior to salting (Beddows *et al.*, 1979). However, the derivation of the 'meaty' aroma of nam-pla is not known and is a complex mixture (Dougan & Howard, 1975). The flavour is salty and depends to a certain degree on the aroma compounds. The full process of manufacture can take over a year, (Saisithi *et al.*, 1966).

It would be advantageous if (a) the fermentation period could be shortened so that a reduction in capital cost would result, (b) if the amount of insoluble protein converted to the soluble form, could be increased and (c) if other 'waste' fish species could be utilized to replace the fish now used in its production, to 'extend' the product in the same way as 'Meiki' is used at present.

A variety of methods to increase the rate have been investigated, such as increase of temperature (Amano, 1962), the addition of proteolytic enzymes such as papain (Santos, 1968), 'pronase' (Sulit & Tiongson, 1967) and an enzyme from *Bacillus subtilis* (Amano, 1962). The processes using these enzymes and the quality of the products have not been fully reported. Fresh pineapple juice, presumably with bromelain activity, has been reported to be added to eviscerated fish in Cochin-China (Chevey, 1930–1). The addition of papain has been investigated fully by others for the actual production of fish sauce having the characteristic aroma. It is reported that the method produced a satisfactory sauce after 4 months and was economically viable. However, it has not been accepted by the manufacturers as yet (Sukhumavasi, 1977, personal communication).

The use of trash fish to produce an acceptable fish sauce by traditional methods has also been investigated but did not meet with any success (Sukhumavasi, 1977, personal communication).

All these are modifications of the traditional process. The addition of bromelain to mackerel to produce a fish sauce was investigated by Beddows, Ismail & Steinkraus (1976) and met with limited success because some of the aroma constituents did not appear without a period of bacterial spoilage before the addition of salt.

It is more feasible to use proteolytic enzymes to provide a fish hydrolysate with a high nitrogen content, which can be added to the traditionally prepared sauce to extend it in the same manner as 'meiki' is used in Thailand at present. It would be preferred that such a proteolysate had very little aroma that could interfere with the particular aroma of fish sauce. Such a method would have an advantage in that other fish species or 'trash' fish might well be utilized. However, for the present investigation only *stolephorus* sp. (Ikanbilis), a major

species of fish utilized in the traditional method, was used. Also this would have the advantage of reducing the production period of a certain amount of fish sauce, and reduce capital costs.

It was decided to investigate the effectiveness of the enzymes on fish : salt mixtures that are being prepared regularly, so as to keep to the traditional method as far as possible. This would reduce the possibility of detrimental microbial spoilage. The investigation was also limited to the plant enzymes, bromelain, ficin and papain, which could prove to be economically viable in the present regions of fish sauce production. However, bacterial enzymes are becoming more readily available and may be worth investigating at a later date.

If the proteolysate is to be added to traditional sauces (with aroma thresholds above the minimum), the criteria for evaluating the production of proteolysates would be based upon (a) the volume yield and (b) the effective conversion of insoluble to soluble protein. The economic aspects of such a process are also of fundamental consideration, but it would be impossible to carry out such an evaluation without a more detailed investigation of local conditions.

Materials and methods

Materials

Bromelain (BDH Ltd., Poole, Dorset, U.K.) (originally isolated from pineapple stem) was used. Its activity was 1200 GDU per gram (1 unit is equivalent to the liberation of 1 mg of amino nitrogen liberated from a gelatine solution after 20 min at 45°C and pH 4.5).

Papain (Sigma Chemicals Co., St. Louis, Missouri) having an activity of 1.3 units/mg solid (1 unit will hydrolyse 1 mmol of α N-benzoyl-L-arginine ethyl ester (BAEE) per min at pH 6.2 and 25°C) was used.

Ficin (Sigma Chemical Co.) was used and had an activity of 0.32 units/mg of solid (1 unit will produce a change of 1.0 UV units/minute at 280 nm, at pH 7.0 and 37°C when measuring TCA soluble products from casein in a final volume of 10 cm^3).

Stolephorus (Ikanbilis) was obtained in a plate-frozen condition from Kuala Trengannu, Malaysia.

α-N-benzoyl-L-arginyl-p-nitroanilide ester (BAEE) and other chemicals were purchased from BDH Ltd.

Enzyme assays All the enzymes were assayed by the method of Erlanger, Kokowsky & Cohen (1961) using BAPNA in order to obtain some indication of their relative proteolytic activities.

Enzymic hydrolysis

A series of flasks were prepared each of which contained 100 g of whole or minced Ikanbilis and salt (66 g) and enzyme (ficin, papain or bromelain) at the appropriate relative concentration, and incubated at 33°C. The bromelain samples also contained 1 cm^3 of 0.6 M cysteine.

Flasks were removed after 1, 2, 4, 7, 14, 18, 21, 24 and 28 days. Each batch was examined for the volume of supernatant liquor by filtering initially through a Büchner filter funnel and then through Whatman No. 1 filter paper. The supernatant liquor was examined further for, (i) pH using a Pye Unicam model No 292 pH meter, (ii) the extent of hydrolysis by the formol titration (Beddows, Ismail & Steinkraus, 1976), (iii) the nitrogen content by the modified Kjeldahl method (Beddows *et al.*, 1976), (iv) volatile nitrogen, (ammonia and trimethylamine) (Beddows *et al.*, 1979), (v) soluble protein by adding TCA to give 5%, filtering and analysing the filtrate for nitrogen content by the Kjeldahl method, (vi) the amino-acid nitrogen by the ninhydrin method (Moore & Stein, 1954).

Further investigation of the factors influencing the rate of reaction with bromelain

(a) *Effect of enzyme concentration.* A number of flasks each containing minced Ikanbilis (100 g) and salt (66 g) were set up as before. Different concentrations of bromelain up to 1.65 g were added, with 1 ml of 0.6 M cysteine in each case. The flasks were incubated at 33°C. Flasks, at each enzyme concentration, were removed at intervals and filtered as before; the volume of supernatant liquor was measured and its nitrogen content determined by the Kjeldahl method. The amount of hydrolysis was determined by the formol titration as before.
(b) *Effect of co-enzyme.* A number of flasks were set up as in (a) each containing 0.8% bromelain, but the concentration of cysteine was adjusted to values between 0 and 0.048 M. The supernatant liquors were analysed as in (a) after 3 weeks incubation at 33°C.
(c) *Effect of salt.* Eight flasks were set up each with 100 g minced Ikanbilis, 0.8 g bromelain and 1 cm^3 of 0.6 M cysteine. A range of salt concentrations between 10 g and 70 g were added to various flasks and incubated at 33°C. The supernatant liquors were analysed as in (a) after 3 weeks.
(d) *Effect of temperature.* A number of flasks were set up, each containing 100 g minced Ikanbilis, 66 g of salt, 0.8% bromelain and 1 ml of 0.6 M cysteine. Some of the flasks were incubated at 30°C and some at 50°C. Batches were removed after 3 and 8 weeks and analysed as in (a).
(e) *Time before addition of salt.* Flasks, each containing 100 g of minced Ikanbilis, 0.8% bromelain and 1 ml 0.6 M cysteine, were incubated for 0 or 24 or 48 hours before the addition of salt (66 g). Flasks were removed after 3 weeks and analysed as in (a) above.

Results and discussion

In order that some comparison could be made between the three enzymes, it was necessary to obtain some idea of their relative activities with a particular substrate, so that the addition of the same amount of proteolytic activity to the fish could be made. The activities with a particular substrate do not however mean that they would be in the same order of activity with another substrate.

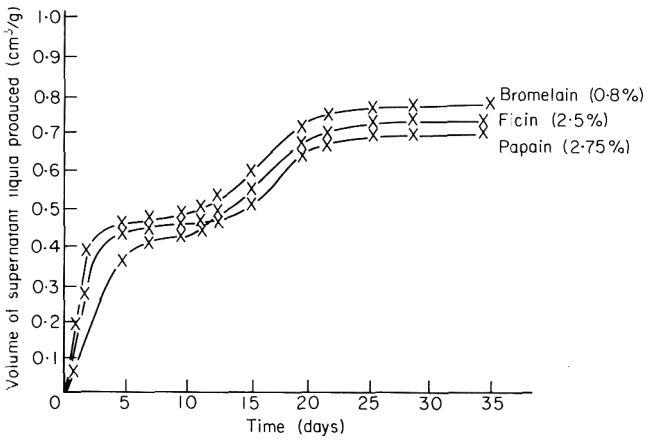

Figure 1. Rate of increase in the volume of supernatant liquor formed (in cm^3/g of fish) when added enzymes were incubated with a mixture of *Stolephorus* and salt (ratio 3:2) at 33°C.

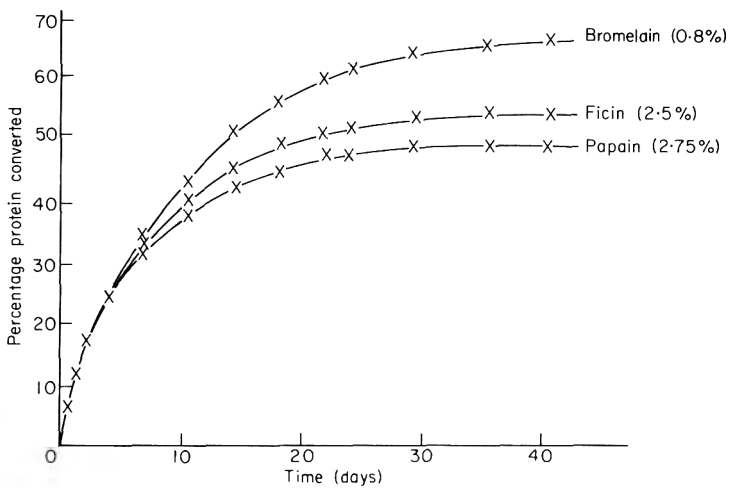

Figure 2. The percentage protein conversion with time, when proteolytic enzymes were incubated with a mixture of *Stolephorus* and salt (3:2) at 33°C.

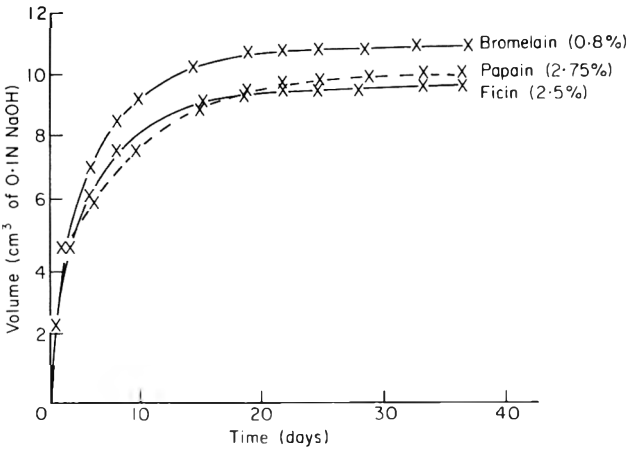

Figure 3. Formol titration of the supernatant liquors produced at intervals of time when proteolytic enzymes were added to mixtures of *Stolephorus* and salt (3:2) and incubated at 33°C.

Since the enzymes purchased have very different activities some correlation was considered to be desirable.

The substrate BAPNA was used and it was found that 1 mg of bromelain had the same activity as 3.1 mg of ficin and 2.9 mg of papain. These values were taken into account when the enzymes were added to the fish.

One of the criteria by which the effectiveness of the enzymes can be measured, that is the ability to convert insoluble protein into the soluble form, can be defined as:

$$\text{percentage protein conversion} = \frac{\text{volume of supernatant liquor} \times \text{N content of liquor (g/cm}^3) \times 100}{\text{wt of fish used} \times \text{N content of the fish (\% g/g)}}$$

The nitrogen value for the fish was found to be 0.0248 g/g (wet weight).

The rate of increase in the volume of the supernatant liquor is compared for the three enzymes in Fig. 1. Only slight differences were apparent.

The results after 18–21 days showed that 0.75 cm^3/g supernatant resulted from the bromelain treatment, 0.69 cm^3/g from ficin and 0.65 cm^3/g from papain. The natural fermentation of 'budu' gives 0.75 cm^3/g of fish. (Beddows *et al.*, 1979). A comparable yield of liquid is only obtained from the bromelain treated sample after 21 days.

The extent of conversion of insoluble to soluble protein with time for the three enzymes are given in Fig. 2. Minced *Stolephorus* was used as the substrate. Although the initial rates were similar, bromelain gave a greater percentage conversion. The hydrolysis appears to have been completed within 18–21 days. This is reflected in the formol titration of the liquors (Fig. 3).

Table 1. Distribution of the nitrogenous compounds formed (in g/100 cm^3) in the supernatant liquors obtained after 21 days from the incubation of proteolytic enzymes with mixtures of minced *Stolephorus* and salt (3:2) at 33°C

Supernatant liquor	Total-N	Protein-N	Amino-N	Volatile-N		Polypeptide-N*
				NH_3	TMA	
Bromelain	2.21	0.09	1.51	0.12	0.036	0.45
Papain	2.11	0.12	1.33	0.11	0.034	0.52
Ficin	1.88	0.08	1.29	0.11	0.035	0.37
Budu†	1.77	0.01	1.17	0.09	0.028	0.48

*found by difference and could include nucleic acid bases and other breakdown products.
†from Beddows *et al.*, 1979.

The natural fermentation of 'budu' gave a conversion of about 56% (Beddows *et al.*, 1979); bromelain gave a 65% conversion. The conversion rates for papain and ficin were lower. However, it must be remembered that the hydrolysis is taking place under saturated salt conditions which could be detrimental to the proteolytic activity of all three enzymes.

Although a slightly better conversion was generally obtained in experiments with bromelain, an increase in the concentration of the other enzymes could improve the net conversion of insoluble to soluble protein for these enzymes.

As would be expected, the conversion within 21 days of insoluble to soluble protein was lower for whole fish than for the minced fish. Overall the maximum conversion obtained for bromelain was 57%, papain 45% and ficin 47%.

The nitrogenous compounds formed in the supernatant liquors could have an effect on consumer acceptability. However, the supernatant liquors produced by each enzyme were tasted and no particularly strong or unpleasant flavour was found with any of them.

The distribution of the nitrogenous compounds in the supernatant liquors was analysed after 21 days. (Table 1). It is interesting to note that the volatiles, ammonia and trimethylamine (TMA), are all at about the same level for each of the enzymes.

Table 2. The percentage distribution of the nitrogenous compounds formed in the supernatant obtained after 21 days from the incubation of proteolytic enzymes with mixtures of minced *Stolephorus* and salt (3:2) at 33°C

Supernatant liquor	Amino-N %	Volatile-N %	Protein-N %	Polypeptide-N %
Bromelain	68.3	7.1	4.1	20.5
Papain	63.1	6.6	5.7	24.6
Ficin	68.6	7.6	4.2	19.6
Budu*	66.3	6.6	0.56	26.5

*from Beddows *et al.*, 1979.

Table 3. The rate of formation of the nitrogenous compounds in the supernatant liquors (in g/100g cm^3) obtained from the incubation of 0.8% bromelain with mixtures of minced *Stolephorus* and salt (3:2) at 33°C

Days	Total-N	Amino-N	Protein-N	Volatile-N		Polypeptide-N*
				NH_3	TMA	
1	0.87	0.53	0.0	0.01	0.0	0.33
2	0.97	0.70	0.004	0.02	0.002	0.21
4	1.30	1.05	0.01	0.04	0.011	0.30
7	1.78	1.24	0.044	0.07	0.021	0.40
14	2.22	1.43	0.080	0.09	0.031	0.59
18	2.05	1.44	0.081	0.11	0.035	0.47
21	2.21	1.51	0.092	0.12	0.036	0.45
24	2.25	1.51	0.090	0.12	0.037	0.46
35	2.26	1.53	0.81	0.12	0.038	0.50

*found by difference and could include nucleic acid bases and other breakdown products.

The percentage distribution of the nitrogenous compounds in these liquors (Table 2) are compared to the supernatant liquid obtained from the manufacture of 'budu'. (Beddows *et al.*, 1979). The amounts of free amino acids seem to be comparable. The most marked difference appears to be in the amount of protein. Presumably in the natural sauce this has been broken down more fully to polypeptides. However the actual concentrations of protein are low and are of no real significance.

The rate of formation of the nitrogenous compounds was followed with each of the enzymes. Only the results for bromelain are given (Table 3) as being representative. As would be expected the initial addition of salt caused the appearance of amino acids by osmotic action. The protein and polypeptides became more appreciable in concentration within 4 days. This was followed by the hydrolysis of the fish by the protease giving amino acids and some polypeptides. The rate of appearance of amino-N is much faster than that in 'budu'

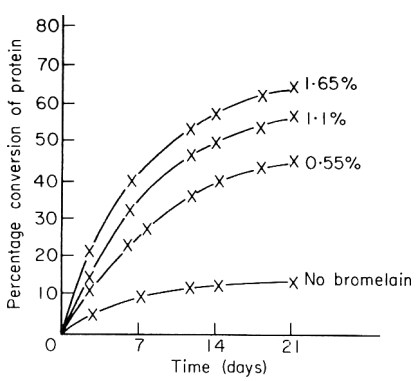

Figure 4. Percentage protein conversion with different concentrations of bromelain incubated with mixtures of minced *Stolephorus* and salt (3:2) at 33°C with time.

Table 4. The effect of the salt concentration on the hydrolysis of a mixture of salt and minced *Stolephorus* (2:3) incubated with bromelain (0.8% w/w) at 33°C for 3 or 7 weeks

Salt added (% w/w)	Volume of supernatant liquor cm^3/g fish		Formol titration cm^3 of 0.1N NaOH		Percentage protein conversion	
	3 weeks	7 weeks	3 weeks	7 weeks	3 weeks	7 weeks
10	0.67	0.69	11.7	10.3	54.2	56.1
30	0.69	0.73	11.9	12.6	55.8	59.2
50	0.71	0.71	11.1	12.2	52.2	62.0
70	0.74	0.74	12.9	13.8	63.7	67.4

manufacture; 0.65 g/100g was produced after 14 days with budu (Beddows *et al.*, 1979); whereas with bromelain, 1.43 g/100 g was produced. As bromelain showed slightly better results than the other two enzymes, this enzyme was investigated further.

An increase in the enzyme concentration increased the rate of percentage protein conversion as would be expected (Fig. 4) but the relationship is not directly proportional. Bromelain requires a sulphydryl co-enzyme: consequently cysteine was used. The rate of hydrolysis increased with increase in co-enzyme concentration up to 0.025 M with 0.8% bromelain. The addition of cysteine to the ficin and papain hydrolysis mixtures had little effect on protein conversion after 21 days.

The effect of salt concentration was more complex. Low salt concentrations would allow spoilage to take place. In earlier work, it was shown that the proteolysis was more rapid with mackerel if the addition of the salt was delayed (Beddows *et al.*, 1976). With minced *Stolephorus* the protein conversion increased from 51% to 67% if a 24 hr salt-free incubation period was allowed. This increased to 71.8% with a 48 hr incubation period. With each, salt (66% w/w) was added and the incubation continued for a period of 3 weeks. However, the aroma became very strong and suggested that spoilage was occurring. Nevertheless, no apparent spoilage occurred with a salt concentration of 10% or more. The results (Table 4) show that the percentage protein conversion increased with time and surprisingly, with higher salt concentrations. This may be due, in part, to the osmotic action of the salt. However, the proteolytic action appears to be enhanced and this is further demonstrated by the increased formol titration (Table 4). An increase of temperature to 50°C increased the protein conversion from 62.5% to 67.2%. However, it is doubtful if the small increase would warrant the investment in heating equipment for production purposes, but the rate of hydrolysis was more rapid.

Conclusions

Bromelain, ficin and papain all produced a hydrolysate, with large quantities of salt present, that would be usable as an additive to fish sauce. Each hydrolysate was clear, slightly brown, with a high protein content and little aroma. The

distribution of nitrogen compounds in the hydrolysates were very similar to the distribution found in a natural fish sauce. In each case, the proteolysis was complete within 21 days, using the normal salt:fish ratio.

A slight advantage occurred with bromelain in that it tended to give higher conversions of insoluble to soluble protein. However the activity does improve if a sulphydryl co-enzyme is present. The amount of fish protein that hydrolysed to soluble nitrogen compounds increased with an increase in enzyme concentration although the relationship is not simple, and is probably due in part to the heterogeneous nature of the reaction.

The hydrolysates were produced in the presence of salt in order to eliminate microbial action. This was also shown to assist in improving the concentration of nitrogen compounds in the supernatant liquor, possibly because of the osmotic action at the high salt concentration.

The effect of an incubation period prior to the addition of salt did increase the amount of protein hydrolysed but bacterial spoilage occurred.

A higher temperature improved the extent of hydrolysis with bromelain a little and increased the rate of hydrolysis. The product from the bromelain hydrolysis could be used to add to traditionally produced fish sauce without any loss of nutritional quality and with an increased saving in capital costs.

Acknowledgments

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The production of soluble fish protein solution for use in fish sauce manufacture

II. The use of acids at ambient temperature

C. G. BEDDOWS AND A. G. ARDESHIR

Summary

When batches of Ikanbilis were acidified using hydrochloric acid to pH 2.0–3.5 at 30°C, the rate and extent of hydrolysis of the fish protein and the rate of formation of supernatant liquor were increased.

The effect of pH and salt concentration was investigated. Although the presence of salt generally decreased the extent of proteolysis, it made the mixtures easier to filter. The optimum conditions were either pH 2.0 and 10% salt (w/w) or pH 3.0 and 15% salt (w/w). The extent of fish protein hydrolysed was comparable to the natural fermentation and the concentrations and distribution of soluble nitrogen were very similar to those obtained in the traditionally produced sauces. This liquor was produced within 6 days. The natural fermentation takes 4–9 months.

The use of formic acid was investigated and showed no particular advantage.

When the pH of the acid ensiled mixtures was adjusted above 4.2, calcium phosphate precipitated; this was removed by filtration.

The effect of adding alkaline ensiled fish to acid ensiled fish mixtures (the pH of which had been re-adjusted) was investigated. The amount of insoluble protein hydrolysed increased to a limited extent.

As the solutions produced by acid ensilation had very little aroma or taste but had a high soluble nitrogen content, they could be used to add to traditionally prepared fish sauce in order to increase the net volume which would increase the rate of production.

Introduction

The principle of adding aroma free, protein rich solutions to the liquid produced in fish sauce manufacture in order to increase the volume has been discussed

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previously (Beddows & Ardeshir, 1979a). The technique is already in use in Thailand, where 'Meiki', the concentration from the bacterial production of monosodium glutamate, is added. (Sukhumavasi, 1977, personal communication). In the previous paper the use of added proteolytic enzymes to produce a protein rich solution from fish was reported (Beddows & Ardeshir 1979a).

However, other methods of breaking down fish protein are in use; the addition of acid at ambient temperature allows the proteolytic enzymes of the fish to work at their optimum pH value (Bakhoff, 1976). The technique has been used for many years to provide animal feedstuffs (Peterson. 1951). However, the product is often a colloidal suspension which can be used directly or mixed with other foodstuffs (cereals etc). Generally inorganic acid has been used, but methods involving organic acids which also have a preservative action, (e.g. 3% formic acid) have also been evaluated (Disney & Hoffman, 1974).

The technique can be used to obtain a fish proteolysate for addition to fish sauce. To be of value, the proteolysate would need to be clear, odourless and have a fairly high soluble nitrogen content (e.g. over 1.7% w/w). The distribution of nitrogen compounds should not be too dissimilar to that found in natural fish sauce. The method is of particular value if hydrochloric acid is used, as adjustment to the pH of fish sauce with sodium bicarbonate gives sodium chloride, which is already present in fish sauces in high concentrations (Van Veen, 1965).

The rapid production of a proteolysate could considerably reduce capital costs and the method could lend itself to the hydrolysis of low quality or trash fish.

This investigation is concerned with the use of Ikanbilis (*Stolephorus* sp.) which is used in fish sauce manufacture in Thailand (nuoc-mam), Malaysia (budu) and other S.E. Asian countries. The traditional method hydrolyses 56% of the insoluble protein to soluble nitrogen compounds (Beddows, Ardeshir & Daud, 1979b). To be competitive it would be preferable to obtain a similar extent of hydrolysis.

It has been shown that a greater hydrolysis of fish protein can be obtained if some of the fish is ensiled at an alkaline pH and is then added to the acid-ensiled fish mixture that has been neutralized, and further incubation is allowed (Raa, 1977, personal communication). This modification depends on the fact that the fish contain proteases with different pH optima; the alkaline enzymes having a preference for certain proteins that are unaffected by the acid proteases.

Materials and methods

Materials

Ikanbilis (*Stolephorus* sp.) were obtained in a plate frozen condition from Kuala Trengannu, Malaysia. All chemicals (Analar grade) were purchased from BDH Ltd., Poole, Dorset, U.K.

Effect of acid on the extent of proteolysis

Triplicate batches (100 g) of minced Ikanbilis were adjusted to specified pH values of 1.0, 2.0, 2.5, 3.0, 3.5, and 4.0, with 50% HCl and incubated at 30°C for 6 days. The mixtures were then filtered. The volume of filtrate was measured and the total nitrogen content determined by the modified Kjeldahl method (Beddows, Ismail & Steinkraus, 1976).

Effect of salt and acid on the extent of proteolysis

Triplicate batches (100 g) of minced Ikanbilis were adjusted to pH values of 1.0, 2.0, 3.0, 4.0 or 5.0 with 50% hydrochloric acid. Salt was added to give a range of concentrations 0, 5, 10, 15, and 20% w/w at each pH value. The mixtures were incubated for 6 days at 30°C and then filtered through Whatman No. 1 filter paper. The volume of filtrate was measured and the total nitrogen content determined as before. The amino-nitrogen was determined with ninhydrin by the method of Moore & Stein (1954).

The soluble protein content of some of the filtrates was estimated by adding TCA to 5% (w/v) filtering and measuring the nitrogen content of the filtrate by the modified Kjeldahl method as before. The ammonia and trimethylamine concentrations were determined (Beddows *et al.*, 1979b) to give the volatile nitrogen content.

The polypeptide content was found by difference.

In some cases, the salt concentration of the filtrate was determined (Beddows *et al.*, 1979b).

The residue from a few of the mixtures was examined visually and dried at 110°C to give the residual weight.

The effect of time on the extent of the proteolysis

Duplicate batches of minced Ikanbilis (100 g) were adjusted to pH 2.0 with 50% hydrochloric acid in the presence of 10, 15 or 20% salt and incubated at 30°C. The mixtures were filtered after 2, 4, 6 and 8 days. The volume of each filtrate was measured; and the nitrogen content, and extent of hydrolysis by the formol titration (Beddows, Ismail & Steinkraus, 1976) were determined.

The effect of formic acid on the extent of the proteolysis

Batches of minced Ikanbilis (100 g) were adjusted to pH values of 1.0, 2.0, 2.5, 3.0, 3.5, 4.0, and 5.0 with 50% hydrochloric acid.

Formic acid was added to a similar series to give 3% w/w before the pH of the mixtures were adjusted. After 6 days, the mixtures were filtered and the volume and nitrogen content of the filtrates were determined as before.

Larger scale preparations

A batch of minced Ikanbilis (500 g) was mixed with 10% salt (w/w) and the pH adjusted to 2.0. Another batch of minced Ikanbilis (500 g) was prepared with 15% salt and adjusted to pH 3.0. Both mixtures were incubated at 30°C for 6 days. The mixtures were filtered. The filtrates were examined, as before, for volume, extent of hydrolysis (formol titration) and total nitrogen content.

Calcium

Calcium was determined qualitatively by using picrolonic acid (Nördbo, 1939) and quantitatively using EDTA with murexide as indicator (Belcher & Nutten, 1960). Phosphate was determined gravimetrically as the magnesium ammonium phosphate in the presence of citrate. (Belcher & Nutten, 1960).

Effect of addition of fish incubated at pH 8.0 on the extent of proteolysis of acid treated Ikanbilis

(a) Several batches of minced Ikanbilis (20 g) were mixed with salt (10% w/w) and the pH was adjusted to pH 8.0. The mixtures were incubated for 6 days. Two of the flasks were removed and filtered; the filtrates were measured for their volume, and their total nitrogen content.
(b) The other flasks were added to batches of minced Ikanbilis, containing 15% salt (w/w) that had been incubated at pH 3.0 for 6 days and the pH of which had then been re-adjusted to 8.0. Two different concentrations of alkaline treated fish (10 g/100 g acid treated and 20 g/100 g acid treated fish) were investigated. Flasks were removed after time intervals of 0, 5, 10 and 15 days. The mixtures were filtered and the volume of each filtrate was measured and its total nitrogen content was determined.

Results and discussion

To be of use as an additive to fish sauce, the solution produced must be clear, without any detrimental aroma and with a high soluble nitrogen content. Also if the fish is the same as that used in normal production, then the amount of soluble nitrogen obtained by the process must be similar to that formed in the traditional fermentation in order for it to be economically competitive.

The ability of the process to convert the insoluble protein into the soluble form can be defined as:

$$\text{Percentage protein conversion} = \frac{\text{volume of liquid produced} \times \text{its N content (g/g)} \times 100}{\text{wt of fish used} \times \text{its N content (g/g)}}$$

The nitrogen content of the fish was found to be 0.0248 g g/g wet weight.

Table 1. The effect of pH on the extent of formation of supernatant liquor and protein converted when *Stolephorus* was incubated for 6 days at 30°C

pH	Volume of liquid produced (cm^3/g)	Percentage protein conversion
1.0	0.44	54
2.0	0.50	57
2.5	0.51	54
3.0	0.56	53
3.5	0.52	52
4.0	0.45	45.1

(a) *Effect of pH*

An initial experiment was set up in which batches of Ikanbilis (*Stolephorus* sp.) were adjusted to specified pH values and incubated for 6 days. The results (Table 1) show that only a small difference occurred in the percentage protein hydrolysed; the values were similar to the percentage conversion obtained with the natural fermentation of 'budu' after 4 months which occurs at pH 5.65 (Beddows *et al.*, 1979b). However, the supernatant liquor was extremely difficult to decant as the fish residue was very gelatinous and presumably reduced the volume of liquid obtained. The natural fermentation of 'budu' gave 0.75 cm^3/g of fish.

Spoilage occurred at pH 3.5 and the solution at pH 4.0 had an unpleasant aroma also.

(b) *Effect of salt and pH*

In order to improve the filtering properties and to extract more liquid, experiments were carried out with salt present. At high salt concentrations, the residue was easily filtered. However the percentage protein converted was reduced (Table 2). The salt also acts as an antimicrobial agent at higher pH

Table 2. The effect of salt concentrations and pH on the percentage nitrogen conversion when *Stolephorus* was incubated for 6 days at 30°C

Percentage salt added	Percentage nitrogen conversion			
	pH 2.0	pH 3.0	pH 4.0	pH 5.0
0	54.3	40.0	—	—
5	70.7	49.9	54.0	34.4
10	50.8	49.8	49.0	—
15	45.2	47.3	28.5	27.8
20	39.1	41.7	35.2	39.5

Table 3. The effect of salt concentration on the distribution of the soluble nitrogen compounds after 6 days incubation of *Stolephorus* at pH 2.0 and 3.0 at 30°C, compared to a natural fermentation

pH	Percentage salt added	Total-N (%)	Protein-N (%)	Volatile-N NH_3 (%)	Volatile-N TMA (%)	Amino-N (%)	Polypeptide* (%)
2.0	0	2.096	0.092	0.091	0.031	1.456	0.49
	5	2.352	0.080	0.082	0.029	1.470	0.67
	10	1.776	0.091	0.084	0.034	1.148	0.40
	15	1.712	0.073	0.078	0.032	1.120	0.41
	20	1.664	0.032	0.091	0.029	1.106	0.39
3.0	10	2.063	0.081	0.083	0.026	1.302	0.63
	15	1.79	0.069	0.090	0.034	1.190	0.40
Budu**	Saturated	1.770	0.010	0.090	0.30	1.173	0.475

*polypeptide found by difference and could include nucleic acid breakdown products.
**from Beddows *et al.*, 1979b.

values. The results indicate that pH 2.0 and 5% salt gave the best percentage protein conversion, although a larger volume of acid is required to adjust the pH (9.8 cm^3 of 50% hydrochloric acid/100 g fish) compared with pH 3.0 (4.1 cm^3/100 g). The supernatant liquors produced at the higher salt concentrations (10–15%) decanted relatively easily. Thus the mixtures at pH 2 and 10% salt and pH 3 and 10% salt gave clear liquids with a nett percentage protein conversions similar to the natural process; 0.67 cm^3/g and 0.63 cm^3/g of liquid were obtained respectively. High salt concentrations (20%) decreased the net percentage protein conversion in the 6-day period, presumably due to the lowering of the proteolytic activity.

The effect of salt at pH 2 and 3.0 on the hydrolysis of the protein to the various soluble nitrogen fractions is given in Table 3. The solutions are slightly higher in soluble nitrogen concentration than with the traditional 'budu' fermentation, but the percentage distribution of nitrogenous compounds is similar (Table 4), with mainly amino acids being formed.

Table 4. Comparison of the percentage distribution of soluble nitrogen compounds of some of the supernatant liquors produced by acid-ensiling Ikanbilis

	Percentage salt added initially	Protein-N	Amino-N	Volatile-N (total)	Polypeptide-N
pH 2	10	5.2	64.7	7.2	22.8
	15	4.7	65.4	7.0	23.9
pH 3	10	3.9	63.1	5.3	27.9
	15	3.8	66.5	6.9	22.3
Budu	—	0.6	66.3	6.6	26.5

Table 5. The rate of hydrolysis of *Stolephorus* incubated with 10% salt at pH 2.0 and 30°C, as determined by the volume of supernatant liquor produced, its formol titration value and percentage nitrogen content and the percentage protein conversion

Time (days)	Volume produced (cm^3/g)	Formol (cm^3 NaOH)	Percentage nitrogen content (g/100 cm^3)	Percentage protein conversion
2	0.62	7.4	1.580	39.5
4	0.68	8.0	1.664	45.6
6	0.66	8.2	1.776	47.2
8	0.67	8.3	1.790	48.2

In general, the percentage nitrogen concentration of the solutions produced, decreased with increase in salt concentration.

It would appear that for practical purposes, the pH 3 at 10% or 15% salt offered the best advantage (although pH 2 gave slightly better results with proteolysis). The residue from a pH 2 (10% salt) 6-day hydrolysis was dried and weighed. After hydrolysis the original 100 g fish gave 11.76 g solid, which contained 10.9% nitrogen. This indicated that 51.8% of the fish protein had not been hydrolysed under acid conditions.

(c) *Effect of time of the hydrolysis*

A number of fish:salt mixtures were set up at pH 2.0 and two each of these were harvested at intervals of 2 days. The results are given in Table 5. The proteolysis increased with time; the 4-day solutions were not as clear after filtering as the 6-day samples. Very little proteolysis occurred after 6 days.

In order to verify the findings, two larger scale experiments were carried out at pH 2 and 10% salt and pH 3 with 15% salt, 500 g of minced Ikanbilis being used in each. The batch at pH 2 produced 293 cm^3 of solution ($\equiv$ 0.58 cm^3/g) containing 2.11 g% nitrogen; the net protein conversion was 49.7% and the batch at pH 3 produced 265 cm^3 of solution ($\equiv$ 0.53 cm^3/g) containing 2.24 g % nitrogen: the net conversion was 47.9%.

All these experiments were carried out on minced fish; however, when whole fish were adjusted to pH 3 in 15% salt, only a little proteolysis (28% protein conversion) occurred in 6 days.

One advantage of the method is that lower concentrations of salt could be achieved with the pH 3, 15% hydrolysate, the final solution containing only 16.9% salt whereas 'budu' and other sauces are saturated at approximately 26%. The pH 2.0 (10% salt) solution contained 13.9% salt.

Fish sauces are used as a condiment and often the salt concentration is important in the quantity of fish sauce consumed. Thus the nitrogen consumption could be increased. However the solutions produced would be mixed with fish sauce and an overall increase in the salt content would occur.

Table 6. The comparison of hydrochloric acid and a mixture of hydrochloric and formic acids, at the same pH values, on the volume of supernatant liquor produced and the percentage protein converted, when *Stolephorus* was incubated for 6 days at 30°C

	HCl only		HCl + 3% HCOOH	
pH	Volume (cm^3/g) produced	Percentage protein conversion	Volume (cm^3/g) produced	Percentage protein conversion
1.0	0.44	54.1	0.56	53.1
2.0	0.50	55.6	0.56	57.1
2.5	0.51	54.0	0.59	54.6
3.0	0.56	53.6	0.56	51.1
3.5	0.52	53.2	0.57	50.6
4.0	0.48	50.7	0.56	48.7
5.0	—	—	0.59	38.7

(d) *Use of formic acid*

As formic acid has been used for producing proteolysates for animal feedstuffs, an experiment was carried out to establish if the use of formic acid has any particular effect on percentage protein conversion. The results (Table 6) show that very little difference occurred and formic acid has no specific action. However higher pH values could be used because of the preservative effect. The use of formic acid is not advocated.

(e) *Re-adjustment of the pH*

To be used, the solutions produced using hydrochloric acid to lower the pH value, must be re-adjusted to pH 5.65, (the pH of naturally produced fish sauce). When this was attempted, a precipitate of calcium phosphate started to form at pH 4.4. It was partially soluble at pH 5.65. The precipitate was identified by filtering an alkaline solution. The concentration was 2.49 g/100 cm^3. A portion of the precipitate (70.1 mg) was ashed at 450°C to give 60.3 mg. The original precipitate did not contain nitrogen (Kjeldahl) but calcium was identified using picrolonic acid (Nördbo, 1939) and phosphate using ammonium molybdate. The calcium was analysed quantitatively with EDTA and the phosphate was determined gravimetrically. Some 95% of the dried residue was calcium phosphate.

The precipitate, which presumably derives from the bones and need not be considered as nutritionally detrimental, would be a problem for consumer acceptance since clear solutions are preferred. Addition of citrate did not prevent precipitate formation. However, the adjustment of the fish mixture to pH 6, and then filtering, removed all the precipitate and gave a clear solution, when readjusted to pH 5.65.

Table 7. The effect of adding pH 8.0 incubated *Stolephorus* to acid ensiled *Stolephorus* (after incubation at 30°C for 6 days followed by re-adjustment of the pH to 8.0) on the percentage protein conversion, after further incubation at 30%C for up to 15 days.

	Time (days)	Percentage protein conversion
100 g of acid	0	48.7
treated fish	5	49.9
+ 10 g of alkaline	10	54.3
treated fish	15	53.8
100 g of acid	0	47.6
treated fish +	5	50.2
20 g of alkaline	10	51.3
treated fish	15	51.4

(f) *Aroma of the proteolysates*

The volatile nitrogen compounds, NH_3 and TMA, formed to the same extent as in fish sauce. The pH 3 (15% salt) and pH 2 (10% salt) solutions were analysed for volatile fatty acids, which are often present in fish sauce. No detectable acids other than ethanoic were present. The ethanoic was at less than 0.01 mg/cm^3, whereas in fish sauce it would be at the 2 mg/cm^3 level. Subjective assessment of the pH 2 and pH 3 hydrolysates after adjustment to pH 5.6 indicated that no strong aroma was present and the taste of both was salty with no other taste being apparent.

(g) *Addition of fish incubated at pH 8.0 to acid treated Ikanbilis*

The percentage protein conversion with the low pH treatment was only just comparable to that of the natural fish sauce fermentation.

When the Ikanbilis was incubated at pH 8.0 in 10% salt, only 23.1% of the fish protein was hydrolysed into the soluble form.

When the pH of the acid treated Ikanbilis was adjusted to pH 8.0, and 10 or 20% of the pH 8.0 treated fish was added, further hydrolysis did take place. (Table 7). Initially the addition gives a dilution effect but after 15 days some improvement had occurred with both concentrations of pH 8 treated fish added.

Fish that had been allowed to hydrolyse at pH 3 in the presence of 15% salt for 6 days, was adjusted to pH 8.0. A sample was removed, filtered, and the nitrogen content of the filtrate determined. This was repeated after 15 days. The nitrogen content changed from 1.87 g/100 cm^3 to 1.89 g/100 cm^3 showing that virtually no hydrolysis was taking place at pH 8.0 after the acid treatment. Presumably the acid and the action of the acid proteases destroyed the enzymes that are active at pH 8.0.

It is possible that the use of the pH 8.0 treatment could give a greater increase in the percentage protein converted. A number of variables exist. However, to optimize the process, it would be preferable to use fresh Ikanbilis or other species, rather than the plate-frozen fish used in these experiments.

Conclusions

The use of low pH values increased the rate and extent of proteolysis of Ikanbilis (*Stolephorus* sp.); the amount of soluble protein being hydrolysed to give soluble nitrogen compounds was comparable to that obtained with the traditional fermentation.

Although the presence of salt generally decreased the extent of the proteolysis, it did make the mixtures far easier to filter. The optimum conditions were either pH 2.0 with 10% salt w/w or pH 3.0 with 15% salt w/w.

The solutions obtained were clear but slightly brown in colour and contained a distribution of nitrogen compounds similar to that found in the traditionally prepared sauce. The solutions had little aroma or taste, although the volatile nitrogen compounds had developed to the same extent as in 'budu'.

When the pH was adjusted to 5.65 calcium phosphate precipitated. This could be filtered off, and the liquid produced could be added to fish sauce, in order to increase the volume.

Adding fish that had been allowed to hydrolyse at pH 8.0 to the low pH hydrolysed fish increased the amount of protein hydrolysed to a limited extent.

The methods investigated present some problems but they do offer a feasible method of increasing the rate of fish sauce production in that a hydrolysate was produced which was suitable for adding to fish sauce, produced by the traditional method, providing that the traditional sauce had a sufficiently high concentration of aroma constituents to keep a product acceptable to the consumer.

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The prediction of water activity in aqueous solutions in connection with intermediate moisture foods

I. a_w Prediction in single aqueous electrolyte solutions

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Summary

A system of theoretical equations for the osmotic coefficient in aqueous electrolyte solutions, developed by Pitzer (1973), has been used for predicting the water activity (a_w) in aqueous solutions of thirty different 'food additive-like' strong electrolytes. Water activity versus solute concentration curves have been plotted for the range of a_w of most interest for intermediate moisture foods (IMF). The Raoult's law behaviour of several of the above electrolytes has also been examined. The results are discussed in connection with the formulation of IMF.

Introduction

Kaplow (1970) has defined an intermediate moisture food (IMF) as one that is moist enough to be eaten without rehydration and yet is shelf stable without thermal processing or refrigeration. The overall stability and quality of an IMF is achieved by reduction of product water activity (a_w), addition of specific antimicrobial agents and often addition of chemical agents to prevent or reduce chemical deterioration during storage. IMF usually have moisture contents in the range of 25–50% (wet basis) and water activity in the range of 0.70–0.90, this depression of a_w being achieved by adding soluble substances to the aqueous phase of the food. Several solutes have been incorporated in IMF to lower the a_w to the desired range. These solutes, sometimes called humectants, include salts, sugars and polyols (Kaplow, 1970; Bone, 1973; Heidelbaugh & Karel, 1975). Among compounds most used to depress a_w are sodium chloride, glycerol, propylene glycol, sucrose, corn syrup, sorbitol and dextrose. Recently, Flink (1978) made a survey of the humectants most cited in the literature for depressing a_w in IMF.

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Ever since the commercial success of IM pet foods, food technologists have attempted to develop IMF for human consumption and IM technology has been the subject of numerous technical and scientific articles in the last decade (Heidelbaugh & Karel, 1975; Davis, Birch & Parker, 1976; Flink, 1977; Flink, 1978). The results, however, have not been successful, basically because essentially all solutes used for a_w lowering yield products with objectionable flavour to human taste, e.g. the sweet-bitter taste of glycerol, the sweetness of sucrose or the saltiness of sodium chloride. This is mainly due to the fact that humectants in use today must be used in high concentrations to depress the water activity, a fact which strongly contributes to their negative influence on product flavour. For instance, glycerol and sucrose concentrations well above 30% of the finished IMF are usually reported in the literature as well as sodium chloride concentrations in excess of 4–5% of product weight (Kaplow & Halik, 1973; Collins *et al.*, 1972). These figures easily explain the unfamiliar and unacceptable flavour usually found in many IMF intended for human consumption.

In 1973, Bone asked the following question: 'How do we obtain solute concentrations that are compatible with flavour and other technical aspects of the food?'. This question still constitutes a challenge for the food technologist interested in the development of IMF for human consumption. One of the ways to find an answer is to look for new solutes for a_w lowering. Flink (1978) has also stressed that future developments in the IMF area will require new humectants which give a_w lowering without adverse effects on taste and other properties of the food. To find new solutes means looking for compounds which exhibit very large deviations from Raoult's law, thus depressing a_w at much lower concentrations than expected, with the subsequent reduced flavour impact. Of course, the selection of a new humectant for the formulation of IMF is not solely a problem of a_w lowering at reduced solute concentration, but also involves considerations about texture, cost, safety, nutrition, pH, compatibility with food components, FDA status and shelf life (Bone, 1973). Nevertheless, the examination of a_w-lowering capacity of new solutes is one of the preliminary needs to be fulfilled in order to reach the desired objective.

The purpose of the present work is to carry out a systematic survey of water activity lowering in single electrolyte aqueous solutions as well as an examination of the theoretical aspects of a_w predictions. It is hoped that this work may constitute a preliminary contribution towards solving the complex problem of finding new solutes for the formulation of IMF. The problem of a_w prediction in multicomponent electrolyte solutions as well as a_w prediction in non-electrolyte solutions is discussed in subsequent papers.

Results and discussion

The prediction of a_w in single aqueous electrolyte solutions

We are here interested in finding an equation which reproduces measured properties of electrolyte solutions within experimental accuracy and which is also convenient in the sense that only a few parameters need to be tabulated for

each substance and the mathematical calculations are relatively simple. It would also be important that those parameters have physical meaning as far as possible, in order to reduce the risk of extrapolations, which is usually very high when working with purely empirical equations.

The thermodynamic properties of aqueous strong electrolyte solutions have been extensively studied both experimentally and theoretically. Excellent summaries may be found in the standard monographs of Harned & Owen (1958) and Robinson & Stokes (1965). Various extensions of the Debye-Hückel model have been proposed to fit the colligative data of single aqueous electrolytes but usually these break down at moderate or high solute concentrations; empirical or semi-empirical fits have also been attempted (Teng & Lenzi, 1974). The situation up to 1968 was clearly reviewed by Scatchard (1968) who pointed out the basic principles of the modern theoretical formulations of electrolyte solutions. The excess Gibbs energy of the solution is separated in a Debye-Hückel (or electrostatic) term which provides the correct theoretical limit at infinite dilution, and an additional term which considers the short range interactions between ions. Most theoretical models developed since 1970 are different variations of this basic formulation. A good example of the 'modern' theory of solutions may be found in the works of Scatchard, Rush & Johnson (1970), Reilly, Wood & Robinson (1971), Pitzer (1973), Bromley (1973), and Pitzer (1975), among others.

Pitzer (1973) developed a system of equations for the thermodynamic properties of electrolytes on the basis of an improved analysis of the classical Debye-Hückel model. By modifying the usual second virial coefficients to include the recognition of an ionic strength dependence of the effect of short-range forces in binary interactions, he obtained a system of equations which yield agreement within experimental error up to concentrations of several mol/kg. The equations developed by Pitzer (1973) were very successfully applied to 227 pure aqueous electrolytes by Pitzer & Mayorga (1973). The equations used in representing the properties of 1–1 and 2–1 electrolytes proved to be equally effective for 3–1, 4–1 and even 5–1 type solutes.

After examination of the literature it was concluded that Pitzer's (1973) model represents one of the most convenient and accurate representations of data in connection with IM technology and this was selected. Reasons for this choice may be summarized as follows: (a) Only a few parameters need to be used to characterize the behaviour of each substance, (b) The equation was developed on rigorous theoretical grounds which give a physical meaning to their parameters, (c) The equation represents experimental data substantially within the experimental error from dilute solutions up to an ionic strength varying from case to case but typically 6 M. The fit is particularly good for 1–1 and 2–1 electrolytes, which are the ones of most concern in this work. It has to be stressed that the ability of Pitzer's model to describe experimental data at high solute concentration is most useful to our purposes because the a_w's required for microbial inhibition are associated with moderate and usually high solute concentrations.

Pitzer's (1973) model

The osmotic coefficient, φ, is given by the equations developed by Pitzer (1973) as:

$$\varphi - 1 = Z_M Z_X | f + m \left(\frac{2\nu_M \nu_X}{\nu} \right) B_{MX} + m^2 2 \frac{(\nu_M \nu_X)^{3/2}}{\nu} C_{MX} \quad (1)$$

where, ν_M and ν_X are the number of M and X ions in the formula and Z_M and Z_X give their respective charges in electronic units; also, $\nu = \nu_M + \nu_X$. The other quantities have the form:

$$f = -A \left[\frac{I^{1/2}}{1 + b I^{1/2}} \right] \quad (2)$$

$$B_{MX} = \beta_{MX}^{(0)} + \beta_{MX}^{(1)} \cdot \exp(-\alpha I^{1/2}) \quad (3)$$

where,

I : ionic strength $= 1/2 \Sigma m_i z_i^2$

A is the Debye-Hückel coefficient for the osmotic function and has a value of 0.392 at 25°C.

Constant b was taken equal to 1.2 for all solutes and also the value of $\alpha = 2$ was found by Pitzer & Mayorga (1973) to be satisfactory for all solutes considered in the present work. Pitzer & Mayorga (1973) noted that for each substance the two parameters, $\beta_{MX}^{(0)}$ and $\beta_{MX}^{(1)}$ define the second virial coefficient and C_{MX} defines the third virial coefficient, which is usually very small.

Pitzer & Mayorga (1973) evaluated the best values of $\beta^{(0)}$, $\beta^{(1)}$ and C from a diversity of literature experimental data. They listed those parameters together with the maximum molality for which agreement was attained to 0.01 in φ or for which data were available, and the standard deviation of the fit. In some cases where the data seemed less accurate the allowable error in φ was increased to 0.02; however, this was the case only for two of the thirty electrolytes examined in this work.

Pitzer & Mayorga's (1973) parameters were used to evaluate the osmotic coefficient for each particular system and concentration. These data were then used to calculate the water activity, a_w, through the relationship:

$$a_w = p/p_0 = \exp(-\varphi m_1 m \nu) \quad (4)$$

where,

φ : osmotic coefficient,
m_1 : number of kg per mol of water,
m : molality of solute,
ν : number of moles of all species which give 1 mol of solute in solution.

Bromley's model (1973)

Bromley (1973) independently developed a system of representation and estimation for thermodynamic properties of strong electrolytes, which was described by Pitzer & Mayorga (1973) as a simplification of Pitzer's model. The third virial coefficient is omitted and the second virial coefficient is modified into a form still dependent on ionic strength but with a single parameter B. Pitzer & Mayorga (1973) indicated that, although less accurate than their equations, Bromley's (1973) model was also quite effective. For this reason and considering that Bromley's model was also satisfactory for high solute concentrations (typically 6 M), it was used to describe the a_w lowering effect for a few solutes for which Pitzer & Mayorga (1973) did not report data. Bromley's (1973) equation for the osmotic coefficient, φ, is:

$$\varphi = 1 - 2.303 \left\{ A_\gamma |Z_+Z_-| \frac{I^{1/2}}{3} \sigma(\rho I^{1/2}) - (0.06 + 0.6B)|Z_+Z_-| \frac{I}{2} \psi(aI) - B\frac{I}{2} \right\} \quad (5)$$

where
$A\gamma = 0.511$ kg$^{1/2}$ mol$^{1/2}$ at 25°C,

$|Z_+Z_-|$ = product of the ions charges in e$^-$ units,

$$\sigma(\rho I^{1/2}) = \frac{3}{(\rho I^{1/2})^3} \left[1 + \rho I^{1/2} - \frac{1}{1 + \rho I^{1/2}} - 2 \ln(1 + \rho I^{1/2}) \right]$$

ρ is taken to be 1 for all solutes,
B is a parameter for each solute,

$$\psi(aI) = \frac{2}{aI} \left[\frac{1 + 2aI}{(1 + aI)^2} - \frac{\ln(1 + aI)}{aI} \right]$$

$$a = \frac{1.5}{|Z_+Z_-|}$$

Choice of solutes and representation of data

The model of Pitzer (1973) with parameters reported by Pitzer & Mayorga (1973) and of Bromley (1973) in a few cases, were applied to thirty different single strong electrolyte solutions for calculating a_w as a function of solute

Table 1. List of electrolytes studied

(1)	Calcium chloride ($CaCl_2$) – (P)
(2)	Calcium iodide (CaI_2) – (P)
(3)	Dipotassium hydrogen phosphate (Na_2HPO_4) – (P)
(4)	Disodium fumarate (Na_2-fumarate) – (P)
(5)	Disodium hydrogen phosphate (Na_2HPO_4) – (P)
(6)	Ferrous chloride ($FeCl_2$) – (P)
(7)	Magnesium acetate (Mg-acetate) – (B)
(8)	Magnesium chloride ($MgCl_2$) – (P)
(9)	Magnesium iodide (MgI_2) – (P)
(10)	Manganese chloride ($MnCl_2$) – (P)
(11)	Monopotassium adipate (KH-adipate) – (P)
(12)	Monopotassium malonate (KH-malonate) – (P)
(13)	Monopotassium succinate (KH-succinate) – (P)
(14)	Monosodium adipate (NaH-adipate) – (P)
(15)	Monosodium malonate (NaH-malonate) – (P)
(16)	Monosodium succinate (NaH-succinate) – (P)
(17)	Potassium acetate (K-acetate) – (P)
(18)	Potassium carbonate (K_2CO_3) – (B)
(19)	Potassium chloride (KCl) – (P)
(20)	Potassium dihydrogen phosphate (KH_2PO_4) – (P)
(21)	Potassium iodide (KI) – (P)
(22)	Potassium pyrophosphate ($K_4P_2O_7$) – (P)
(23)	Sodium acetate (Na-acetate) – (P)
(24)	Sodium butyrate (Na-butyrate) – (B)
(25)	Sodium caproate (Na-caproate) – (B)
(26)	Sodium chloride (NaCl) – (P)
(27)	Sodium dihydrogen phosphate (NaH_2PO_4) – (P)
(28)	Sodium formate (Na-formate) – (P)
(29)	Sodium propionate (Na-propionate) – (P)
(30)	Sodium valerate (Na-valerate) – (B)

concentration in the a_w range of most interest for intermediate moisture foods. Solute concentration was expressed in total weight basis instead of molality, because it may be more useful for the specific purposes of this work. Electrolytes studied include inorganic compounds of the 1–1 type (e.g. KCl), salts of carboxylic acids (e.g. Na acetate), inorganic compounds of 2–1 type (e.g. $MgCl_2$), organic electrolytes of 2–1 type (e.g. Na_2 fumarate) and also one compound of the 4–1 type ($K_4P_2O_7$). Table 1 lists the thirty electrolytes studied. Letters within brackets following each solute indicate whether Pitzer's (P) or Bromley's (B) model was used to generate the a_w data. Most of the solutes listed in Table 1 are included in the U.S. government lists of frequently employed direct food additives (Furia, 1972). Its regulatory status (always subjected to changes) includes GRAS (generally recognized as safe) compounds such as $CaCl_2$, KCl, NaCl, KI, Na-acetate, K_2CO_3, etc., as well as approved food additives (e.g. Na_2-fumarate, K-acetate, Na-propionate, etc.) and Na or K salts of approved or GRAS mono and di-carboxylic organic acids (e.g. Na caproate,

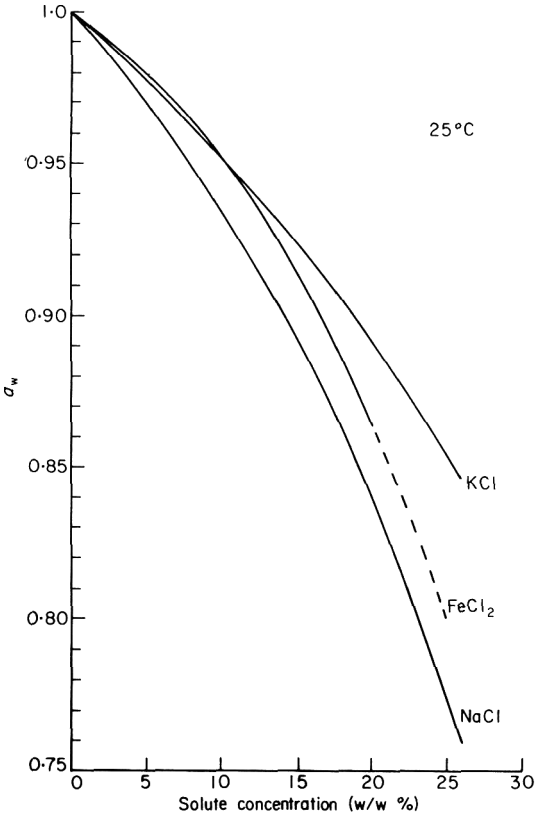


Fig. 1

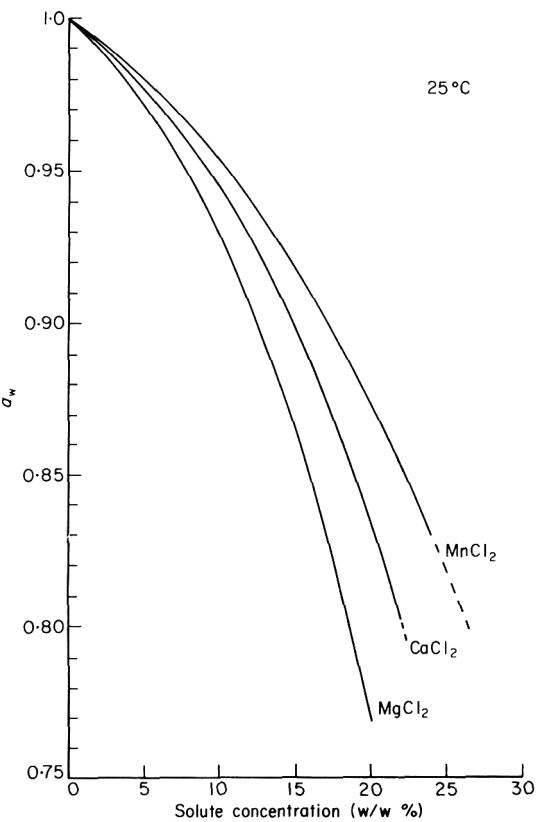


Fig. 2

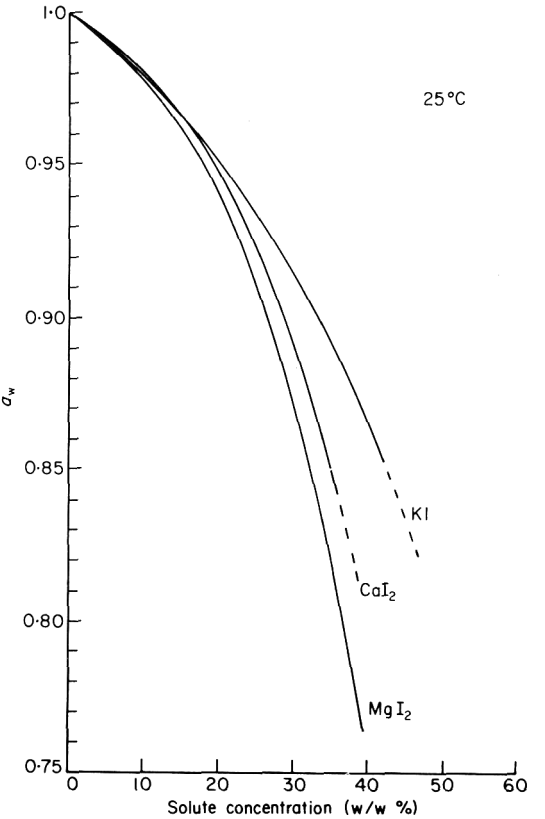


Fig. 3

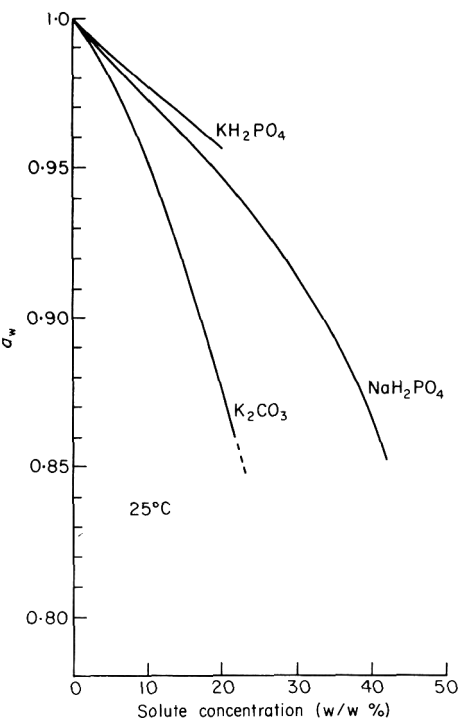


Fig. 4

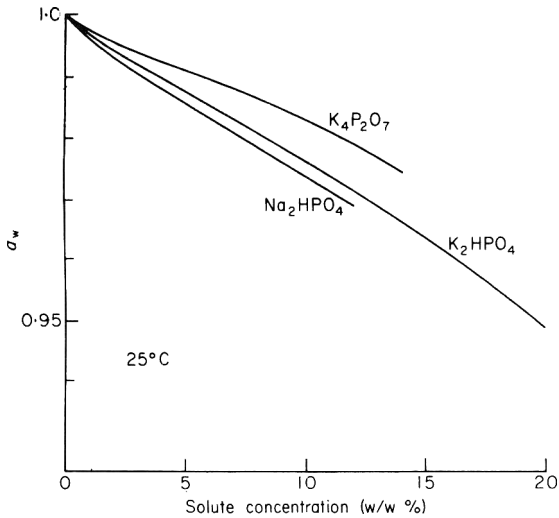
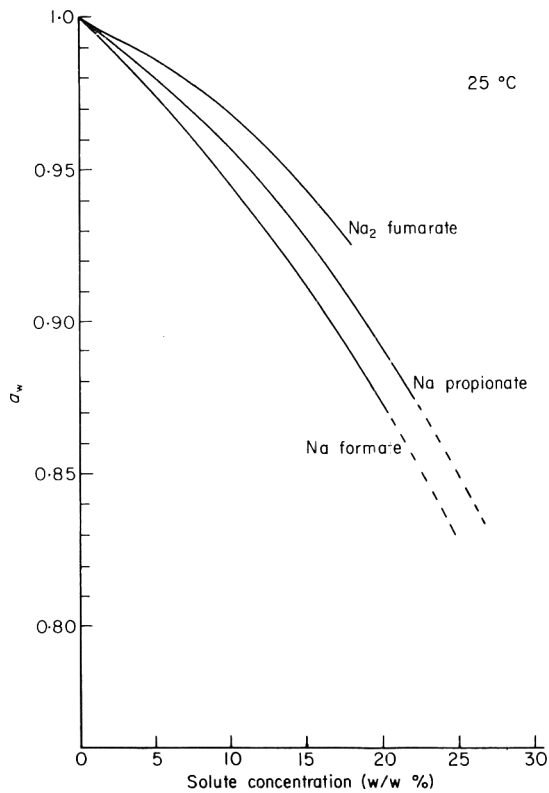


Fig. 5



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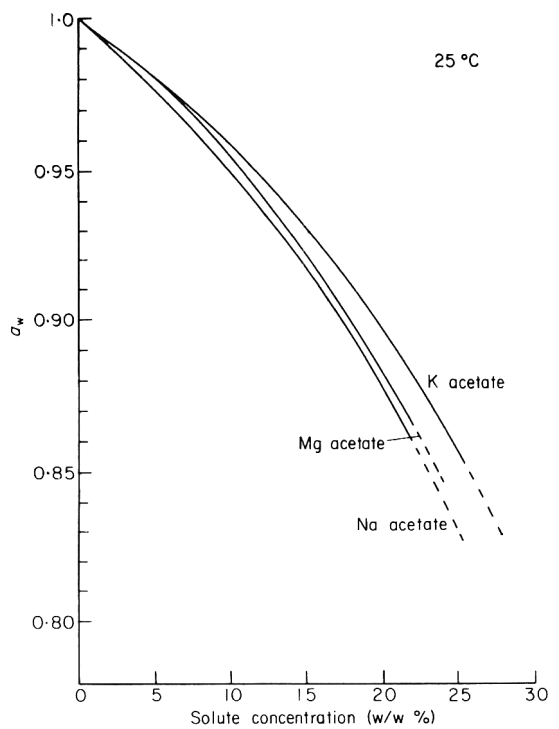

Fig. 6

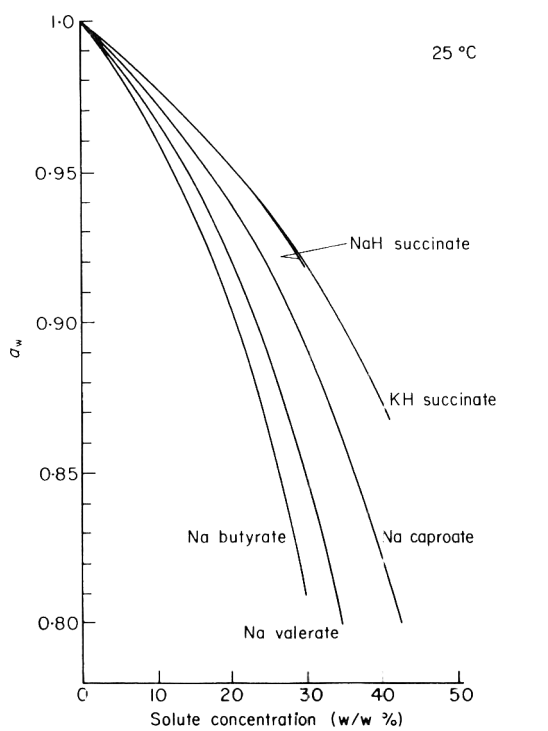

Fig. 8

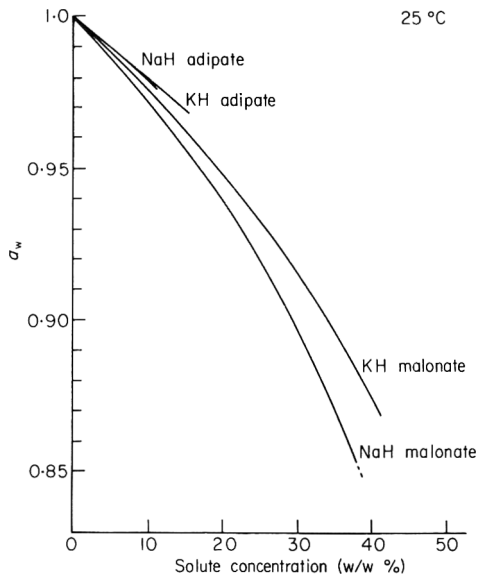

Fig. 9

Figures 1–9. Predicted water activity as a function of solute concentration for various single aqueous electrolyte solutions at 25°C.

Na formate, Na butyrate, NaH-adipate, etc.). Of course, this is not to say that any of the solutes here examined may be used as a_w lowering agents in IMF, but at least they are 'food additive models' which may be used to study the characteristics of a_w lowering. For instance, alkaline salts (like K_2CO_3) are not likely to be used in IMF and neutral or slightly acid ones are to be preferred.

Figures 1 to 9 show the variation of a_w with solute concentration for the different electrolytes at 25°C. The curves were drawn in most cases only up to the maximum concentration indicated by Pitzer & Mayorga (1973) or Bromley (1973) respectively, and this is shown by the full lines. In some cases, however, the curves were somewhat extrapolated (using the corresponding equation) and this is indicated using dotted lines. It is noteworthy that Tran & Lenzi (1974) reviewed the methods of estimating the water activity of supersaturated solutions and found that extrapolation of Pitzer (1973) equation was a safe and convenient procedure. Thus, it appears that little risk of error exists in the relatively small extrapolations here performed in some cases.

The data of a_w lowering versus solute concentration as shown in Figs. 1 to 9 are of direct use for the food technologist. There are several observations which can be made about the nature of the a_w *vs*. concentration curves. Perhaps, the first one refers to the solute concentration needed to inhibit microbial growth through a_w lowering. Of course any microbial effect independent of a_w which a particular solute may have (e.g. Na propionate) is not considered here. It is well known that a_w lowering is not the only means of insuring microbial product stability in IMF (Karel, 1976). Although there exist critical levels of a_w at which an IMF product can be considered microbiologically stable, these levels are

Table 2. Solute concentration (w_s) in single aqueous electrolyte solutions having an a_w = 0.85 at 25°C

Solute	w_s (%, w/w)
$MgCl_2$	15.8
$CaCl_2$	18.7
NaCl	19.0
$FeCl_2$†	21.2
$MnCl_2$	22.2
Na-Formate‡	22.6
K_2CO_3*	22.7
Na-Acetate*	23.0
Na-Propionate‡	25.0
KCl	25.5
K-Acetate*	25.7
Na-Butyrate	26.2
Mg-Acetate†	27.3
Na-valerate	29.6
MgI_2	32.4
NaH-malonate*	34.2
CaI_2	35.0
Na-caproate	36.3
KI*	42.4
NaH_2PO_4	42.6

* v. slightly extrapolated
† slightly extrapolated
‡ extrapolated

influenced by several factors, like product pH, type of microorganism, temperature, air or vacuum packaging, etc. Nevertheless, it is usually accepted that the highest limit of a_w to inhibit bacterial growth in most foods is about 0.85, while the growth of yeasts and moulds is inhibited by adding specific components (typically potassium sorbate). Table 2 shows the weight concentration of each individual electrolyte needed to achieve a 'safe' water activity of 0.85. The concentration values range from a minimum of 15.8% (w/w) for $MgCl_2$ up to 42.6% (w/w) for NaH_2PO_4. It is interesting to note that two solutes, $MgCl_2$ and $CaCl_2$, are able to depress a_w to 0.85 at a lower weight concentration than NaCl (the 'traditional' a_w lowering solute) despite their considerably higher molecular weights. If 0.85 is taken as the highest limit of a_w for safe IMF, Table 2 reveals that for all electrolytes examined, the solute concentration in the aqueous phase appears to be too high in the sense that they may adversely affect taste and other physical properties of the food. Of course, the a_w giving safe products can be raised if means exist to separately deal with the microorganisms which would grow at low levels of a_w. Raising the minimally required a_w will

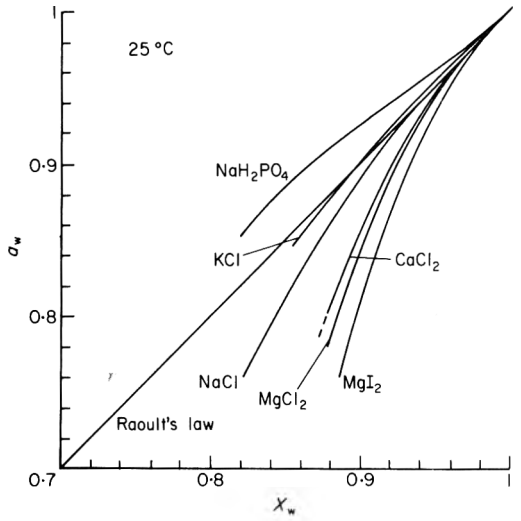

Fig. 10

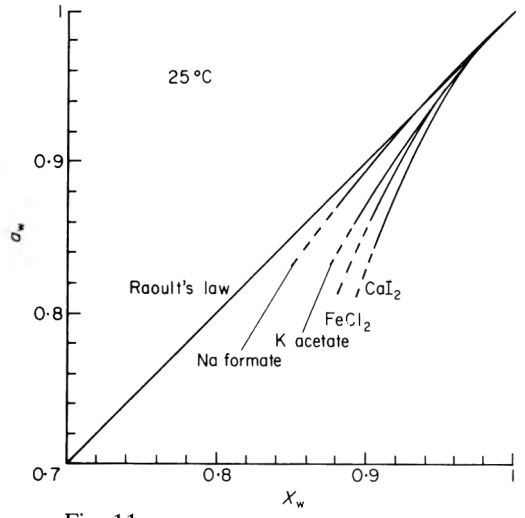

Fig. 11

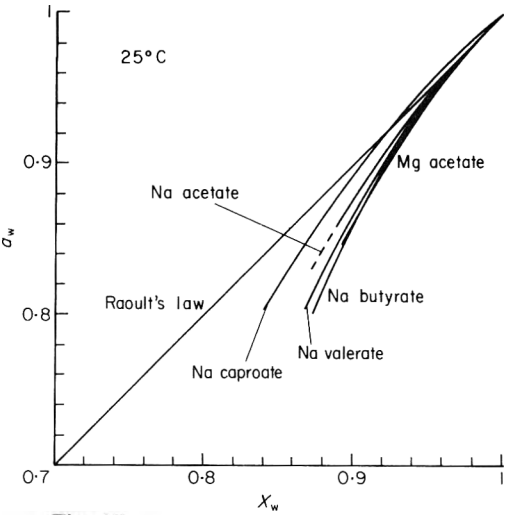

Fig. 12

Figures 10–12. Comparison of a_w depression by various electrolytes with depression expected from Raoult's law at 25°C.

give a more organoleptically acceptable product. The complex interactions which will influence microbial growth in IMF have been discussed by Leistner & Rodel (1976).

Comparison with Raoult's law

One of the reasons for this search for new a_w lowering additives consisted in finding solutes which exhibit very large negative deviations from Raoult's law,

thus depressing a_w at lower concentrations than expected. The behaviour of various of the solutes here studied, with respect to that predicted by Raoult's law, is shown in Figs. 10, 11 and 12. For these calculations it was assumed that each electrolyte was completely dissociated and total number of ionic species computed for Raoult's law calculation of water activity. It can be seen that with one exception all electrolytes examined showed negative deviations from ideality; i.e. they depress a_w at lower concentrations than expected. The large deviations exhibited by CaI_2, MgI_2, $MgCl_2$ and $CaCl_2$, among others, are particularly noticeable and will be discussed in a companion paper.

Conclusions

The theoretical model of Pitzer (1973) – and in a few cases the one of Bromley (1973) – have been used to calculate water activity as a function of solute concentration in aqueous solutions of thirty different 'food additive like' electrolytes. Their Raoult's law behaviour has been examined and it was found that almost all compounds tested presented negative deviations in the range of a_w at which microbial inhibition occurs. The results here obtained suggest that if an a_w of 0.85 is accepted as a highest limit for safe IMF, the aqueous solute concentration appears to be too high to be compatible with taste and other physical properties of the food, even for the 'best performing' electrolytes.

At this point we may not draw definite conclusions because other electrolytes of better performance may exist whose a_w lowering behaviour have not been examined. However, from present results it appears that it will be difficult to find a single 'food additive like' electrolyte with such a large negative deviation from Raoult's law that can lower a_w to 0.85 without impairing food characteristics.

The subject of Raoult's law deviation will be discussed in detail in a subsequent paper.

Acknowledgments

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The prediction of water activity in aqueous solutions in connection with intermediate moisture foods

II. On the choice of the best a_w lowering single strong electrolyte

C. FERRO FONTAN*‡, J. CHIRIFE†AND E. A. BENMERGUI*

Summary

A theoretical study is made of the a_w lowering ability of single strong electrolytes on the basis of a thermodynamic model equation developed by Bromley (1973). This study allowed us to make predictions and draw conclusions about the possibility of finding new solutes (electrolytes) with better a_w lowering abilities than those in use in present IMF technology.

Introduction

As discussed in part I of this work, one of the reasons why IMF for human consumption have not been successful is because humectants in use today must be used in high concentrations to depress water activity.

It is the purpose of the present investigation to study theoretically the a_w lowering ability of single electrolytes based on a thermodynamic model equation developed by Bromley (1973) and already introduced in part I (Benmergui, Ferro Fontán & Chirife, 1979). This study allows us to make predictions and draw conclusions on the possibility of finding new electrolytes with better a_w lowering abilities than those in present use in intermediate moisture food (IMF) technology.

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Results and discussion

Bromley's (1973) equation for the osmotic coefficient is:

$$\varphi = 1 + 2.303 \left[-A_\gamma |Z_+ Z_-| \frac{I^{1/2}}{3} \sigma(\rho I^{1/2}) \right.$$

$$\left. + (0.06 + 0.6B)|Z_+ Z_-| \frac{I}{2} \psi(aI) + B \frac{I}{2} \right] \quad (1)$$

where I is the ionic strength of the solution and

$$\sigma(X) = \frac{3}{X^3} \left[1 + X - \frac{1}{1+X} - 2 \ln(1+X) \right],$$

$$\psi(X) = \frac{2}{X} \left[\frac{1+2X}{(1+X)^2} - \frac{\ln(1+X)}{X} \right]$$

The numerical values of the parameters are as follows:

$\rho = 1$; $a = 1.5/|Z_+ Z_-|$; $A_\gamma = 0.511$ kg$^{1/2}$ mol^{-1} at 25°C

and B is a constant for each electrolyte, which measures the strength of solute-solute interactions. Most of this paper is devoted to a discussion of this relevant parameter in connection with intermediate moisture food (IMF) technology.

The osmotic coefficient is particularly useful for our purposes since water activity (a_w) is simply given by,

$$a_w = \exp \left\{ -0.018 (\Sigma m_i) \varphi \right\} \quad (2)$$

where Σm_i is the dissociated molality of the solution. Thus, a large osmotic coefficient corresponds to a low water activity. From eqn. (1) one concludes that, other things being equal, a solute with high B number has better water activity-lowering properties. This is due to the fact that φ is a linear function of B with positive slope at all ionic strengths.

Our choice of Bromley's (1973) equation as a theoretical tool for the selection of the best a_w lowering electrolytes stands on this property: only one parameter is needed to rank different substances according to their a_w lowering ability. This equation (eqn 1) belongs to a large family of models inspired by modern developments in the cluster theory of solutions. For a formal presentation of this subject we refer the reader to Friedman's (1962) book. To the same category pertains Pitzer's (1973) equation which we used extensively in part I of this paper (Benmergui *et al.*, 1979). As Pitzer & Mayorga (1973) pointed out, eqn (1) is a simplification of Pitzer's model which takes advantage of the fact that some parameters in Pitzer's (1973) equation are actually correlated and that the third virial coefficients are usually negligible. Notwithstanding its simplicity, eqn (1) is still quite effective in fitting data up to high concentrations.

In his paper, Bromley (1973) examined over 175 strong electrolyte compounds and determined their second virial coefficients, B, at 25°C by the

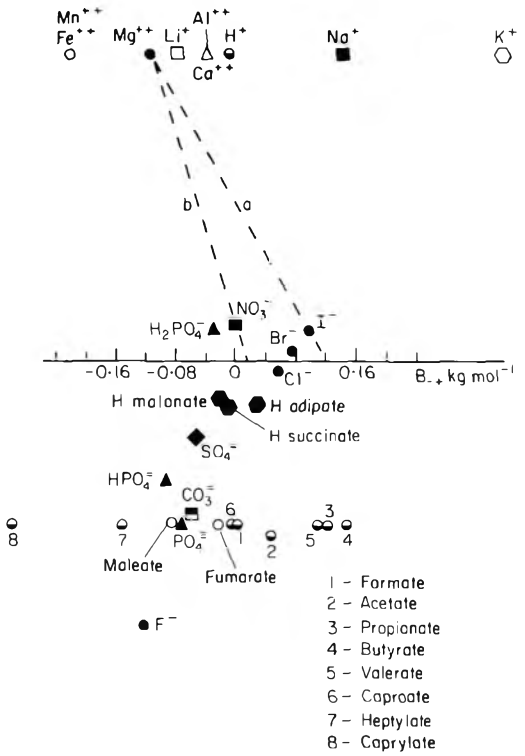

Figure 1. *Nomogram for evaluating the B number.*

method of least squares. Compounds examined included 1–1 (e.g. NaCl), 1–2 (e.g. K_2CO_3), 1–3 (e.g. Na_3PO_4), 2–1 (e.g. $CaCl_2$), 2–2 (e.g. $MgSO_4$), 3–1 (e.g. $AlCl_3$), 3–2 (e.g. $Al_2(SO_4)_3$) and 4–1 (e.g. $ThCl_4$) type, electrolytes. The values of B range from -0.479 kg/mol^{-1} for Na caprate to 0.227 kg/mol^{-1} for $UO_2(ClO_4)_2$. Moreover, he observed a very important correlation among these values which can be stated as follows:

$$B = B_{\text{cation}} + B_{\text{anion}} + \delta_{\text{cation}} \cdot \delta_{\text{anion}} \tag{3}$$

Namely, the second virial coefficient is an almost additive function of the individual ions, except for an interaction term. This composition rule is perhaps the most remarkable virtue of Bromley's (1973) classification, since it allows systematic estimation of a_w lowering properties for compounds that have never been measured. In special graphic form, eqn (3) reveals at a glance which are the most favourable associations of ions from the point of a_w lowering ability. Consequently, a nomogram (Figs. 1 and 2) was devised, whose use will be best explained with two examples. First, eqn (3) is re-written in one of the equivalent forms,

$$B = B_+ + B_{-+}\,;\; B_{-+} = B_- + \delta_+ \delta_- \tag{4a}$$

$$B = B_- = B_{+-}\,;\; B_{+-} = B_+ + \delta_+ \delta_- \tag{4b}$$

Let us suppose we want to know which is the best anion to be used in association with the Mg^{++} cation to depress a_w. Obviously it is the one with the highest B_{-+} in eqn (4a). The nomogram in Fig. 1 automatically adds to B the effect of the interaction between the chosen cation and any other partner in such a way that a straight line issued from the Mg^{++} cation (in this example) to its associated anion intersects the horizontal axis at the corresponding value of B_{-+}. Thus, Fig. 1 shows that $I^-(a)$ is a better partner for Mg^{++} than $NO_3^-(b)$.

If one has to deal with a specific anion the task is to find the cation which in association with it, will give the highest B value and thus the lower a_w (at same ionic strength). In this case, Fig. 2 gives the answer. For instance the nomogram in Fig. 2 reveals that $Li^+(b)$ is a better partner for Cl^- than $Na^+(a)$. The nomograms only include cations and anions which may be considered as 'food additive' like species (with some exceptions included for illustration).

The nomograms are self explanatory and need no more comments. They summarize in a pictorial way the tendency of cations and anions to enhance or to disrupt the solvent structure. A discussion on these basis has been advanced by Pitzer & Mayorga (1973) in the conclusions of their paper. We shall not repeat it since the main aim of the present work is to emphasize the practical aspects of the question rather than the physico-chemical ones. We merely observe that some striking correlations can be drawn from the aforementioned nomograms. For example, the trend exhibited by ions belonging to the same group of the periodic table, or the behaviour of constant B with the number of carbon atoms in the sodium salts of fatty acids (Fig. 3). It turns out that the optimum size of the molecule with regard to its a_w lowering ability corresponds to the butyric radical.

All the previous considerations focused on the B number as one of the relevant parameters characterizing the a_w lowering ability of an electrolyte as IMF additive. However this parameter alone is not sufficient. In fact, what we are looking for are compounds which are able to depress a_w at reduced weight rather than molal concentrations, so the 'natural' composition of the finished IMF is altered as little as possible. Consequently, the molecular weight of the solute is also a parameter of relevant importance. For example, sodium butyrate (mol.wt = 110) having a B number as high as 0.15 kg mol^{-1} is a 'poorer' additive – in the sense that a higher weight concentration is needed – than sodium chloride (mol.wt = 58.5) having a much lower B (0.057 kg mol^{-1}). In order to simplify the survey of solutes for IMF we have prepared a chart shown in Fig. 4 in which both B and molecular weight of the compound are considered together, and in which their relative contributions are adequately displayed. In Fig. 4 is plotted a_w calculated from eqns (1) and (2) as a function of B number and molecular weight (mol.wt) at a fixed concentration by weight, namely 20% w/w (which correspond to an a_w of about 0.84 for NaCl). This choice however, is not relevant and could be changed to any other convenient value since Fig. 4 is only intended for comparison among different electrolytes. The mol.wt. scale is logarithmic and covers the range of most practical interest; only positive B numbers have been represented since we are primarily interested in compounds

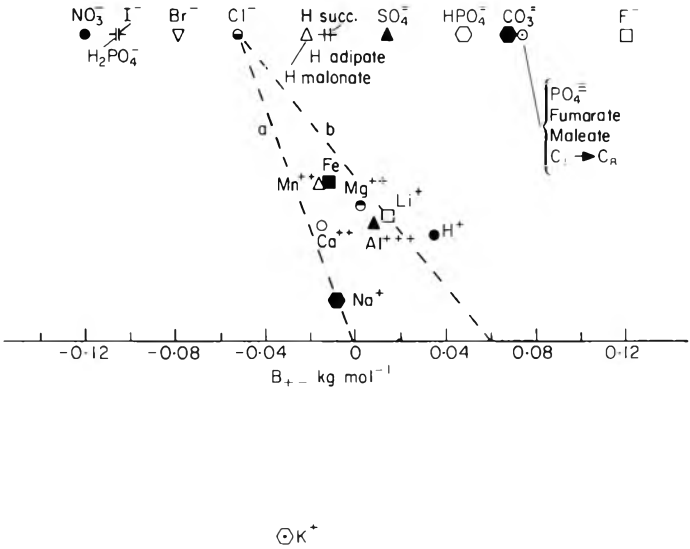

Figure 2. Nomogram for evaluating the B number.

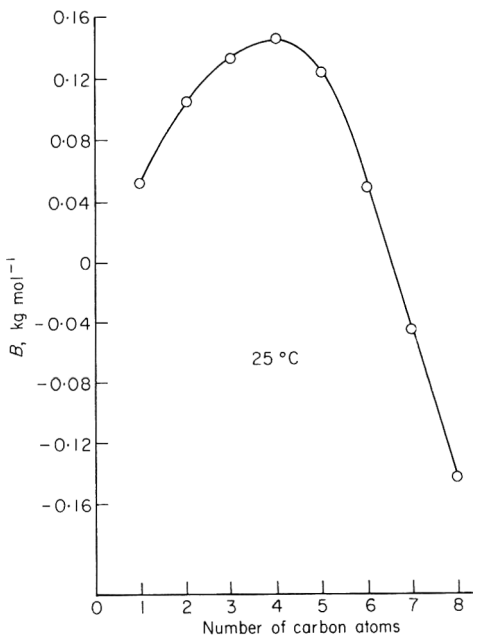

Figure 3. Effect of number of carbon atoms on B number for sodium salts of various fatty acids.

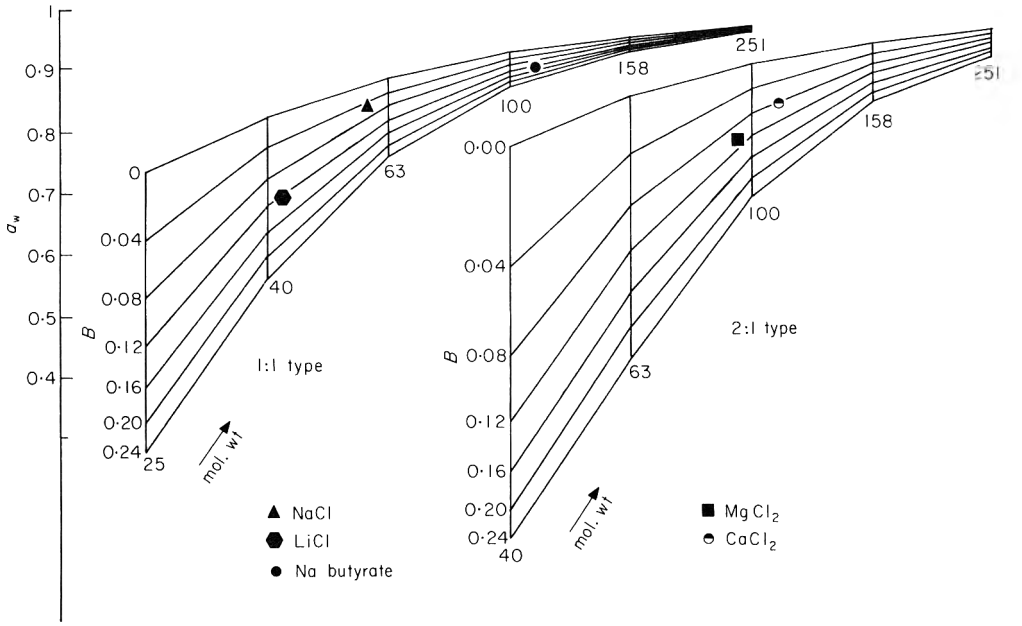

Figure 4. Influence of B number and mol.wt. on a_w lowering ability of 1–1 and 2–1 electrolytes.

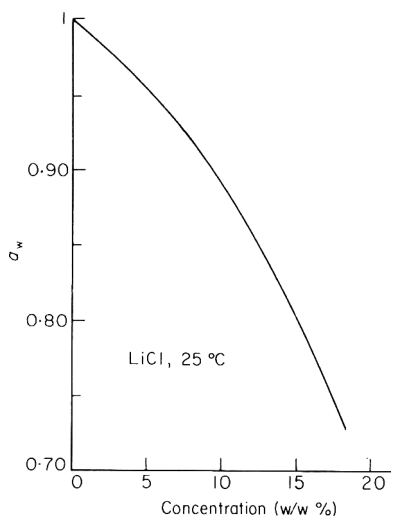

Figure 5. Predicted water activity as a function of solute concentration for LiCl at 25°C.

with high a_w lowering abilities. To each type of electrolyte corresponds a set of curves. The sets for 1–1 and 2–1 solutes have been separated for convenience but maintaining the same vertical scale for a_w. Fig. 4 allows us to make useful predictions concerning the choice of the best a_w lowering electrolyte for IMF; that is, the one which is able to depress a_w with a minimum of added substance. Perhaps the most important one is that the relative influence of B number on a_w lowering (at constant weight concentration) strongly diminishes when increasing molecular weight.

Electrolytes of 2–1 type exhibit a better relation between molecular weight and a_w than those of 1–1 type, due to their larger ionic strength at comparable molality. Thus, $MgCl_2$ (mol.wt. = 95) performs better than NaCl in part due to its good B value, 0.113 kg mol^{-1}, but also because of a doubling of its ionic strength at equal molality. It follows that when looking for new solutes (electrolytes) for IMF one should not consider compounds with molecular weights above certain values in order to have reasonable chances of finding a substance with the desired properties. For example, if we accept as maximum B number the value of 0.24 kg mol^{-1} (this point will be discussed later), there is not any 1–1 electrolyte of mol.wt. $\geqslant$ 90, or 2–1 electrolyte of mol.wt. $\geqslant$ 160 which can match the a_w lowering properties (on weight basis) of NaCl. Of course, this presupposes that electrolytes with a B number higher than 0.24 Kg mol^{-1} do not exist. Although this can not rigorously be demonstrated it is supported by the following fact. Bromley (1973) reported individual ion values of B and δ for thirty-six cations and forty anions (of inorganic as well as of organic nature). We have worked out his data and calculated the B values which may be expected for a very large number of combinations of cations and anions (leading to several hundreds compounds) and found that the highest value was 0.227 kg mol^{-1} corresponding to $UO_2(ClO_4)_2$. Thus, it appears safe to say that electrolyte compounds with a B value higher than 0.24 (used in Fig. 4) are not likely to exist, or if so, they are unlikely to be used as a food additive.

Summarizing, in looking for new solutes (electrolytes) for depressing a_w in IMF one should select compounds of high B values guided by the nomograms of Figs. 1 and 2. Moreover, one should only try electrolytes of mol.wt. below certain values as indicated by Fig. 4. It is important to stress that there is no hope to improve on solutes of high mol.wt. by looking for better B values.

Guided by these principles, an examination of all available data lead us to the following conclusion. It is unlikely to find a non-volatile single electrolyte which may be able to depress a_w at lower weight concentrations than LiCl (mol.wt. = 42.5, B = 0.128 kg mol^{-1}), whose a_w-concentration curve is shown in Fig. 5. Of course, we are not proposing to use LiCl as an IMF additive, but merely showing that no electrolyte food additive is expected to depress a_w at a lower weight concentration than indicated in Fig. 5. For instance, a 12.5% w/w concentration is needed to depress a_w to 0.85. There is no possibility to beat this value and IMF technology should consider this as the 'theoretical' limit of a_w lowering ability.

Acknowledgments

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Book reviews

Candies and Other Confections. By Martha T. Gillies.
New Jersey: Noyes Data Corporation, 1979. Pp. XI + 354, US $36.00.

This is another of the well known series of Noyes Data Corporation publications dealing with technology and apparatus, and with formulation of original products described in the Patent literature.

The topics covered include chocolate candy, other candies, frozen desserts, other desserts such as jellies, starch based milk puddings and dry mixes, confectionery ingredients, icings and toppings, chewing gum, and speciality products.

Full indexes by company, inventor and U.S. patent number are included.

The publication, like the others in the series, is useful for reference particularly for those engaged in new product formulation and in R & D situations.

J. R. Rothwell

Strategy for the U.K. Dairy Industry.
Reading: Centre for Agricultural Strategy, University of Reading. 1978, Pp. 186. £2.95.

It is not generally realized that the United Kingdom is one of the leading dairying countries of the world, coming sixth after U.S.A., U.S.S.R., France, Italy and Poland. Although only a small, densely populated island, we produce more milk and make more cheese than Australia, Canada, or New Zealand. Dairy products account for 15% of household food costs, contribute 22% of food energy and 24% of protein.

The present structure of our dairy industry has evolved over many years and is based mainly on a constant and fairly high liquid consumption, all produced at home, and importation of most of our butter and about half or our cheese. There was no problem with our traditional dairy industry, because it was based on farmhouse practice and produced milk, cream, butter and cheese. There were no wasteful by-products because skim milk and whey were ideal foods for pigs and other farm animals. It is our modern large unit dairy industry which has created the whey problem and produced the skimmed milk powder mountains.

In 1975 the Nuffield Foundation established a Centre for Agricultural Strategy at Reading University. The team under Professor J. C. Bowman has now published *Strategy for the U.K. Dairy Industry*. This stimulating book is packed with statistical information about the industry and, irrespective of

whether one agrees with the authors or not, will be of the greatest interest to all connected with the dairy industry in this country. As might be expected from the background of the authors, the report is mainly concerned with the milk production side of the industry, the composition of milk and the principle of the method of utilization. It is divided into six main sections, milk in the U.K. economy, milk and the national interest, milk and the individual, milk production and processing, the proposed strategy and the implementation of the strategy.

Milk is the most important single product of farms in the U.K. and in spite of our very efficient dairy industry we import much of our butter and cheese, the two most important consumer milk products. After a detailed discussion of what the industry produces in milk and its products, the authors discuss the data in relation to the quantities of the three major milk ingredients which are produced (lactose, fat and protein), their contribution to our consumption of the various dairy products as at present, and how this would be affected by such changes as a reduction in the consumption of liquid milk, partial skimming of retail milk, reduction in butter consumption and changes in cheese consumption. The obvious and outstanding fact of our present milk supply is that we produce more lactose than required but not enough fat. Economically it would be desirable to so modify the composition of our milk that the fat content would be increased and the lactose content reduced, but it would take a long time to achieve any substantial change in this direction.

On this basis the authors suggest a strategy for the amount and type of milk that should be produced and the use that should be made of it. The strategy is based on the concept of self-sufficiency, and the authors point out that for every tonne of butter, 2 tonnes of unwanted lactose and protein are produced. The demand for skimmed milk powder is low and the price correspondingly poor. The authors claim that by changing the composition of milk, all the demand for dairy products in the U.K. could be met by the present gross production of milk. They claim that the proposed strategy is biologically and technologically feasible, would not require any increase in agricultural resources, would not produce any unwanted by-products and would have economic advantages.

Dairy technologists and farmers may be sceptical in respect of some of these claims. The statement that 'the by-products from the manufacture of cheese and butter are unwanted and, if possible, should not be produced', will appear strange to the dairy technologist today. There are countless ways of utilizing milk protein and lactose for human food or other products, and the food and other industries have only just started working seriously on the problem. If millions of pounds can be spent in successfully persuading people to buy things which are of little value, or even harmful, surely money could be spent on persuading them to buy things which are beneficial!

In one sense the proposed strategy has an air of unreality about it. The U.K. dairy industry has settled down over a long period to a system of milk production in the grassland regions of the country with an inevitable seasonal bulge but a constant demand for liquid milk. The industry has spent vast sums in making

provision for this state of affairs by building and equipping manufacturing creameries, which become more economic as milk production increases and the relative proportion of milk allocated to manufacture also increases. The British farmer and housewife are both very conservative in outlook, and this outlook and the structure of the dairy industry will not be altered in a hurry. It may be possible in time to produce a milk much higher in fat and much lower in lactose, but such a milk would cost more to produce. Manufacture is already concentrated in high production areas, and standardization of the fat content would cost money, and homogenization (an inevitable consequence) is also expensive and gives a peculiar taste to milk. If we accept medical and nutritional advice to reduce our consumption of animal fats, the logical thing would be to eat less butter and more soft margarine (i.e. containing a high proportion of polyunsaturated fats). Milk fat remains milk fat in whatever form it is consumed. If butter consumption were halved, our health would be improved according to some medical theories and there would be no need to effect any major change in the composition of our milk supply. However, not all specialists accept this theory and the Royal College of Physicians Report is very guarded in its conclusions. For most people milk fat constitutes only about one third of the total fat intake.

One topic which should be considered in any strategy for British agriculture is that of self sufficiency in food production. The aspect which is of perennial interest in this respect is whether we should produce all or at least as much as possible of our food from plants. It is easy to quote figures showing the greater amounts of food which can be produced in this way, for example two tonne of cereals and up to 20 tonnes of roots per acre compared with the much smaller amounts of animal foods produced. The conversion efficiencies for passing plant foods through animals vary from 5 to 30%, but, apart from energy, the balance of nutrients not utilized to produce animal food is returned to the soil. The dairy cow scores highly in this respect with a conversion efficiency of about 25%.

Agriculturalists and economists tend to classify foods as plant or animal, but the consumer thinks of food in terms of what he likes. Although the British dairy industry may not wax enthusiastic about the proposals of this book, we should all be grateful to Professor Bowman and his colleagues for giving us such a stimulating and provocative work.

J. G. Davis

Lipids as a Source of Flavor. A.C.S. Symposium Series No. 75. Ed. by M. K. Supran.
Washington: American Chemical Society, 1978. Pp. ix + US $17.50.

This volume is a collection of eight papers presented at a symposium held in Chicago in August 1977. It is a pot-pourri of articles ranging from a straightforward experimental report of a synthesis of jasmine-like substances to a wide-ranging contribution on soybean, oil and meal.

It suffers from the disadvantage of all symposium proceedings in that it cannot be used easily as a student text because, though it gives most of the basic theory and equations, they are scattered over two or three chapters.

The chapter on positive and negative food flavour contains several useful tables (some a little confusing) which show the major classes of molecules giving rise to flavour and how the flavour alters with increasing chain length. The chemistry of deep fat fried flavour is covered well in two chapters dealing with corn, soybean and coconut oils, trilinolein, and triolein.

One of the chapters deals with the experimental analysis of volatiles which is valuable but some more recent work with HPLC has widened the horizons of this work.

The article on oxidation of lipids could have been placed first to good effect as it explains clearly the basic mechanisms of hydroperoxide formation and its subsequent decomposition. This chapter also discusses the lipid oxidation in various flesh foods.

The final chapter deals with the microbiological breakdown of lipids from the effect of lipases to the decomposition of individual fatty acids.

This book will find favour with the specialist in the food industry who has to deal with the complicated formation of flavours when fats, amino acids and sugars come into contact under oxidative conditions. Apart from a few minor misprints, the book is adequately presented and at $17.50 is a reasonable buy.

R. J. Hamilton

Progress in Flavour Research. Ed. by D. G. Land & H. E. Nursten. London: Applied Science Publishers Ltd. Pp. xiv + 371. £22.00.

This book comprises the thirty papers presented at the second Weurman Flavour Research Symposium held at the University of East Anglia, England, 2–6 April, 1978. The number of participants at this symposium was restricted (the word is the editors' own) to seventy-five. It is good that the formal papers should have a wider audience.

The layout and presentation of the papers is excellent; each one is set in the same type-face and the proofs appear to have been examined carefully—much to the editors' credit. The standard of the papers is, almost uniformly, very high.

Of particular interest is the thirtieth and last paper in which Professor Nursten reviews progress in flavour research since the first Weurman Symposium in 1975. He uses many of the other papers to chart this progress — perhaps he saw the preprints. The programme was planned to cover recent developments in four major areas: sensory aspects, analytical and instrumental techniques, formation of flavour substances and aspects relating to consumer quality.

The papers are in fact presented in order of those four topics, logically enough, but a little explanation of this arrangement into topics would perhaps

have made the book even more useful to students and others not already familiar with flavour chemistry.

Among the papers covering sensory aspects, J. F. Clapperton describes various techniques used in the sensory characterization of beers. D. G. Laing and H. Panhuber include a fascinating review of recent physiological & anatomical studies of odorant and olfactory cell interaction. Basic physical chemistry is not neglected by D. G. Land, one of the editors, in relating Raoult's Law, and deviations from it, to the concentration of flavour volatiles above foods.

In the section on analytical and instrumental techniques R. Self gives a comprehensive review of developments in mass-spectrometry. A. M. Humphrey describes some very relevant examples of the use of nitrogen specific (NPD) gas-chromatographic detector and he also reminds the reader that gas and thin-layer chromatography are not incompatible techniques. S. Evans and R. Skinner describe negative/positive ion chemical ionization mass-spectrometry, although the relevance to flavour research is not stated.

There are eleven papers on the formation of flavour substances, and three different authors consider in some depth the formation of flavour components by chemical and biosynthetic routes and by micro-organisms. There are then some papers on specific types of food e.g. G. G. Freeman on the growth, storage and processing of vegetables and J. P. Dumont and J. Adda on flavour formation in dairy products. P. A. T. Svoboda and K. E. Peers propose a mechanism for the formation of metallic taints by selective lipid oxidation.

Consumer quality is more difficult to define, particularly if it is to be kept separate from the first group of papers on sensory aspects. This is reflected in the smaller number of papers although those by A. A. Williams on fruit quality and D. N. Rhodes on meat flavour are relevant and interesting. An earlier paper by P. Booth describes the problems to be overcome when the consumers are cats or dogs.

The book ends with an index. This is a good idea but it is not comprehensive and thus could be misleading. For example: octa-1, cis-dien-3-one is listed both under its own name and under dairy products, while 2-nonenal which is mentioned in at least two papers gets no reference.

It has only been possible to mention a few of the thirty papers but that is no criticism of the others. Despite one or two minor qualifications the reviewer thoroughly recommends this well-produced book to all readers who have any interest in flavour chemistry — from student to research worker.

J. T. Davies

Saving of energy in the production of cold.
Paris: International Institute of Refrigeration, 1978. Pp. 267. FFr 50.

'While I would not wish to split hairs', said G. Lorentzen, Past President of the Scientific Council of IIR in his concluding summary of the meeting of Commis-

sion B2 (Refrigerating Machinery) at Delft in September 1978, 'we cannot save energy since, according to the first law of thermodynamics, it does not disappear and is merely changed into other forms. In more concrete terms, we can save fuel'. The papers and discussions of that meeting have been published under three headings, namely, 'Improvement of current Refrigerating Systems' (twelve papers), 'Application of Energy Saving Methods to Refrigeration' (three papers), 'Unorthodox Systems of Refrigeration' (six papers).

Disregarding correct terminology, only about 2% of the World's energy demand is spent on refrigeration including air conditioning. It therefore appears doubtful whether food technologists and biologists will be able to contribute significantly to fuel savings. It is rather the retailer and consumer who will have to be educated to use appliances more economically and to buy fuel saving appliances rather than being tempted by a low purchase price. Unless new ways will be discovered to make potential new sources of energy available, extensive savings of energy are the only answer to the severe shortage which is bound to occur in the nineties.

F. Levy

Books received

The Value of Food. By P. Fisher and A. Bender.
Oxford: Oxford University Press, 1979. Pp. x + 208. £5.00, Paperback £2.25.

This is the third edition of an introductory textbook on nutrition intended for students of home economics, social science, food technology and hotel and institutional management. It has been revised and brought up to date.

The Importance of Vitamins to Human Health. Ed. By T. G. Taylor.
Lancaster: MTP Press, 1979. Pp. xiii + 178. £7.95.

Proceedings of a symposium held in London in 1978.

Report of the Government Chemist 1978.
London: H M S O, 1979. Pp. 170. £4.25.

An interesting review of the work of the Laboratory of the Government Chemist which covers many areas of food science and technology.

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

SI UNITS

gram	g	Joule	J
kilogram	kg = 10^3 g	Newton	N
milligram	mg = 10^{-3} g	Watt	W
metre	m	Centigrade	°C
millimetre	mm = 10^{-3}m	hour	
micrometre	μm = 10^{-6} m	minute	
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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