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Prediction of toxin production by *Clostridium botulinum* in pasteurized pork slurry

T. A. ROBERTS, ANGELA M. GIBSON AND A. ROBINSON*

Summary

A model pork slurry system was used to study factors controlling the growth of *Clostridium botulinum* types A & B (Roberts, Gibson & Robinson, 1981a,b). The following factors were studied in combination: sodium chloride (2.5, 3.5, 4.5% w/v on water); sodium nitrite (100, 200, 300 μg/g); sodium nitrate (0, 500 μg/g); sodium isoascorbate (0, 1000 μg/g); polyphosphate (Curaphos 700, 0, 0.3% w/v); heat treatment (none, 80°C/7 min, 80°C/7 min + 70°C/1 hr); at two pH levels and stored at: 15, 17.5, 20 or 35°C.

Analyses of results yielded a statistical model providing two formulae (for 'low' and 'high' pH slurries) which estimate the probability of toxin production in the pork slurry system within the limits defined above.

Introduction

Clostridium botulinum spores are occasionally present in pork (Roberts & Smart, 1977), and outbreaks of botulism from home-cured pork where levels of curing salts are inadequately controlled occur from time to time (Sébald, 1970; Gonzalez & Gutierrez, 1972; Korsukewitz, Lenk & Schneider, 1977).

The wish to reduce, or prohibit completely, the use of nitrite in cured meats has led to considerable interest in their microbiological safety. In addition to imparting to cured meats the traditional colour (Fiddler *et al.*, 1973; Fujimaki, Emi & Okitani, 1974) and flavour (Cho & Bratzler, 1970; Wasserman & Talley, 1972; Kueper & Trelease, 1974; Mottram & Rhodes, 1974) nitrite is important in controlling microbial growth, particularly that of *Cl. botulinum* (Status Report, 1972; Krol & Tinbergen, 1974; Tinbergen & Krol, 1977; Gray & Randall, 1979; Roberts, Jarvis & Rhodes, 1976).

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Bulman & Ayres (1952) showed that concentrations of curing salts used commercially, when considered individually, did not control growth of a putrefactive anaerobe, but the same levels used in combination were more inhibitory. Interaction of factors in the control of clostridial outgrowth was shown to be significant by Riemann (1963) and Baird-Parker & Freame (1967) and the triple interaction of pH × salt × nitrite in controlling growth of *Cl. botulinum* in a laboratory medium was illustrated semiquantitatively by Roberts & Ingram (1973). Hence, in principle, a similar degree of control of growth of *Cl. botulinum* may be obtained by many different combinations of pH, salt and nitrite. A model pork slurry system devised by Rhodes & Jarvis (1976) was used to study the combined effect of salt, nitrite and storage temperature on the growth of *Cl. botulinum* types A and B, in heated and unheated systems using several levels of inoculum (Roberts, Jarvis & Rhodes, 1976). The effect of a broader range of factors on growth and toxin production by *Cl. botulinum* types A and B have since been reported (Roberts, Gibson & Robinson, 1981a, b) and, using that large data base a model has been constructed which estimates the probability of toxin production by *Cl. botulinum* in the presence of any combination of factors tested in the model system.

Materials and methods

Meat

Experiments were divided into two groups: in the first the pH level of the meat was not controlled, and the final pH level of the slurries ranged from 5.5 to 6.3 (designated 'low' pH) (Roberts *et al.*, 1981a). In the second group of experiments only meat with a pH level of 6.3 or above was used. The final pH level of slurries prepared from this batch of meat was 6.3–6.72 (designated 'high' pH) (Roberts *et al.*, 1981b). Full details of the pork slurry system, the preparation of the inoculum and the treatments are given in Roberts *et al.* (1981a,b). Data were collected and analysed statistically in two groups designated 'low' and 'high' pH.

Experimental plan

The experimental plan is described in full in Roberts *et al.* (1981a,b).

NaCl (% w/v on water)	2.5, 3.5, 4.5
$NaNO_2$ (μg/g slurry)	100, 200, 300
$NaNO_3$ (μg/g slurry)	0, 500
sodium isoascorbate (μg/g slurry)	0, 1000
polyphosphate (Curaphos* 700 % w/v)	0, 0.3
inoculum (spores/bottle)	10, 1000
heat treatment	0, 80°C/7 min, 80°C/7 min + 70°C/1 hr
storage temperature (°C)	15, 17.5, 20, 35
initial pH level of slurry	'low' (5.54–6.36), 'high' (6.3–6.72)

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Results

Toxin tests were carried out as described by Roberts *et al.* (1981a) and an example of the toxin data is presented in Table 1. The full data consists of twenty-five such tables and copies are available from the authors at a nominal handling charge.

Predicting toxin production

Our aim was to assess the probability of toxin production for various combinations of treatments in our model system. The appropriate method for doing this is logistic regression; however, the system was so complex that fitting a logistic regression model *ab initio* is inefficient. A preliminary prediction to identify the most significant factors for subsequent use in a logistic regression was carried out:

(1) *Preliminary prediction based on the analyses of variance published by Roberts* et al. *(1981a,b).* The proportion (p) of samples containing *Cl. botulinum* toxin was calculated to provide a single representative value for each treatment combination. To use these data in subsequent analyses of variance, the angular transformation given by:

$$y = \frac{180}{\pi} \sin^{-1} p^{1/2}$$

was applied to overcome the heterogeneity in the error of the original data (see Roberts *et al.*, 1981a).

The models allowed for interactions of up to three factors and fitted a linear model of the form

$$\begin{aligned} y_{ijk\ldots rst} = {} & \text{mean} + (\text{nitrite})_i + (\text{nitrate})_j + (\text{polyphosphate})_k \\ & \ldots + (\text{salt})_r + (\text{isoascorbate})_s + (\text{heat treatment})_t + \\ & (\text{nitrite/nitrate})_{ij} + \ldots + (\text{isoascorbate/heat treatment})_{st} \\ & + (\text{nitrite/nitrate/polyphosphate})_{ijk} + \ldots \\ & + (\text{salt/isoascorbate/heat treatment})_{rst} + \epsilon_{ijk\ldots rst} \end{aligned}$$

In the above model $y_{ijk\ldots rst}$ represents the response to the combination of the factor levels on the right of the equation where the subscripts indicate the appropriate levels. For example, i may take the values 100, 200, 300, and j may take the values 0 or 500. $y_{ijk\ldots rst}$ is defined by $y_{ijk\ldots rst} = \frac{180}{\pi} \sin^{-1} p^{1/2}_{ijk\ldots rst}$

where $p_{ijk\ldots rst}$ is the observed proportion of toxin positive bottles which were subjected to the treatment combination defined by the subscripts $ijk\ldots rst$.

Values for the parameters μ, (nitrite_i), (nitrate_j), etc. were found by least-squares, referred to as $\hat{\mu}$, $(\hat{\text{nitrite}}_i)$ etc. and the appropriate fitted values, $\hat{y}_{ijk\ldots rst} = \hat{\mu} + \hat{\text{nitrite}}_i + \hat{\text{nitrate}}_j + \ldots$, were calculated. Then the inverse

Table 1. An example of toxin data accumulated after 6 months' storage. Five replicates per treatment combination, slurry heated 80°C/7 min. The entries are the number of replicates containing toxin at a particular spoilage score

Slurry No.	NaCl (% on water)	$NaNO_2$ (μg/g)	Sodium isoascorbate (μg/g)	Polyphosphate (% w/v)	$NaNO_3$ (μg/g)	A	15°C										17.5°C										20°C										35°C									
						B	10^1										10^2										10^1										10^2									
						C	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
77	4.5	100	—	0.3	—			0					0					0					2					1					0	3		1		0			0	1					5
78	4.5	300	1000	—	—			0					0					0					0					0					0	0					0					0				1
79	4.5	300	1000	0.3	—		0	0					1					0					1	1				0	0					1	4				0					0	0			
80	4.5	300	1000	—	—			0					0	0				0	0				0					0					0	0				0					0				0
81	4.5	300	1000	0.3	—		0						0					0					0					0					0					0					0				
82	4.5	300	—	0.3	—		0						0					2					0						3	1			0	0	0								0	2		
83	4.5	300	—	0.3	—		0						0					4					0						4	1			0	0	0				0				0	1		
84	2.5	300	—	0.3	—		2	0				2	1					0	0					1	0			0	0			2					4		0			3				5
85	2.5	100	—	—	—		0					2						0					2								5		0		3		0		4		0			3	
86	2.5	100	1000	0.3	—		0					2	0					0					0	0		1	0	1	0						1	3		0					0			2	
87	2.5	300	—	—	—		0	0				0	0					0					1	2				0	0						2	1		0	0				0	0		2	
88	2.5	300	1000	0.3	—			0					3					0					0		3			0	0							5		0	0		1		0			3	
89	4.5	100	—	—	—			0					0	0				0					0					0	0						1		0			2	0				3	
90	3.5	100	—	0.3	—			0					3					1	1					5				0		4					1	4				5					4	
91	3.5	200	—	0.3	—		0	0				0	2				0	1	2				0	3	1			0	1	2	1				1	4			0		3		0	4	
92	3.5	300	—	0.3	—		0	0				0	0					2	1				0					0		1	2	0			0	3	0	0	0		0	0	0		

A = storage temperature; B = inoculum level; C = spoilage score.

angular transformation $\hat{p}_{ijk\ldots rst} = \sin^2\left(\frac{\pi}{180}\hat{y}_{ijk\ldots rst}\right)$, was applied to produce an estimate of the expected proportion toxin positive at each treatment combination.

The values of the $\hat{p}$'s (expected proportion toxin positive) were not expected to be accurate in any absolute sense, but it was hoped that they might give some indication of the relative degree of protection against toxin production afforded by the different treatment combinations. In particular an idea of the protection against toxin production may be obtained by observing the exponent of $\hat{p}$ (i.e. $-1, -2, -3$ etc.).

Table 2 presents an example of the fitted values for the proportion (p) of toxin positive samples. Lower numbers indicate a lower probability of toxin production, e.g. in Table 2, 2.2–2 ($= 2.2 \times 10^{-2}$) represents a greater likelihood of toxin production than the lower 1.7–4 ($= 1.7 \times 10^{-4}$).

The exponents of the fitted values were related to the actual toxin results to give a simple calibration of the probability of the absence of toxin in any replicate the exponent of which was less than a given value (Table 3).

2. Logistic regression (Plackett, 1974). Having identified the most significant factors a logistic regression was carried out where the model was of the form

$$p_{ijk\ldots rst} = \frac{1}{(1 + e^{-\mu})}$$

where $p_{ijk\ldots rst}$ is the probability of toxin production under the combination of treatments defined by the subscripts $_{ijk\ldots rst}$ as in the preliminary analysis—see (1) above—and μ is an expression similar to the right hand side of the equation in preliminary analysis above, but here some of the individual terms may correspond to regression on a quantitative variable which varies continuously over some range e.g. such a term might be $\beta_{nitrite} \times$ (level of nitrite), which allows us to estimate the probability of toxin production for values intermediate to those used in the experiment. This procedure was not adopted for additives which were tested either 'present' or 'absent' i.e. isoascorbate (0 or 1000 μg/g), polyphosphate (0 or 0.3%), nitrate (0 or 500 μg/g). These parameters were calculated for slurries of 'low' and 'high' initial pH, using the GLIM computer package (NAG Central Office, 7 Banbury Rd., Oxford). The resulting model for meat of 'high' initial pH after LOW or HIGH heat treatment is:

$\mu = 4.679$	
$-(1.47 \times N)$	where $N = NaNO_2$ μg/g $\times 10^2$
$-(1.104 \times S)$	where S = NaCl % w/v on the water
$+(0.1299 \times T)$	where T = storage temperature °C
$-(2.09) + (0.67 \times N)$	if 500 μg/g nitrate added
$-(6.238) + (0.8264 \times S)$	if 1000 μg/g isoascorbate added
$-(1.7049) + (0.3987 \times N)$	if 0.3% polyphosphate* added
$-(1.771) + (0.3997 \times N)$	if heat treatment HIGH
$-(0.01937 \times N \times T)$	
$-(1.2824)$	if nitrate *and* polyphosphate added
$+(0.99)$	if nitrate added *and* heat HIGH

*Curaphos 700
HIGH heat = 80°C/7 min + 70°C/1 hr

Table 2. Fitted values for toxin data (see key) normal pH (5.6–6.0) inoculum ten spores/bottle

Slurry No.	NaCl (% on water)	$NaNO_2$ (μg/g)	Isoascorbate (μg/g)	Polyphosphate (% w/v)	$NaNO_3$ (μg/g)	Unheated				LOW Heat				HIGH Heat			
						15	17.5	20	35	15	17.5	20	35	15	17.5	20	35
3	2.5	100	—	—	—	*2.2–2	1.8–1	5.2–1	7.1–1	4.6–1	7.3–1	9.6–1	9.1–1	1.5–1	4.2–1	6.9–1	7.2–1
5	2.5	100	1000	—	—	8.3–3	7.5–2	1.2–1	1.2–1	3.0–1	3.7–1	5.3–1	2.5–1	2.0–1	2.4–1	4.3–1	1.5–1
6	2.5	100	—	0.3	—	5.0–1	9.8–1	1.0–1	9.3–1	7.7–1	1.0–1	9.5–1	9.7–1	1.6–1	4.7–1	5.9–1	8.1–1
4	2.5	100	1000	0.3	—	4.4–1	9.6–1	7.4–1	9.1–1	5.3–1	8.3–1	7.9–1	7.0–1	1.8–1	3.4–1	2.4–1	2.8–1
11	2.5	200	—	—	—	2.8–2	1.5–2	2.8–2	3.2–1	4.3–2	7.5–2	2.5–1	1.9–1	3.6–4	2.4–2	1.2–1	2.1–1
15	2.5	200	1000	—	—	1.6–2	2.0–2	1.4–2	9.8–2	1.3–3	2.0–3	2.0–3	4.9–4	8.2–3	8.7–4	2.9–2	2.1–2
13	2.5	200	—	0.3	—	2.0–2	2.9–2	1.4–1	4.8–1	1.1–1	2.0–1	5.4–1	3.8–1	2.4–2	3.9–3	1.9–2	1.1–1
16	2.5	200	1000	0.3	—	1.4–2	5.0–2	9.7–6	2.7–1	2.8–3	1.5–2	2.8–2	2.5–2	1.6–2	2.1–2	2.4–2	1.7–3
25	3.5	100	—	—	—	8.4–2	1.2–1	3.2–1	1.4–1	6.3–2	2.5–1	3.1–1	2.0–1	4.0–2	1.0–1	1.5–1	9.8–2
27	3.5	100	1000	—	—	1.6–6	4.2–3	1.8–2	1.3–2	7.5–4	7.5–3	4.5–2	2.5–3	2.5–2	2.7–2	6.5–2	1.0–5
26	3.5	100	—	0.3	—	3.1–1	7.7–1	8.5–1	5.3–1	2.7–2	2.4–1	4.8–1	1.8–1	5.6–4	3.7–2	4.7–2	1.3–2
28	3.5	100	1000	0.3	—	2.1–1	7.4–1	5.3–1	2.9–1	8.4–4	1.4–1	1.6–1	3.9–2	7.3–3	6.6–2	2.5–2	4.7–4
29	3.5	200	—	—	—	4.6–3	1.8–3	2.6–2	2.2–2	6.2–3	3.0–2	4.6–2	6.9–3	1.0–3	2.1–2	7.9–3	4.0–3
30	3.5	200	1000	—	—	1.9–2	1.3–2	2.1–2	1.5–4	2.4–2	1.1–2	9.4–3	4.2–3	2.1–8	4.5–4	1.2–3	5.0–4
31	3.5	200	—	0.3	—	7.9–4	9.6–2	2.0–1	2.9–2	3.0–3	6.6–2	2.5–1	4.0–3	1.0–2	1.7–4	5.6–5	1.3–2
32	3.5	200	1000	0.3	—	1.7–4	1.6–1	6.0–2	7.3–2	1.1–2	2.1–2	4.5–2	4.6–3	1.2–5	1.2–2	7.1–3	3.4–3
52	4.5	100	—	—	—	3.5–3	2.2–3	2.8–2	1.0–2	2.1–5	3.8–4	5.9–3	1.7–1	3.7–3	2.0–2	4.4–3	1.3–1
73	4.5	100	1000	—	—	5.3–3	1.7–3	8.9–6	6.7–2	1.8–2	7.1–3	5.6–3	5.1–2	1.6–2	9.1–3	3.2–3	2.7–4
74	4.5	100	—	0.3	—	5.5–2	3.0–1	2.9–1	1.6–1	2.0–4	2.4–2	3.4–2	1.5–1	2.5–4	1.3–2	8.2–3	5.7–2
72	4.5	100	1000	0.3	—	2.2–2	3.3–1	6.3–2	3.1–3	3.0–4	2.2–2	2.5–3	1.3–2	3.2–4	3.1–3	7.4–2	1.9–2
53	4.5	200	—	—	—	3.2–3	1.7–3	4.6–2	4.6–3	6.5–4	6.9–3	8.6–6	3.1–3	6.8–3	1.8–2	1.0–2	2.9–2
57	4.5	200	1000	—	—	3.2–3	1.0–3	1.7–2	1.3–4	2.6–4	3.5–3	3.6–4	1.6–3	6.7–3	4.4–3	9.5–3	2.7–4
58	4.5	200	—	0.3	—	3.6–4	2.5–2	1.2–1	3.9–4	3.1–3	1.0–3	6.0–2	9.7–4	1.9–2	1.3–2	1.2–2	5.0–3
56	4.5	200	1000	0.3	—	4.8–3	5.3–2	1.2–2	1.2–2	6.1–3	9.9–4	3.0–4	6.8–3	4.2–4	2.9–4	6.6–3	1.5–2

Table 3. Estimation of probability (p) of *no* toxin in any replicate sample from 'Fitted values' data

(a) Group 1 slurries ('low pH)				x			
10 spores/bottle	*−1	−2	−3	−4	−5	−6	−7
p (see footnote)	0.64	0.85	0.92	0.96	0.95	1.0	1.0
(b) Group 1 slurries ('low' pH)				x			
1000 spores/bottle	*−1	−2	−3	−4	−5	−6	−7
p	0.375	0.8	0.92	0.96	1.0	1.0	1.0
(c) Group 2 slurries ('high' pH)				x			
10 spores/bottle	*−1	−2	−3	−4	−5	−6	−7
p	0.7	0.96	0.96	0.98	1.0	1.0	1.0

*x is the negative power given in Table 2, i.e. $-1 \equiv 1.0 \times 10^{-1} - 9.9 \times 10^{-1}$.
p = Probability that an observation whose exponent (fitted value) is ≤ to x contains *no* toxin in any of its samples.

For meat of initial 'low' pH without heating or after LOW or HIGH heat treatment the model is:

$\mu = 5.750$

$-(3.25 \times N)$	where $N = NaNO_2\ \mu g/g \times 10^2$
$+(0.04834 \times T)$	where T = storage temperature °C
$-(1.854 \times S)$	where S = NaCl % w/v on the water
$+(1.846)$	if inoculum 1000 spores/bottle
$-(4.074) + (1.222 \times N) + (0.4861 \times S)$	if nitrate added
$-(2.23) + (0.3617 \times N) + (0.3174 \times S)$	if isoascorbate added
$+(2.577) - (0.3615 \times S)$	if polyphosphate* added
$+(0.4233 \times N \times S)$	
$+(2.154) - (0.4510 \times T) - (0.2555 \times S)$	if heat treatment LOW
$-(0.01424) - (0.0214 \times T) + (0.1256 \times S)$	if heat treatment HIGH
$-(0.532)$	if heat LOW and isoascorbate added
$+(0.05193)$	if heat HIGH and isoascorbate added
$+(0.4618)$	if heat LOW and polyphosphate added
$-(1.548)$	if heat HIGH and polyphosphate added
$+(1.025)$	if heat LOW and nitrate added
$-(1.048)$	if heat HIGH and nitrate added

*Curaphos 700
LOW heat = 80°C/7 min
HIGH heat = 80°C/7 min + 70°C/1 hr

Key for Tables 4–12

(1) Inoculum consists of a mixed suspension of equal numbers of spores of five strains of *Cl. botulinum* type A and type B.

(2) Slurries prepared from pork of 'low' pH (range 5.34–6.36) were mixed with water (ratio meat:water 1:1.5). Those prepared from pork of 'high' pH (range 6.3–6.72) were mixed in the ratio meat:water 1:1.8.

(3) The polyphosphate used in the slurries was Curaphos 700 (Fibrisol Service Ltd., Colville Road, London W3 8TE).

(4) Heat treatments: unheated
LOW heat = heated in water at 80°C for 7 min which raised the centre temperature to 70°C
HIGH heat = heated at 80°C for 7 min plus 70°C for 1 hr.

(5) + = present at stated level
− = absent

The percentage probabilities are rounded to the nearest whole number.

Table 4. Probability (%) of toxin production by *Clostridium botulinum* types A and B[1], in pork slurry[2], 'low' pH meat, ten spores/bottle

NaCl (% on water)	$NaNO_2$ (μg/g)	Polyphosphate (0.3% w/v[3])	Sodium isoascorbate (1000 μg/g)	$NaNO_3$ (μg/g)	Unheated[4] Storage temperature (°C)				LOW heat[4] Storage temperature (°C)				HIGH heat[4] Storage temperature (°C)			
					15	17.5	20	35	15	17.5	20	35	15	17.5	20	35
2.5	100	−	−	−[5]	41	44	47	65	62	62	62	63	41	42	44	54
2.5	100	−	−	+[5]	12	13	15	26	47	47	47	49	4	5	5	7
2.5	100	−	+	−	19	21	23	39	25	25	25	26	20	21	22	30
2.5	100	−	+	+	4	5	6	11	15	15	15	16	2	2	2	3
2.5	100	+	−	−	79	81	83	91	85	85	85	85	44	46	47	57
2.5	100	+	−	+	42	45	48	66	75	75	75	76	5	5	6	8
2.5	100	+	+	−	56	59	62	77	52	52	53	54	22	23	24	32
2.5	100	+	+	+	20	22	24	40	37	37	38	39	2	2	2	3
2.5	200	−	−	−	7	8	9	17	15	15	16	16	7	8	8	12
2.5	200	−	−	+	5	6	6	12	25	25	25	26	2	2	2	3
2.5	200	−	+	−	4	4	5	9	5	5	5	5	4	4	4	6
2.5	200	−	+	+	2	3	3	6	9	9	9	9	1	1	1	2
2.5	200	+	−	−	30	32	35	52	38	38	38	39	8	9	9	13
2.5	200	+	−	+	22	24	26	42	53	53	53	55	2	2	2	3
2.5	200	+	+	−	17	19	21	35	15	15	15	16	4	5	5	7
2.5	200	+	+	+	12	13	15	26	24	25	25	26	1	1	1	2
2.5	300	−	−	−	1	1	1	2	2	2	2	2	1	1	1	1
2.5	300	−	−	+	2	2	2	5	11	11	11	12	1	1	1	1
2.5	300	−	+	−	1	1	1	2	1	1	1	1	1	1	1	1
2.5	300	−	+	+	1	2	2	4	5	5	5	5	0	1	1	1
2.5	300	+	−	−	4	5	6	11	6	6	6	7	1	1	1	2
2.5	300	+	−	+	9	11	12	22	30	30	30	31	1	1	1	1
2.5	300	+	+	−	3	4	4	8	3	3	3	3	1	1	1	1
2.5	300	+	+	+	7	8	9	16	15	15	15	16	1	1	1	1

After the terms in these models have been estimated, the predicted or fitted values of the p's are calculated. These values, being estimates of probabilities, are more easily interpreted than the exponents of $\hat{p}$ above, but note that extrapolation outside the limits of the experiment is not recommended as the estimated probabilities are subject to considerable errors.

Tables 4–12 show the calculated probabilities of toxin production (expressed as a percentage) for every combination and level of salt, nitrite etc. tested: values being rounded to the nearest whole number.

Table 5. Probability (%) of toxin production by *Clostridium botulinum* types A and B[1], in pork slurry[2], 'low' pH meat, ten spores/bottle

NaCl (% on water)	$NaNO_2$ (μg/g)	Polyphosphate (0.3% w/v[3])	Sodium isoascorbate (1000 μg/g)	$NaNO_3$ (μg/g)	Unheated[4]				LOW heat[4]				HIGH heat[4]			
					Storage temperature (°C)				Storage temperature (°C)				Storage temperature (°C)			
					15	17.5	20	35	15	17.5	20	35	15	17.5	20	35
3.5	100	–	–	–[5]	14	16	18	31	23	23	23	24	16	17	18	24
3.5	100	–	–	+[5]	5	6	6	12	21	21	21	22	2	2	2	3
3.5	100	–	+	–	7	8	9	17	8	8	8	8	8	9	10	14
3.5	100	–	+	+	2	3	3	6	7	7	7	7	1	1	1	2
3.5	100	+	–	–	38	41	44	62	41	42	42	43	13	14	14	20
3.5	100	+	–	+	17	18	20	34	38	39	39	40	2	2	2	3
3.5	100	+	+	–	23	25	27	44	16	16	16	17	7	7	8	11
3.5	100	+	+	+	8	9	11	20	15	15	15	15	1	1	1	1
3.5	200	–	–	–	3	3	4	7	5	5	5	5	3	3	4	5
3.5	200	–	–	+	3	3	4	7	13	13	14	14	1	1	1	2
3.5	200	–	+	–	2	2	2	5	2	2	2	2	2	2	3	4
3.5	200	–	+	+	2	2	3	5	6	6	6	6	1	1	1	1
3.5	200	+	–	–	10	11	12	22	11	11	11	11	2	3	3	4
3.5	200	+	–	+	10	11	13	23	26	27	27	28	1	1	1	2
3.5	200	+	+	–	7	7	8	16	5	5	5	5	2	2	2	3
3.5	200	+	+	+	7	8	9	17	12	13	13	13	1	1	1	1
3.5	300	–	–	–	0	1	1	1	1	1	1	1	1	1	1	1
3.5	300	–	–	+	2	2	2	4	8	8	8	9	1	1	1	1
3.5	300	–	+	–	0	1	1	1	0	0	1	1	1	1	1	1
3.5	300	–	+	+	2	2	2	4	5	5	5	5	1	1	1	1
3.5	300	+	–	–	2	2	2	5	2	2	2	2	0	0	0	1
3.5	300	+	–	+	6	7	8	15	17	17	17	18	1	1	1	1
3.5	300	+	+	–	2	2	2	4	1	1	1	1	0	0	0	1
3.5	300	+	+	+	6	7	8	14	11	11	11	11	1	1	1	1

In most cases the fitted (calculated) values mimic closely the actual experimental data. By way of example in Fig. 1 fitted values are plotted against observed values for the 'high' pH slurries, which were inoculated with ten spores per bottle. In that figure 50% of the data for each observed value lies within the box, half above and half below the line dividing the box. If a line is taken by eye through the boxes, the slope shows that the general relationship between fitted and observed values (i.e. experimental results) is reasonable. However, some lack of fit is inevitable in such a system, particularly in experiments over such a long period and using different batches of pork, where the only common factor was the pH value. The extent of the extremes of lack of fit is illustrated in Fig. 1 by the lines projecting beyond the boxes to the point of the worst agreement between fitted and observed values. Such variation is not uncommon, even in

Table 6. Probability (%) of toxin production by *Clostridium botulinum* types A and B[1], in pork slurry[2], 'low' pH meat, ten spores/bottle

NaCl (% on water)	$NaNO_2$ (μg/g)	Polyphosphate (0.3% w/v[3])	Sodium isoascorbate (1000 μg/g)	$NaNO_3$ (μg/g)	Unheated[4] Storage temperature (°C)				LOW heat[4] Storage temperature (°C)				HIGH heat[4] Storage temperature (°C)			
					15	17.5	20	35	15	17.5	20	35	15	17.5	20	35
4.5	100	−	−	−	4	4	5	10	5	5	5	6	5	5	5	8
4.5	100	−	−	+	2	2	3	5	7	7	8	8	1	1	1	2
4.5	100	−	+	−	3	3	3	6	2	2	2	2	3	4	4	6
4.5	100	−	+	+	1	1	2	3	3	3	3	3	1	1	1	1
4.5	100	+	−	−	9	11	12	21	8	8	8	9	3	3	3	5
4.5	100	+	−	+	5	6	6	12	12	12	12	12	0	1	1	1
4.5	100	+	+	−	6	7	8	15	3	3	3	4	2	2	2	3
4.5	100	+	+	+	3	4	4	8	5	5	5	5	0	0	0	1
4.5	200	−	−	−	1	1	1	3	1	1	1	2	1	1	1	2
4.5	200	−	−	+	2	2	2	5	7	7	7	7	1	1	1	1
4.5	200	−	+	−	1	1	1	2	1	1	1	1	1	1	1	2
4.5	200	−	+	+	2	2	2	4	4	4	4	4	1	1	1	1
4.5	200	+	−	−	3	3	3	7	2	2	2	2	1	1	1	1
4.5	200	+	−	+	5	5	6	11	10	10	10	11	0	0	1	1
4.5	200	+	+	−	2	3	3	6	1	1	1	1	1	1	1	1
4.5	200	+	+	+	4	5	5	10	6	6	6	6	0	0	0	1
4.5	300	−	−	−	0	0	0	1	0	0	0	0	0	0	0	1
4.5	300	−	−	+	2	2	2	4	6	6	6	6	1	1	1	1
4.5	300	−	+	−	0	0	0	1	0	0	0	0	0	1	1	1
4.5	300	−	+	+	2	2	3	5	5	5	5	5	1	1	1	2
4.5	300	+	−	−	1	1	1	2	1	1	1	1	0	0	0	0
4.5	300	+	−	+	4	5	5	10	9	9	9	10	0	0	0	1
4.5	300	+	+	−	1	1	1	2	0	0	0	1	0	0	0	0
4.5	300	+	+	+	5	6	7	13	7	7	7	8	1	1	1	1

Table 7. Probability (%) of toxin production by *Clostridium botulinum* types A and B[1], in pork slurry[2], 'low' pH meat, 1000 spores/bottle

NaCl (% on water)	$NaNO_2$ (μg/g)	Polyphosphate (0.3% w/v[3])	Sodium isoascorbate (1000 μg/g)	$NaNO_3$ (μg/g)	Unheated[4] Storage temperature (°C)				LOW heat[4] Storage temperature (°C)				HIGH heat[4] Storage temperature (°C)			
					15	17.5	20	35	15	17.5	20	35	15	17.5	20	35
2.5	100	–	–	–[5]	82	83	85	92	91	91	91	92	81	82	83	88
2.5	100	–	–	+[5]	46	49	52	70	85	85	85	86	23	24	25	34
2.5	100	–	+	–	60	63	66	80	67	68	68	69	61	63	64	73
2.5	100	–	+	+	23	25	27	44	53	53	53	54	10	10	11	16
2.5	100	+	–	–	96	96	97	98	97	97	97	97	83	84	85	89
2.5	100	+	–	+	82	84	85	92	95	95	95	95	25	27	28	37
2.5	100	+	+	–	89	90	91	96	87	87	88	88	64	66	67	75
2.5	100	+	+	+	61	64	67	81	79	79	79	80	11	11	12	17
2.5	200	–	–	–	33	36	39	57	54	54	54	55	33	34	36	46
2.5	200	–	–	+	25	27	30	46	68	68	68	69	10	11	11	16
2.5	200	–	+	–	20	22	24	39	25	25	25	26	20	21	22	30
2.5	200	–	+	+	14	15	17	30	38	38	38	39	6	6	6	9
2.5	200	+	–	–	73	75	77	87	79	80	80	80	36	37	39	49
2.5	200	+	–	+	64	66	69	82	88	88	88	88	11	12	13	18
2.5	200	+	+	–	57	59	62	77	53	53	53	54	22	23	25	33
2.5	200	+	+	+	46	49	52	69	67	67	68	69	6	7	7	10
2.5	300	–	–	–	5	6	7	13	11	11	12	12	5	6	6	9
2.5	300	–	–	+	11	12	14	25	45	45	45	46	4	4	5	7
2.5	300	–	+	–	4	4	5	9	5	5	5	5	4	4	4	6
2.5	300	–	+	+	8	9	10	19	25	25	25	26	3	3	4	5
2.5	300	+	–	–	23	25	27	44	30	30	31	32	6	6	7	10
2.5	300	+	–	+	40	43	46	64	73	73	73	74	5	5	5	8
2.5	300	+	+	–	17	19	21	35	15	15	15	16	4	5	5	7
2.5	300	+	+	+	32	35	37	55	53	53	53	54	3	4	4	6

Table 8. Probability (%) of toxin production by *Clostridium botulinum* types A and B[1], in pork slurry[2], 'low' pH meat, 1000 spores/bottle

NaCl (% on water)	$NaNO_2$ (μg/g)	Polyphosphate (0.3% w/v[3])	Sodium isoascorbate (1000 μg/g)	$NaNO_3$ (μg/g)	Unheated[4]				LOW heat[4]				HIGH heat[4]			
					Storage temperature (°C)				Storage temperature (°C)				Storage temperature (°C)			
					15	17.5	20	35	15	17.5	20	35	15	17.5	20	35
3.5	100	−	−	−[5]	52	55	58	74	66	66	66	67	54	56	58	67
3.5	100	−	−	+[5]	25	28	30	47	63	63	63	64	12	12	13	18
3.5	100	−	+	−	33	36	39	57	34	35	35	36	37	35	40	50
3.5	100	−	+	+	14	15	17	29	32	32	32	33	6	6	7	10
3.5	100	+	−	−	80	82	83	91	82	82	82	83	48	50	52	62
3.5	100	+	−	+	56	59	61	77	80	80	80	81	9	10	11	15
3.5	100	+	+	−	65	68	70	83	55	55	56	57	32	33	35	44
3.5	100	+	+	+	37	40	43	61	52	52	52	54	5	5	6	8
3.5	200	−	−	−	15	17	19	32	25	25	25	26	17	18	19	26
3.5	200	−	−	+	16	18	29	34	49	50	50	51	7	8	8	12
3.5	200	−	+	−	11	12	13	24	11	11	12	12	13	13	14	20
3.5	200	−	+	+	12	13	14	26	28	28	28	29	5	5	6	8
3.5	200	+	−	−	40	43	46	64	43	43	44	45	14	15	14	21
3.5	200	+	−	+	42	45	48	66	70	70	70	71	6	6	6	9
3.5	200	+	+	−	31	34	37	54	23	23	23	24	10	11	11	16
3.5	200	+	+	+	33	36	38	56	47	48	48	49	4	4	5	7
3.5	300	−	−	−	3	3	4	8	5	5	5	6	3	4	4	6
3.5	300	−	−	+	10	11	13	23	36	36	36	38	4	4	5	7
3.5	300	−	+	−	3	3	4	7	3	3	3	3	3	4	4	6
3.5	300	−	+	+	10	11	12	22	24	24	25	25	4	5	5	7
3.5	300	+	−	−	10	12	13	23	12	12	12	12	3	3	3	4
3.5	300	+	−	+	30	32	35	52	57	57	57	58	3	4	4	6
3.5	300	+	+	−	10	11	12	23	7	7	7	7	3	3	3	5
3.5	300	+	+	+	29	31	34	52	43	43	43	44	3	4	4	6

Table 9. Probability (%) of toxin production by *Clostridium botulinum* types A and B[1], in pork slurry[2], 'low' pH meat, 1000 spores/bottle

NaCl (% on water)	$NaNO_2$ (μg/g)	Polyphosphate (0.3% w/v[3])	Sodium isoascorbate (1000 μg/g)	$NaNO_3$ (μg/g)	Unheated[4]				LOW heat[4]				HIGH heat[4]			
					Storage temperature (°C)				Storage temperature (°C)				Storage temperature (°C)			
					15	17.5	20	35	15	17.5	20	35	15	17.5	20	35
4.5	100	−	−	−[5]	20	22	25	40	24	26	26	27	24	26	27	35
4.5	100	−	−	+[5]	12	13	14	26	34	34	34	35	5	6	6	9
4.5	100	−	+	−	14	16	17	30	12	12	12	12	18	19	20	27
4.5	100	−	+	+	8	9	10	18	16	16	16	17	4	4	4	6
4.5	100	+	−	−	40	43	46	63	37	37	37	38	15	16	17	23
4.5	100	+	−	−	25	28	30	47	45	45	46	47	3	3	4	5
4.5	100	+	+	−	30	32	35	53	18	18	18	19	11	11	12	17
4.5	100	+	+	+	18	20	22	36	24	24	24	25	2	2	2	4
4.5	200	−	−	−	6	7	8	15	8	8	9	9	8	8	9	13
4.5	200	−	−	−	10	12	13	23	31	31	31	32	5	5	6	8
4.5	200	−	+	−	6	6	7	14	5	5	5	5	8	8	8	12
4.5	200	−	−	−	10	11	12	22	20	20	20	21	5	5	5	8
4.5	200	+	−	−	15	16	18	31	13	13	13	14	4	5	5	7
4.5	200	+	−	−	23	25	28	44	42	42	43	44	3	3	3	5
4.5	200	+	+	−	14	15	17	29	8	8	8	8	4	5	5	7
4.5	200	+	+	+	22	24	26	42	28	29	29	30	3	3	3	4
4.5	300	−	−	−	2	2	2	4	2	2	2	2	2	2	2	4
4.5	300	−	−	+	9	10	12	21	28	29	29	30	4	5	5	7
4.5	300	−	+	−	2	3	3	6	2	2	2	2	3	3	3	5
4.5	300	−	+	+	12	13	15	26	24	24	24	25	6	6	7	10
4.5	300	+	−	−	4	5	5	11	4	4	4	4	1	1	1	2
4.5	300	+	−	+	21	23	25	41	39	39	40	41	2	3	3	4
4.5	300	+	+	−	6	6	7	14	3	3	3	3	2	2	2	3
4.5	300	+	+	+	26	28	31	48	34	34	34	35	3	4	4	6

Table 10. Probability (%) of toxin production by *Clostridium botulinum* types A and B[1], in pork slurry[2], 'high' pH meat, ten spores/bottle

NaCl (% on water)	$NaNO_2$ (μg/g)	Polyphosphate (0.3% w/v[3])	Sodium isoascorbate (1000 μmg/g)	$NaNO_3$ (μg/g)	LOW heat[4] Storage temperature (°C)				HIGH heat[4] Storage temperature (°C)			
					15	17.5	20	35	15	17.5	20	35
2.5	100	−	−	−[5]	89	92	93	99	68	73	78	95
2.5	100	−	−	+[5]	67	72	78	95	58	64	70	93
2.5	100	−	+	−	11	14	18	54	3	4	5	23
2.5	100	−	+	+	3	4	5	22	2	3	4	16
2.5	100	+	−	−	69	75	79	95	36	43	50	84
2.5	100	+	−	+	13	16	21	58	9	12	15	48
2.5	100	+	+	−	3	4	6	24	1	1	1	7
2.5	100	+	+	+	0	0	0	2	0	0	0	1
2.5	200	−	−	−	59	64	69	90	35	40	46	77
2.5	200	−	−	+	40	46	51	81	41	46	52	81
2.5	200	−	+	−	2	3	3	12	1	1	1	5
2.5	200	−	+	+	1	1	2	6	1	1	2	6
2.5	200	+	−	−	36	42	47	78	18	21	25	57
2.5	200	+	−	+	7	9	11	32	7	9	11	32
2.5	200	+	+	−	1	1	1	5	0	0	1	2
2.5	200	+	+	+	0	0	0	1	0	0	0	1
2.5	300	−	−	−	20	23	26	51	12	14	16	37
2.5	300	−	−	+	18	21	25	49	26	29	33	59
2.5	300	−	+	−	0	0	1	2	0	0	0	1
2.5	300	−	+	+	0	0	0	1	1	1	1	2
2.5	300	+	−	−	13	15	17	38	8	9	11	26
2.5	300	+	−	+	4	4	5	14	5	6	8	19
2.5	300	+	+	−	0	0	0	1	0	0	0	1
2.5	300	+	+	+	0	0	0	0	0	0	0	0

Table 11. Probability (%) of toxin production by *Clostridium botulinum* types A and B[1], in pork slurry[2], 'high' pH meat, ten spores/bottle

NaCl (% on water)	$NaNO_2$ (μg/g)	Polyphosphate (0.3% w/v[3])	Sodium isoascorbate (1000 μmg/g)	$NaNO_3$ (μg/g)	LOW heat[4] Storage temperature (°C)				HIGH heat[4] Storage temperature (°C)			
					15	17.5	20	35	15	17.5	20	35
3.5	100	−	−	−[5]	73	78	83	96	41	48	55	86
3.5	100	−	−	+[5]	40	47	53	86	31	37	44	80
3.5	100	−	+	−	9	11	14	47	2	3	4	18
3.5	100	−	+	+	2	3	4	18	2	2	3	13
3.5	100	+	−	−	42	49	56	87	16	20	25	63
3.5	100	+	−	+	5	6	8	31	3	4	6	24
3.5	100	+	+	−	3	3	4	19	1	1	1	6
3.5	100	+	+	+	0	0	0	2	0	0	0	1
3.5	200	−	−	−	32	37	43	74	15	18	22	52
3.5	200	−	−	+	18	22	26	58	19	22	26	58
3.5	200	−	+	−	2	2	3	9	1	1	1	4
3.5	200	−	+	+	1	1	1	5	1	1	1	5
3.5	200	+	−	−	16	19	23	54	7	8	10	31
3.5	200	+	−	+	2	3	4	13	2	3	4	14
3.5	200	+	+	−	1	1	1	4	0	0	0	2
3.5	200	+	+	+	0	0	0	1	0	0	0	1
3.5	300	−	−	−	7	9	10	25	4	5	6	16
3.5	300	−	−	+	7	8	10	24	10	12	14	32
3.5	300	−	+	−	0	0	0	1	0	0	0	1
3.5	300	−	+	+	0	0	0	1	0	0	1	2
3.5	300	+	−	−	5	5	6	17	3	3	4	10
3.5	300	+	−	+	1	1	2	5	2	2	3	7
3.5	300	+	+	−	0	0	0	1	0	0	0	0
3.5	300	+	+	+	0	0	0	0	0	0	0	0

Table 12. Probability (%) of toxin production by *Clostridium botulinum* types A and B[1], in pork slurry[2], 'high' pH meat, ten spores/bottle

NaCl (% on water)	$NaNO_2$ (μg/g)	Polyphosphate (0.3% w/v[3])	Sodium isoascorbate (1000 μng/g)	$NaNO_3$ (μg/g)	LOW heat[4] Storage temperature (°C)				HIGH heat[4] Storage temperature (°C)			
					15	17.5	20	35	15	17.5	20	35
4.5	100	−	−	−[5]	47	54	61	89	19	23	29	68
4.5	100	−	−	+[5]	18	22	28	67	13	16	21	58
4.5	100	−	+	−	7	9	11	40	2	2	3	14
4.5	100	−	+	+	2	2	3	14	1	2	2	10
4.5	100	+	−	−	20	24	30	69	6	8	10	36
4.5	100	+	−	+	2	2	3	13	1	1	2	9
4.5	100	+	+	−	2	3	3	15	0	1	1	4
4.5	100	+	+	+	0	0	0	1	0	0	0	1
4.5	200	−	−	−	13	16	20	49	6	7	8	27
4.5	200	−	−	+	7	8	10	31	7	9	11	32
4.5	200	−	+	−	1	2	2	7	0	1	1	3
4.5	200	−	+	+	1	1	1	4	1	1	1	4
4.5	200	+	−	−	6	7	9	28	2	3	4	13
4.5	200	+	−	+	1	1	1	5	1	1	1	5
4.5	200	+	+	−	1	1	1	3	0	0	0	1
4.5	200	+	+	+	0	0	0	0	0	0	0	0
4.5	300	−	−	−	3	3	4	10	1	2	2	6
4.5	300	−	−	+	2	3	3	9	4	4	5	14
4.5	300	−	+	−	0	0	0	1	0	0	0	1
4.5	300	−	+	+	0	0	0	1	0	0	0	1
4.5	300	+	−	−	2	2	2	6	1	1	1	4
4.5	300	+	−	+	0	0	1	2	1	1	1	3
4.5	300	+	+	−	0	0	0	1	0	0	0	0
4.5	300	+	+	+	0	0	0	0	0	0	0	0

smaller experiments (Tompkin, Christiansen & Shaparis, 1977; Rhodes & Jarvis, 1976). In our very large experiment (Roberts *et al.*, 1981a) substitution of results from slurries repeated several months later in the overall analysis of variance did not change the main conclusions, although a few minor differences in the two- and three-factor interactions were evident. The reasons for this batch to batch variation of pork are not known and have yet to be associated with a particular factor e.g. breed of pig.

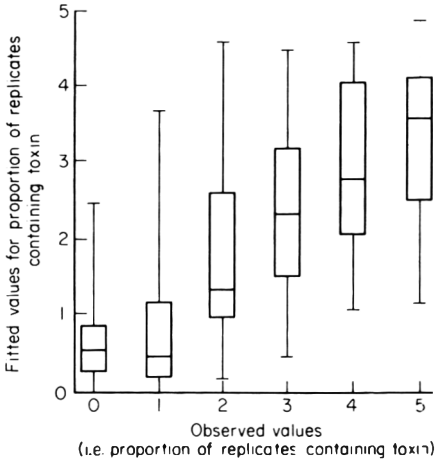

Figure 1. Box plot of fitted values for each observed value for number of replicates (five replicates per treatment combination tested) containing toxin. 'High' pH slurries inoculated with ten spores/bottle. For each observed value (0.1.2.3.4.5) the middle 50% of data lie within the box, half above and half below the line. The vertical lines extend to the most extreme fitted values obtained for each observed proportion of samples toxic.

Discussion

Given any combination of chemical additives within the range tested it is possible from the above model to predict the probability of toxin production by *Cl. botulinum* types A and B in the pork slurry system at three heat treatments and four storage temperatures with an inoculum of ten or 1000 spores/bottle.

By way of example considering only sodium chloride and sodium nitrite, in a slurry containing 2.5% NaCl and subjected to HIGH heat treatment (Table 4) the probability of toxin production can be reduced from *ca* 41–54% if 100 μg/g $NaNO_2$ is initially present to 7–12% by increasing the initial nitrite concentration to 200 μg/g and to *ca* 1% by increasing it to 300 μg/g. If such an increase in nitrite is deemed undesirable, a reduction in the probability of toxin production of the same order can be achieved by increasing the salt level to 4.5% (Table 5). The consequences of manipulating the other additives and conditions tested can be evaluated similarly. Although these probabilities cannot be applied directly to commercial products such as bacon and ham, the relative effect of changes in

concentration of various additives on growth of *Cl. botulinum* may be assessed in terms of increased or decreased safety of the processed product.

The many different product formulations are unlikely to support bacteriological growth identically but it is much less time-consuming and less costly to test whether the response of different products is of the same order as that predicted in the model slurry system than to test each product separately.

Wider use of such a slurry system would enable the effect on growth of *Cl. botulinum* of any new chemical additive, e.g. a substitute for nitrite, to be evaluated. The mathematical model should only be used to assess relative effects of treatment combinations. Extrapolation beyond the limits of the experiments will result in loss of precision. It should not be used to assess which combinations give a 'guaranteed risk' of toxin production, since minor changes in product formulation or its production, or in experimental conditions might significantly alter the ability to support toxin production and the variability of the system.

Acknowledgments

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Assessment of shelf life of fresh water prawns stored at 0°C*

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Summary

The shelf life of fresh water prawns stored at 0°C was judged by a sensory panel and monitored by microbiological and chemical tests. After 14 days' storage, but not after only 10 days, frozen (control sample) prawns were preferred over the former. During this period too, TVBN values of 0°C-stored prawn tails rose from 22 to 31 mg per 100 g, and TBA values rose from 0.25 to 0.65; the total aerobic psychrotrophic plate counts increased by about 2 logarithmic units. The relationship between the results of the various tests is discussed.

Introduction

The giant fresh water prawn (*Macrobrachium rosenbergii*) comes from the Indo-Pacific region. Males reach about 25 cm in length and females 15 cm, both sexes fetching high prices as compared with other seafood (Bardach, Ryther & Melarney, 1972).

The culture of these prawns is largely in the experimental stages in the region mentioned above; yields of from 3 to 4 tons per hectare have been reported from pilot plots. There is very little literature on the technology of post-harvest handling of these crustaceans.

The pilot plant production of these prawns for export was recently instituted in Israel, and trials are in progress regarding methods of feeding, collecting, cleaning and packing, *etc.*

The main objective of the experiments now reported, was to assess the changes in sensory quality with storage at 0°C. Microbiological changes,

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rancidity, total volatile base nitrogen (TVBN) and proximate composition were monitored for comparison.

Materials and methods

The experiments were carried out with *M. rosenbergii* prawns grown in fresh water ponds in northern Israel and fed on 25 g of carp pellets per m^2 of pond per week. Water temperature varied from 23 to 30°C and the ponds were warmed geothermally as required. Five hundred prawns with live weight averaging 28 g were iced and transported to the laboratory within 2–3 hr of collection. On receipt, the prawns were rinsed with water for 1 min and sealed in polyethylene bags. The bags, with about half the quantity of prawns, were packed between layers of crushed ice with good drainage and stored in a 0°C room. The ice was changed daily to avoid compaction and melting away from the bags. The remaining prawns were frozen and stored in polyethylene bags at −35°C, to serve as control samples. Taste evaluation, proximate composition, TVBN, rancidity and microbiological examinations were carried out at 2–4 day intervals for 14 days.

Taste panel evaluation

The prawns were boiled whole for 3 min in salted water, and were then deheaded and shelled. The tails were rinsed and drained and allowed to cool to room temperature before being presented to the taste panel.

Five taste sessions were held, after 2, 4, 7, 10 and 14 days of storage. At the first and second sessions, the samples were presented for comparison as (2 *vs* 3)-pentagonal tests (Basker, 1980) and at the remaining three sessions, as triangular tests (Helm & Trolle, 1946; ASTM Committee E-18, 1968). The statistical significance of any differences between the samples was judged according to the number of assessors who made the correct identifications between the samples.

After identifying (correctly or incorrectly) the sample groups, the assessors were requested to state their preference between the groups: these preferences have meaning only when the identifications were correct.

The assessors were also asked to rate the quality of each sample group on a five-point nonparametric ordinal scale, as 'excellent', 'very good', 'good: usual quality', 'poor' or 'unacceptable'. Even if correct sample identifications were not made, the rating of the smaller group at each presentation may be identifiable. The sample 'identified' as the singlet in a triangular test thus receives an unambiguous rating. The 'pair' in a pentagonal test receives an unambiguous rating only if both members of the 'pair' were indeed from the same sample. When the difference between the samples was found to be statistically not significant ($0.05 < P$), all identifiable ratings were considered (Basker, 1977).

The assessors were further asked to assign a parametric value to the quality of each sample group, on a scale of values from 0 to 10 units. Values were considered for inclusion in calculations in the same way as were the above ratings.

Ten or eleven assessors participated in each panel session. Occasionally some of their assessments were recorded idiosyncratically and were removed from consideration when unclear. The samples, each consisting of a single prawn tail, were coded with two-digit random numbers and were arranged in random order on the tables before seating the assessors. The general procedures for sensory testing are discussed elsewhere (ASTM Committee E-18, 1968).

TVBN

TVBN was determined in duplicate on aliquots of homogenates of the raw, shelled tails of three prawns from each of the two groups of samples (frozen and 0°C stored, respectively), on each occasion. The test was carried out in accordance with the Israel Standard (1973); a weighed sample of homogenate was digested with MgO and water, and the distillate was collected in an excess of standard acid. Back-titration enabled the TVBN evolved to be calculated, expressed as mg N per 100 g sample.

Proximate composition

Moisture, fat and ash contents of the stored raw prawn tails, without shells, were determined with a Ultra-X-70 apparatus (Groner, W. Germany): moisture was determined by infra-red radiant heating at 100°C; the dried material was powdered, repeatedly treated with 40–60°C petroleum spirit to extract fatty material and excess solvent was removed by heating; the residue was ashed at 500°C. The crude protein content was calculated by difference. All measurements were carried out in triplicate, and the mean results are now reported.

Rancidity

Thiobarbituric acid (TBA) and conjugated diene values were determined on the raw shelled tails and hepatopancreas. The increase in malonaldehyde was tested according to Witte, Krause & Bailey (1970); a weighed sample of homogenate was blended with trichloracetic and phosphoric acids and filtered. The colour developed on warming a portion of the filtrate with TBA under standard conditions was measured at 532 nm, and compared with that obtained by reacting TBA with 1,1,3,3-tetraethoxypropane. The TBA values are expressed as mg malonaldehyde per kg sample.

Conjugated dienes

Conjugated dienes were determined according to Tsoukalas & Grosh (1977). The homogenates of the samples were freeze-dried and the lipids were extracted with chloroform–ethanol (1 + 2). After evaporating off the solvent, the fat was dissolved in cyclohexane–diethyl ether (1 + 9). An aliquot of this solution was mixed with methanol and the absorbance of the mixture measured at 234 nm, as a measure of the conjugated dienes present.

Bacteriological analyses

Prawns were deheaded aseptically and composite samples (*ca* 25 g) of either heads or tails were homogenized with 225 ml of 0.1% sterile Bacto-Peptone solution in a blender at 8000 rev/min for 2 min. Appropriate serial decimal dilutions of the homogenate were prepared with 0.1% peptone and used to inoculate the various bacteriological media.

Total aerobic mesophilic (20°C, 3 days) and psychrotrophic counts (7°C, 10 days) were performed by the pour plate method on plate count agar; total Enterobacteriaceae were enumerated in double-poured plates of violet red bile agar with 1% glucose, incubated for 24 hr at 35°C.

Faecal coliforms (confirmed) were enumerated by the 5-tube most probable number (MPN) technique using the procedure described by Fishbein *et al.* (1976).

Coagulase-positive Staphylococci were enumerated by surface plating on Baird-Parker agar, incubating for 24–48 hr at 35°C. Colonies showing typical reaction were selected and tested for coagulase production by the tube method using rabbit plasma containing EDTA.

For the detection of Salmonellae both selenite-cystine (35°C) and tetrathionate bile brilliant green (43°C) broths were used. Following incubation for 24–48 hr, each broth was streaked onto plates of brilliant green sulphadiazine agar (24 hr at 35°C). Since no colonies exhibiting positive reactions were found, further biochemical and serological confirmation were not necessary.

Results

Sensory evaluations

The prawns stored at 0°C are designated as C (= chilled), and those stored at − 35°C are designated as F (= frozen). Results of identification and preference tests are given in Table 1.

In the panel session held after 2 days of storage, only two out of the nine assessors made the correct identifications in a pentagonal test (result not statistically significant). No significant difference was found between the

Table 1. Correct identification and sample preference by panelists for chilled *Macrobrachium* prawns as compared with frozen prawns

Storage (days)	Test design	Number of assessors making correct identification	Statistical significance	Number of assessors preferring frozen sample	Statistical significance (two-tailed)
2	Pentagonal	2 out of 9	$0.05 < P$	1 out of 2	—
4	Pentagonal	2 out of 11	$0.05 < P$	1 out of 2	—
7	Triangular	7 out of 11	$0.02 < P < 0.05$	3 out of 7	$0.20 < P$
10	Triangular	4 out of 10	$0.10 < P$	3 out of 4	$0.20 < P$
14	Triangular	7 out of 11	$0.02 < P < 0.05$	8 out of 9	$0.02 < P < 0.04$

non-parametric ratings of the two samples by Kolomogorov-Smirnov two-sample test ($n_1 = 4$; $n_2 = 6$, $= 0.05 < P$) (Beyer, 1966). No significant difference was found between the parametric quality values of the two samples ($t = 0.74$, d.f. $= 8$, $0.40 < P < 0.50$).

At the second session after a total of 4 days of storage, only two of the eleven assessors made the correct difference identification (result not statistically significant). Sample F was preferred by one assessor and sample C by the other. Because of the very small numbers involved, no decision was possible regarding the significance or otherwise of the differences between the nonparametric ratings of the two samples. No significant difference was found between the parametric quality values of the two samples ($t = 1.48$, c.f. $= 5$, $0.10 < P < 0.20$).

At the third session, after a total of 7 days of storage and using a triangular test, seven out of eleven assessors correctly identified the odd sample (result statistically slightly significant). Three assessors preferred one sample while four preferred the other (result not statistically significant). No significant difference was found between the nonparametric ratings of the two samples by the Kolmogorov–Smirnov two-sample test ($n_1 = n_2 = 7$, $0.05 < P$). No significant difference was found between the parametric quality values of the two samples ($t = 0.09$, d.f. $= 10$, $0.90 < P < 0.95$).

At the fourth session, held after 10 days of storage, only four of the 10 assessors identified the 'odd' sample correctly in a triangular test (result not statistically significant). Three assessors preferred one sample, while one preferred the other (result not statistically significant). No significant difference was found between the nonparametric ratings of the two samples ($n_1 = n_2 = 7$, $0.05 < P$). Likewise, there was no significant difference between the parametric quality values of the two samples ($t = 1.48$, d.f. $= 12$, $0.10 < P < 0.20$).

At the fifth session, after a total of 14 days of storage, seven of the eleven assessors identified the odd sample correctly in a triangular test (result statistically slightly significant). A preference was recorded by nine assessors, eight of whom preferred sample F over sample C (result statistically slightly significant). No significant difference was found between the nonparametric

ratings of the two samples ($n_1 = n_2 = 7$, $0.05 < P$). A significant difference was found between the parametric quality values of the two samples ($t = 3.88$, d.f. = 12, $0.001 < P < 0.005$) with sample F scoring 50% higher than sample C.

The panels' quality assessments of all the samples are summarized in Table 2. The fraction of ratings which were 'Good: usual quality' or better indicate that sample F retained its good quality throughout the storage period, while sample C did so for seven days only. The linear correlation of the quality values with storage time was not significant for sample F ($r = +0.01$, d.f. = 26, $0.10 < P$), but was significant for sample C ($r = -0.43$, d.f. = 27, $0.01 < P < 0.05$).

Table 2. Sensory quality assessments of all samples (F = frozen storage; C = chilled storage)

Storage (days)	Fraction of ratings 'good: usual quality' or better		Mean quality values	
	F	C	F	C
2	1.0	1.0	7.0	8.0
4	1.0	1.0	9.5	7.7
7	0.7	0.6	5.2	5.3
10	0.6	0.3	5.9	3.9
14	1.0	0.4	8.1	5.4

TVBN values

The TVBN values obtained from the tails of the 0°C-stored prawns increased to 24.3 after 7 days and to 30.7 after 14 days (see Table 3). The comparative values from the tails of the frozen prawns were practically constant, varying only from 22.1 to 22.3 per 100 g, throughout the 14 day period.

Table 3. TVBN, TBA and conjugated diene values for 0°C-stored prawns

Storage (days)	TVBN values tail	TBA values		Conjugated diene values	
		Tail	Hepatopancreas	Tail	Hepatopancreas
2	22.7	0.25	0.75	0.20	0.25
4	22.3	0.65	1.30	0.32	0.21
7	24.3	—	—	—	—
10	27.3	0.64	2.00	0.28	0.22
14	30.7	0.66	1.25	0.22	0.23

Proximate composition

The proximate composition found of the tails of the 0°C-stored prawns, is shown in Table 4. An increase was noted in the moisture content on storage, and a decrease in the crude protein content.

Table 4. Proximate composition of 0°C stored prawn tails

Storage (days)	Moisture (%)	Fat (%)	Ash (%)	Crude protein (%) (by difference)
2	79.3	1.9	1.3	17.5
4	79.9	2.9	1.3	15.9
7	80.0	4.3	1.4	14.3
14	80.8	2.4	1.9	14.9

Rancidity

The TBA and conjugated diene values obtained from the 0°C-stored prawns are shown in Table 3. An increase was noted in the TBA value from the tails after 4 days, and from the heads after 10 days' storage followed by a decrease.

Table 5. Bacteriological plate counts for 0°C-stored prawns

	Logarithmic units per g											
	Aerobic mesophiles		Aerobic psychrotrophs		Entero-bacteriaceae		Faecal coliforms		Coagulase-positive Staphylococci		Salmonella	
Storage (days)	Tail	Head	Tail	Head	Tail	Head	Tail	Head	Tail	Head	Tail	Head
0	6.2	6.4	6.1	6.3	4.7	5.3	ND	1.0	1.0	2.1	Absent in 40g	Absent in 40g
5 min.			5.7	5.7	2.6	3.8						
max.			6.0	6.6	2.8	5.0						
9 min.			6.6	6.9	3.6	4.3						
max.			6.9	8.3	3.7	7.0						
14 min.	6.7	8.0	7.8	7.9	4.4	5.0						
max.	8.3	8.3	8.3	8.6	5.4	5.7						

ND = not detected
min. = lowest result
max. = highest result

Bacteriological analyses

Table 5 presents the results of bacteriological analyses of prawn samples rinsed under tap water for 1 min and stored at 0°C. In particular, the total aerobic psychrotrophic count was found to have increased by 0.5 to 2.0 logarithmic units after 9 days, and by 1.7 to 2.2 logarithmic units after 14 days. Within each sample, too, the results were very variable. Counts were consistently higher in the heads than in the tails. In the fresh (unstored) prawns, no Salmonellae were found, and the numbers of faecal coliforms and of coagulase-positive Staphylococci were low.

Discussion and conclusions

Montgomery, Sidhu & Vale (1970), Cobb & Vanderzant (1975), and Cheuk, Finne & Nickelson (1979) assesed the acceptability of ice-stored prawns and shrimp by odour and other tests. Montgomery *et al.* and Cobb & Vanderzant employed trained inspectors as panelists and non-parametric assessment methods, while Cheuk *et al.* used a twelve-member untrained panel and an hedonic assessment scale. Good quality was retained for up to 10 days, after which time flavour was lost, but spoilage odours did not set in until after 16 days on ice. Our own results, obtained with assessors who were not preselected, were very similar; statistically significant differences were not detected after 10 days' storage at 0°C, but were detected after 14 days.

Montgomery *et al.* (1970), Cobb & Vanderzant (1975), Cobb *et al.* (1976), Cobb *et al.* (1977) and Cheuk *et al.* (1979) reported 30 mg TVBN per 100 g prawn meat to be a useful indicator of the limit of prawn and shrimp acceptability. Cheuk *et al.* (1979) found constant TVBN values for brown shrimp during the first 11 days on ice, and during the first 15 days for pink shrimp. Spoilage set in after 16 days for pink shrimp and after 19 days for brown shrimp, when the TVBN values rose to 30 mg per 100 g. In the present trials too, the TVBN values rose to 30.7 after 14 days on ice, and this corresponded to a significant decrease of acceptability as compared with the frozen control samples in which the TVBN values did not rise. Cobb & Vanderzant (1975) found that increased TVBN values were due to bacterial spoilage, and that some bacterial species produced volatile nitrogen more readily than others.

The apparent increase of moisture content (1.5%) during storage at 0°C might have resulted from a deterioration in muscle texture and consequent greater release of water on drying. The apparent decrease of crude protein content (about 3%) during storage at 0°C may have resulted from losses of volatile nitrogen. Bauer & Eitenmiller (1974) reported that intracellular catabolism of protein by enzymes occurred in white shrimp. They suggested that protein as well as non-protein nitrogen from the interstitial fluids were lost through cell breakdown during storage.

In the present trials, the TBA values of the tail meat rose between 2 and 4

days' storage, and then remained unchanged. Bottino, Lilly & Finne (1979), using gas chromatography, found no changes in fatty acid composition or any oxidative deterioration following 18 days' storage on ice. The TBA test is based on the production of malonaldehyde as a result of the dissociation of hydroperoxides formed during fatty acid oxidation. Malonaldehyde is an active compound that can react with various substances, including free amino acids. As free amino acids accumulate during storage at 0°C, it is not impossible that condensation took place with these amino acids or even with proteins (Kanner & Karel, 1976), resulting in lower TBA values.

In the bacteriological tests, the low numbers of faecal coliforms and the absence of Salmonellae indicate that the common enteric pathogens do not constitute a food poisoning hazard. An increase was found in the total aerobic psychrotrophic count after 9 days' storage, and only minor changes in the Enterobacteriaceae counts.

The assessors did not find the tails objectionable with the initially high total plate counts of 6 logarithmic units per g, nor when the TVBN value reached 27 mg after 7 days' storage: this indicates that enzymatic and bacteriological activity were in progress, leading to eventual spoilage, long before the sensory panel could detect it significantly. Significant deterioration was apparent only after 14 days, when the total aerobic psychrotrophic counts had increased by about 2 logarithmic units per g, and the TVBN value to 30 mg per 100 g. Alvarez & Koberger (1979) reported that objectionable odours had developed after 11 days' storage on ice, and Vanderzant, Cobb & Nickelson (1974) found that half the shrimp became unacceptable after 7–14 days on ice.

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Stability of pectins during storage

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Summary

Low methoxyl pectins (LMP) prepared by HCl, NaOH and NH_3 deesterification procedures were stored with or without adding Na_2CO_3 as buffer at room temperature (25–30°C) or exposed to different relative humidities (r.h.) at 37°C. Losses of methoxyl, molecular weight and gelling power were observed in all the pectin samples. During storage, NaOH and NH_3 deesterified LMP were more stable than HCl deesterified LMP with respect to gelling power. The monolayer values (V_m) of 0.5 to 0.7 g per 100 g of LMP showed that even when stored at 11% r.h., the moisture in LMP was present in the multilayer region which was conducive to methoxyl loss.

NH_3 deesterified LMP precipitated at pH 0.5 and 1.5 after saponification, formed good gels similar to the LMP precipitated at pH 0.5, dried and mixed with buffer. NH_3 deesterified LMP precipitated at pH 3.0 and 4.5, on the contrary, formed only coagulated gels.

The loss of gelling power of LMP during storage was due to depolymerization besides gradual transformation of increasing portions of pectinic acid molecules into pectic acid molecules. High methoxyl pectins with or without added sodium carbonate or citrate as buffer stored at 62 and 75% r.h. showed methoxyl loss. Between r.h. of < 0.1 and 49%, methoxyl loss was more in the buffered samples than in the control.

Introduction

The sorption properties play an important role in determining the storage stability of foods. Water activity is one of the basic controlling factors in the prevention of chemical and biological deterioration of foods. The water sorption isotherms show, in a graphical form, the variation in relative humidity (r.h.) with the change in water content of a sample at a specified temperature.

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The isotherms for macromolecular food constituents are generally sigmoid in shape. The isotherms are characteristic of a product and differ with different products (Labuza, 1968).

Although dry pectins are considered to change little during storage (Kertesz, 1951; Doesburg, 1965), in this study a general loss of methoxyl content, gelling power and molecular weight was observed in low methoxyl pectin (LMP) samples stored at 37°C at various r.h. levels, and at room temperature. The factors responsible for such changes are presented in this paper.

Materials and methods

The procedures used for preparing LMP by HCl, NaOH and NH_3 deesterification were essentially the same as described in our earlier paper (Padival, Ranganna & Manjrekar, 1979). The exact conditions used for preparing the LMP samples used in storage studies at room temperature, and their characteristics are given in Table 1. It was ensured that the pectin samples were free of any residual HCl by repeated washing with 70% alcohol until the filtrate was free of chloride ions. The pectin samples were stored at room temperature (25–30°C) in stoppered glass containers.

The effect of storage of LMP at different r.h. levels and at 37°C were investigated as follows.

HCl, NaOH and NH_3 deesterified samples of dry LMP were prepared according to the particulars given in Table 2. The dried pectin samples were mixed with precalculated amounts of anhydrous Na_2CO_3, so that a 1% solution of resulting pectin would have a pH value of ~3.0.

In another study, the pectin extract was deesterified using NH_3 at pH 10.5 and at 25°C for 2 hr. Thereafter, the pH of the saponified extract was adjusted to 0.5,

Table 1. Particulars of LMP used for storage studies at room temperature

	Particulars of deesterification of pectin extract			Characteristics of LMP					
Sl No.	Agent and pH	Temp (°C)	Time (hr)	pH of pptn.	Moisture (%)	Ash (%)	MeO AMFB* (%)	Gel strength (ml H_2O)	Appearance†
1	HCl at pH 0.5	60	8.00	0.5	5.6	0.5	4.2	38	****
2		70	4.00	0.5	7.4	0.5	4.6	36	****
3		80	1.00	0.5	4.4	0.6	5.2	30	***
4	NaOH at pH 11.7	5	0.25	0.5	5.9	0.1	4.7	50	****
5	NH_3 at pH 10.5	25	3.50	0.5	5.8	0.4	3.0	60	****
6		25	2.50	0.5	6.6	0.6	4.3	50	****
7		25	1.50	0.5	7.2	0.7	5.2	40	****

* AMFB—Ash and moisture free basis.

†****Very good gel; ***good gel; **soft gel; *coagulated gel.

Table 2. Particulars of LMP used for storage studies at 37°C and at different r.h. levels

Particulars of deesterification of the extract	Buffer*	Characteristics of LMP				Gel characteristics‡		
		Moisture (%)	Ash (%)	MeO AMFB† (%)	Mol. wt AMFB†	Calcium (mg/g LMP)	Gel strength (ml H_2O)	Appearance§
HCl deesterification at 60°C and pH 0.5 for 8 hr, and pptd. at pH 0.5	−	8.77	0.47	4.23	58860	40	43	++++
	+	8.16	4.58	4.21		40	45	++++
NaOH deesterification at 25°C and pH 11.7 for 15 min, and pptd. at pH 0.5	−	10.40	0.57	4.35	69870	60	52	++++
NH_3 deesterification at 25°C and pH 11.7 for 1 hr, and pptd. at pH 0.5	−	9.26	0.68	4.02	47670	40	54	++++
	+	8.86	1.42	4.01		40	55	++++

*Storage with (+) or without (−) added buffer.

†On ash and moisture free basis.

‡The pH level of the pectin solution was adjusted to 3.0 using sodium citrate or citric acid, and the gels were prepared without sugar using 1.0 g LMP, 100 ml water and calcium chloride solution.

§++++ Very good gel; +++ good gel; ++ soft gel; + coagulated gel.

1.5, 3.0 and 4.5 using HCl, and the LMP was precipitated with alcohol. The precipitated pectin samples were repeatedly washed with 70% alcohol, dried and powdered, and used for storage studies.

Determination of equilibrium relative humidity

Equilibrium relative humidity (e.r.h.) moisture relationships were determined by Wink's (1946) weight equilibrium method. Saturated solutions of different salts having definite r.h. values were used to obtain a range of r.h. from 11.1 to 75.1%. Sulphuric acid was used to obtain a r.h. of less than 0.1%. The LMP samples were taken in Petri dishes, spread uniformly, and exposed to r.h. levels ranging from <0.1 to 75.1% in desiccators maintained at 37 ± 0.1°C in a Hot-Pack walk-in incubator. The initial moisture contents of the samples were determined by drying at 70°C for 16 hr in a hot air oven. The equilibrium moisture contents of the samples were calculated by using the initial moisture content of the sample, and the moisture gained or lost during the equilibration period. The moisture sorption isotherms for the LMP samples were converted into BET isotherms from which the monolayer (V_m) values were calculated using the following equation (Labuza, 1968):

$$\frac{a}{(1-a)V} = \frac{1}{V_m C} + \frac{a(C-1)}{V_m C}$$

where, a = water activity = p/p_o = (r.h./100)

V = volume absorbed in grams per 100 g of dry matter

C = slope, when $\frac{a}{(1-a)V}$ is plotted against a

V_m = monolayer coverage (grams per 100 g of dry matter)

Fractionation of low methoxyl pectins

The stored LMP samples were fractionated into water-soluble and water-insoluble portions according to the procedure of Owens *et al.* (1952).

Moisture, ash and methoxyl contents were determined by the procedures described by Owens *et al.* (1952). Gels were prepared using a 1% LMP solution at pH 3.0 without sugar but using optimum concentrations of calcium. The gel strength was determined using a B.F.M.I.R.A. Jelly Tester with a water flow rate of 75 ml/min at 30° torque. Molecular weight was determined by the viscosity procedure of Smit & Bryant (1967).

Results and discussion

Changes at room temperature

Changes in methoxyl content. Figure 1 shows the pattern of decrease in the methoxyl content of HCl, NaOH and NH_3 deesterified pectin samples. These LMP samples having 4–5% methoxyl were prepared by precipitating the saponified pectin extract at pH 0.5. The methoxyl content decreased slightly during storage up to one year. The LMP samples prepared by adjusting the pH to 4.5 after saponification as recommended by earlier workers (Hills, White & Baker, 1942; Owens, McCready & Maclay, 1949; Graham & Shepherd, 1953), did not improve the stability with respect to methoxyl content when stored under similar conditions.

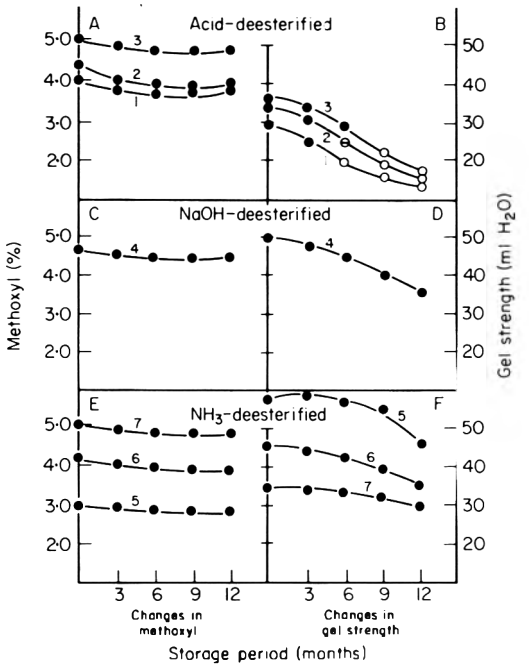

Figure 1. Changes in methoxyl content and gel strength of HCl, HaOH and NH_3 deesterified LMP samples during storage at room temperature. ⊖, Soft gel; ●, good gel; ○, coagulated gel. See Table 1 for particulars of 1–7.

Changes in gel strength. HCl deesterified LMP formed stable gels for up to 6 months of storage, but thereafter yielded only coagulated gels (Fig. 1). This is evident from the loss of gel strength. The NaOH and NH_3 deesterified LMP formed stable gels even after 12 months of storage, but the gel strength decreased.

Changes during storage at different relative humidities in LMP mixed with buffer

Equilibrium moisture content at different relative humidities. The characteristics of the LMP used in these studies are given in Table 2. The moisture sorption isotherms of LMP samples had a sigmoid pattern (Fig. 2). The LMP samples reached moisture equilibrium in 10–15 days. The BET isotherms (Fig. 3) gave V_m values of 0.53, 0.73 and 0.70 g per 100 g of HCl; NaOH and NH_3 deesterified LMP samples respectively. These values corresponded to 5% e.r.h. which showed that even at 11% r.h., the moisture present in LMP was in the multilayer region which rendered the samples conducive to methoxyl loss during storage.

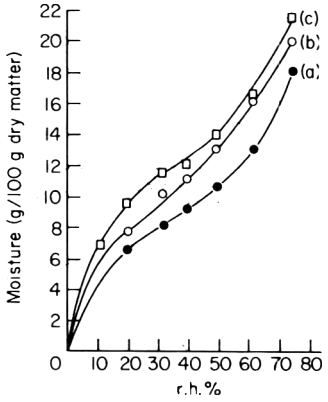

Figure 2. Sorption isotherms of LMP. (a) HCl deesterified LMP; (b) NH_3 deesterified LMP; (c) NaOH deesterified LMP.

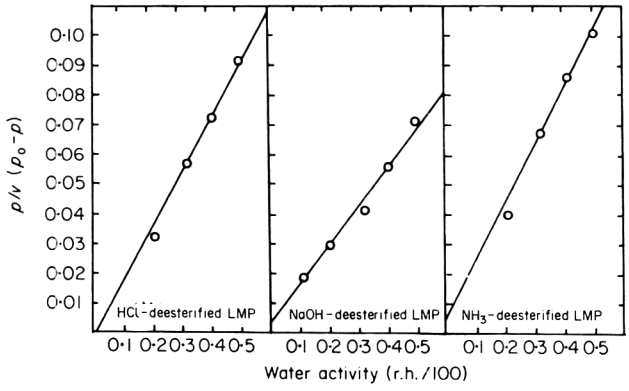

Figure 3. BET monolayer plot of LMP.

Changes in methoxyl content. Between 11 and 75% r.h., the three types of LMP stored with or without added Na_2CO_3 showed loss in the methoxyl content. The loss occurred mostly during the first 2 months of the storage period with little thereafter (Table 3). Methoxyl loss occurred even in the samples stored over conc. H_2SO_4 which would have rendered the LMP almost

Table 3. Changes in methoxyl content and gel characteristics of HCl, NaOH and NH_3 deesterified LMP samples stored at 37°C and at different r.h. levels

		HCl deesterified LMP						NaOH deesterified LMP			NH_3 deesterified LMP					
		Unbuffered (pH 2.4)			Buffered (pH 3.3)			Unbuffered (pH 2.4)			Unbuffered (pH 2.4)			Buffered (pH 3.3)		
r.h. (%)	Storage period (months)	MeO* (%)	Gel strength (ml H_2O)	Appea-rance†	MeO* (%)	Gel strength (ml H_2O)	Appea-rance†	MeO* (%)	Gel strength (ml H_2O)	Appea-rance†	MeO* (%)	Gel strength (ml H_2O)	Appea-rance†	MeO* (%)	Gel strength (ml H_2O)	Appea-rance†
	0	4.23	43[b]	++++	4.21	45[b]	++++	4.35	50[a]	++++	4.02	54[b]	++++	4.01	55[b]	++++
<0.1	2	3.73	22[b]	++	3.81	24[b]	++	4.14	40[a]	++++	3.29	45[c]	++++	3.25	43[c]	++++
	4	3.73	<10[b]	+	3.79	<10[b]	+	4.02	40[a]	++++	3.21	43[c]	++++	3.10	41[c]	++++
11	2	3.77	20[b]	+	3.77	18[b]	+	4.05	40[a]	++++	3.24	45[c]	++++	3.22	44[c]	++++
	4	3.73	<10[b]	+	3.75	<10[b]	+	4.02	40[a]	++++	3.21	45[c]	++++	3.21	40[c]	++++
20	2	3.31	20[c]	+	3.16	19[c]	+	4.02	40[a]	++++	3.15	45[c]	++++	3.23	44[c]	++++
	4	3.27	<10[c]	+	3.15	<10[c]	+	3.95	39[a]	++++	3.11	40[c]	++++	2.94	40[c]	++++
32	2	3.10	20[c]	+	3.00	17[c]	+	3.86	38[b]	++++	3.08	48[c]	++++	3.20	40[c]	++++
	4	2.90	<10[c]	+	2.87	<10[c]	+	3.66	36[b]	+++	3.03	38[c]	+++	2.88	40[c]	++++
40	2	3.11	20[c]	+	2.74	15[d]	+	3.35	30[c]	+++	3.01	47[c]	++++	3.07	40[c]	++++
	4	2.84	<10[c]	+	2.73	<10[d]	+	3.04	25[c]	+++	2.99	35[c]	+++	2.94	40[c]	++++
49	2	3.15	16[c]	+	2.72	<10[d]	+	3.14	30[c]	+++	2.94	42[c]	++++	2.92	42[c]	++++
	4	2.85	<10[c]	+	2.67	<10[d]	+	2.60	25[c]	+++	2.80	32[c]	+++	2.80	37[c]	+++
62	2	2.38	15[d]	+	2.66	<10[d]	+	2.50	25[c]	+++	2.98	35[c]	+++	2.38	27[d]	++
	4	2.33	<10[d]	+	2.65	<10[d]	+	2.35	22[d]		2.68	23[c]	++	2.32	21[d]	+
75	2	2.10	<10[d]	+	2.62	<10[d]	+	1.98	20[d]	++	2.80	32[c]	+++	1.97	25[d]	++
	4	1.83	<10[d]	+	2.62	<10[d]	+	1.90	<10[d]	+	2.70	17[c]	+	1.98	18[d]	+

*On ash and moisture free basis; †++++ very good gel; +++ good gel; ++ soft gel; + coagulated gel.
Optimum concentration of calcium used: [a], 60 mg; [b], 40 mg; [c], 30 mg; [d], 20 mg per g. of LMP used in gels.

completely dry. The extent of methoxyl loss, however, increased with increasing r.h. levels.

Changes in gelling characteristics. At the end of two months of storage, HCl deesterified sample which formed stable gels initially lost its gelling power irrespective of r.h. (Table 3). NaOH deesterified samples stored between 11 and 62% RH retained the gelling power though the gel strength decreased during storage. At the end of 4 months, the samples stored between 11 and 49% r.h. formed good gels while the remaining formed either soft or coagulated gels.

The NH_3 deesterified LMP at the end of 2 months formed stable gels at all the r.h. levels studied, and after 4 months up to 49% r.h.

Changes during storage in ammonia deesterified LMP precipitated at different pH.

Table 4 gives the characteristics of the NH_3 deesterified LMP precipitated at different pH values. The ammonium salt content increased from 0 to 38% with an increase in the pH of precipitation of the saponified extract from 0.5 to 4.5, while the amide content remained more or less the same (3.9–4.2%). The LMP precipitated at pH 3.0 and 4.5, in spite of having a methoxyl content of 4.3, formed only coagulated gels even initially (Table 5). During storage at r.h. ranging from 11 to 62% at 37°C, the methoxyl content decreased only very little, and the gel characteristics remained the same. The LMP precipitated at pH 1.5 formed stable gels up to a storage period of 4 and 2 months at 37°C and r.h. of 20 and 62% respectively. The observations of the LMP precipitated at pH 0.5 were similar to that of LMP precipitated at pH 1.5 except that it formed stable gels up to a storage period of 4 months at 40% r.h.

The above results show that the NH_3 deesterified LMP precipitated at pH 0.5 and mixed with buffer to pH 3.0 after drying is superior to the NH_3 deesterified LMP which had been prepared by precipitating at pH 3.0 or 4.5 with respect to gelling characteristics. The NH_3 deesterified LMP precipitated at pH 1.5 compared well with the LMP mixed with buffer. Hence, to prepare the NH_3 deesterified LMP which would be stable during storage, it is preferable to admix

Table 4. Characteristics of ammonia deesterified LMP precipitated at different pH

Characteristics of the LMP						Gel characteristics		
pH of pptn.	Moisture (%)	Ash (%)	Ammonium salts AMFB* (%)	Amides AMFB (%)	MeO AMFB (%)	Optimum calcium (mg/g of LMP)	Gel strength (ml H_2O)	Appearance†
0.5	15.0	0.51	0.0	4.2	4.23	40	55	++++
1.5	14.1	0.93	5.0	4.0	4.20	40	45	++++
3.0	10.8	1.70	10.5	4.1	4.20	40	10	+
4.5	13.4	3.60	38.1	3.9	4.20	40	10	+

*Ash and moisture free basis.

†++++ Very good gel; +++ good gel; ++ soft gel; + coagulated gel.

Table 5. Changes in methoxyl content and gel characteristics of ammonia deesterified LMP precipitated at pH 0.5, 1.5, 3.0 and 4.5 and stored at different r.h. and 37°C

		LMP precipitated at pH 0.5			LMP precipitated at pH 1.5			LMP precipitated at pH 3.0			LMP precipitated at pH 4.5		
r.h. (%)	Storage period (months)	MeO AMFB* (%)	Gel strength (ml H_2O)	Appearance†	MeO AMFB (%)	Gel strength (ml H_2O)	Appearance†	MeO AMFB (%)	Gel strength (ml H_2O)	Appearance	MeO AMFB (%)	Gel strength (ml H_2O)	Appearance
	0	4.23	50	++++	4.23	42	++++	4.23	< 10	+	4.23	< 10	+
< 0.1	2	3.30	40	++++									
	4	3.12	36	+++									
11	2	3.25	40	++++							4.23	< 10	+
	4	3.10	34	+++							4.21	< 10	+
20	2	3.10	38	++++	3.60	35	+++	4.11	< 10	+	4.21	< 10	+
	4	3.00	33	+++	3.50	30	++++	4.09	< 10	+	4.12	< 10	+
32	2	3.00	33	++++				4.11	< 10	+	4.20	< 10	+
	4	2.95	30	++++				4.00	< 10	+	4.17	< 10	+
40	2	2.95	30	++++	3.29	32	++++	4.11	< 10	+	4.07	< 10	+
	4	2.75	30	+++	3.24	28	++	4.00	< 10	+	4.00	< 10	+
49	2	2.90	30	+++				4.10	< 10	+	4.06	< 10	+
	4	2.65	28	++				3.99	< 10	+	3.99	< 10	+
62	2	2.85	30	+++	3.07	30	+++	4.06	< 10	+	3.98	< 10	+
	4	2.55	20	+	3.06	20	+	3.99	< 10	+	3.78	< 10	+

*On ash and moisture free basis

†++++ Very good gel; +++ good gel; ++ soft gel; + coagulated gel.

the dried LMP prepared by precipitating at pH 0.5 with the buffer, or alternatively, to precipitate at pH 1.5.

Changes in the solubility of low methoxyl pectins during storage. The LMP samples used in this study, irrespective of deesterification procedure, were completely soluble in water initially. After storage, some of the samples became partly insoluble, and lost their methoxyl groups and gelling power to varying degree.

(1) *HCl deesterified pectin*

Table 6 shows the methoxyl content and the molecular weight of the unfractionated LMP, and of the water-soluble and insoluble fractions after 10 months of storage at 37°C. The molecular weight, methoxyl content and the solubility of the LMP decreased with increasing RH during storage. On fractionation, the molecular weight in the soluble fraction was found to decrease with increasing RH of storage and *vice versa* in the insoluble fraction. The methoxyl content of the water-insoluble fraction was 0.5% irrespective of RH of storage while the soluble fraction had 2.93 to 3.95% methoxyl groups.

(2) *NaOH deesterified pectin*

The LMP remained soluble up to 20% r.h. during storage after 10 months, and formed good gels (Table 6). The other observations with respect to decrease in molecular weight, methoxyl content and solubility before and after fractionation were similar to the HCl deesterified LMP.

(3) *NH_3 deesterified pectin*

The LMP yielded good gels up to 40% r.h. during storage after 10 months, though the sample was soluble only to the extent of 70–88% (Table 6). The decrease in molecular weight, methoxyl content and solubility of the LMP before or after fractionation was similar to HCl or NaOH deesterified samples.

The fractionation studies on the HCl, NaOH and NH_3 deesterified LMP samples discussed above show that the loss of gelling power of LMP during storage is not merely due to the random loss of methoxyl groups or the depolymerization of pectin molecules, but also due to the loss of all methoxyl groups from increasingly large portions of the pectin molecular chain (i.e., transformation from pectinic to pectic acids). In the HCl deesterified LMP, the loss in gelling power was exclusively due to the degradation of the molecular chain length even at very low r.h. (11% or below); though the methoxyl content was within the limits for LMP, the gelling ability was completely lost. On the contrary, in NaOH and NH_3 deesterified LMP, up to 11% r.h. depolymerization was not much, and hence the gels had a good set. Thus, it is possible to store the alkali deesterified LMP but not the acid deesterified LMP at low r.h. levels for long periods without much loss in gelling power. At higher r.h. levels, the loss in gelling power is a result of simultaneous depolymerization and demethylation.

Table 6. Changes in molecular weight and methoxyl content of HCl, NaOH and NH_3 deesterified LMP* and of water-soluble and -insoluble fractions formed during storage for 10 months at 37°C and at different r.h.

	Unfractionated LMP					Fractionated LMP					
						Water-soluble fraction			Water-insoluble fraction		
r.h. (%)	Molecular weight	Loss in molecular weight (%)	MeO‡ (%)	Gel strength (ml H_2O)	Appearance§	Extent soluble (%)	Molecular weight	MeO‡ (%)	Extent insoluble (%)	Molecular weight	MeO‡ (%)
HCL deesterified LMP											
‡	58860		4.23	43	++++	100					
<0.1	32120	45.4	3.59	<10	+	90.2	34800	3.95	9.1	10390	0.50
11	30590	48.0	3.52	<10	+	89.0	32680	3.90	10.6	18180	0.50
20	27960	52.5	3.07	<10	+	82.3	29180	3.77	15.1	29010	0.50
32	26630	54.8	2.93	<10	+	79.5	26860	3.81	19.5	26370	0.50
40	25310	57.0	2.71	<10	+	74.4	25390	3.54	24.9	24680	0.50
49	22940	61.0	1.97	<10	+	68.7	22760	2.93	30.1	22120	0.50
62	21450	63.6	1.89	<10	+	59.7	20580	3.12	40.0	23440	0.50
75	18450	68.7	1.73	<10	+	45.0	12990	3.79	51.0	26860	0.50
NaOH deesterified LMP											
‡	69870		4.35	52	++++	100					
<0.1	68760	1.6	4.00	40	++++	100					
11	62900	10.0	3.85	40	++++	100					
20	41300	40.9	3.35	20	++	100					
32	32110	54.1	3.32	<10	+	87.0	33610	3.03	12.2	17850	1.27
40	30420	56.5	2.40	<10	+	78.8	30810	2.36	20.7	28850	1.30
49	23360	66.6	2.04	<10	+	68.2	19890	2.27	29.2	30880	1.52
62	22690	67.5	1.74	<10	+	37.4	11000	2.08	59.8	31090	1.56
75	16010	77.0	1.54	<10	+	32.2	11700	1.93	67.0	18400	1.52
NH_3 deesterified LMP											
‡	47670		4.02	54	++++	100					
11	46400	2.9	3.11	45	++++	88	48000	3.29	10	14900	1.72
40	43900	7.9	2.99	35	+++	70	44200	4.01	27	43780	0.50
62	30790	35.4	2.16	23	++	68	32750	3.22	31	28830	0.50
75	29660	37.8	1.99	17	+	45	30900	4.31	53	29600	0.50

*LMP prepared as described in the text. ‡Initial value. ‡On ash and moisture free basis.
§++++ Very good gel; +++ good gel; ++ soft gel; + coagulated gel.

Storage changes in high methoxyl pectins

As the methoxyl content of LMP decreased considerably during storage at 62 and 75% r.h., the possibility of converting high methoxyl pectins into LMP by storing at high r.h. was investigated. The methoxyl content decreased from 10 to 7% but not below, when stored with or without added sodium citrate (1.6 and 3.2% w/w basis of the sample) or Na_2CO_3 (0.6%) as buffers. At other r.h. (<0.1 to 49%), the methoxyl loss was more in the presence of buffers than in the control sample (Fig. 4). Hence, this cannot be considered as a useful procedure for the preparation of LMP.

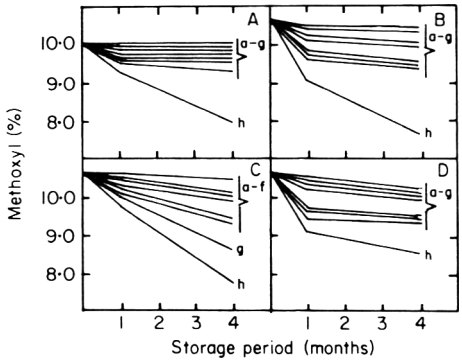

Figure 4. Changes in methoxyl content of high methoxyl pectin samples during storage at 37°C and different r.h. levels.
A, Buffered with 1.6% (w/w) sodium citrate; B, unbuffered; C, buffered with 3.2% (w/w) sodium citrate; D, buffered with 0.6% (w/w) Na_2CO_3; a, < 0.1%; b, 11%; c, 20%; d, 32%; e, 40%; f, 49%; g, 62%; h, 75% r.h.

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Apple juice extraction in a counter-current diffuser

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Summary

Measurements have been carried out on an industrial diffuser for the extraction of apple juice. Concentration profiles in the free juice and in the juice contained in the apple slices were measured at various temperatures and counter-current flow rates. The data can be described by a theoretical model taking into account the time of plasmolysis as a function of temperature. Below around 58°C, the concentration profiles break into two parts—before and after plasmolysis—and the diffuser yield is lowered. The yield is also lowered if the water-to-apple volume ratio is not kept well above unity. Under optimal conditions it is possible to obtain an overall recovery of soluble matter from apple stock to final juice which is above 90%. The axial dispersion coefficient ('eddy diffusion coefficient') of the apple slices was also measured. At a mean speed of apple slices through the diffuser of about 0.06 m/min (holding time = 120 min), the axial dispersion coefficient was around 0.01 m^2/min.

Introduction

Whereas the continuous counter-current solid-liquid extraction process has been widely applied in the sugar beet industry due to the development of the diffuser by De danske Sukkerfabrikker (DdS) in the early sixties (Brüniche-Olsen, 1962), the application to apple juice extraction is quite recent. The firm A/S Rynkeby Mosteri in Denmark was among the first to experiment with modifications of the DdS-diffuser in order to apply that device to continuous apple juice extraction. After successful runs with a double helix diffuser built

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by Rynkeby in 1972 (max. capacity 3000 kg apples/hr), a continuous diffuser was installed in 1975. It has a capacity of about 4–5000 kg apples/hr. The largest industrial diffuser for apple juice was brought into operation by Appletiser Ltd. in South Africa. It has a capacity of 30 metric tons/hr. In Europe the biggest diffuser has been installed by Brunia in Stade in West Germany (10 tons/hr).

In the United States, mechanical pressing of apple juice still seems to prevail. A comparison between pressed juice and juice from a small single helix pilot DdS diffuser was recently published by Binkley & Wiley (1978). A recent review of the state of the art in West Germany confirms that almost all apple juice production in that country is now by the diffusion method (Lüthi & Glunk, 1979). The reason is the much higher recovery of soluble matter by the diffusion method—especially when the raw material is over-ripe. In Denmark, however, Rynkeby is the exception, and all other juice is produced by pressing. There seems to be no appreciable difference in quality and taste between the two types such as observed by Binkley & Wiley (1978). These authors made organoleptic investigations which favoured pressed juice, but this could be ascribed to the quite unstable and poorly reproducible operation of the pilot diffuser apparent from their figures and tables.

With the increased interest in continuous apple juice extraction it becomes important to optimize the production of plants of widely differing sizes. In order to initiate such an optimization we have constructed some mathematical models where the most important features of continuous extraction (solid phase holding time, solid and liquid phase flow, etc.) appear in a few basic dimensionless variables. The method of non-dimensionalization is known from physics and chemistry, as well as from other branches of chemical engineering, to facilitate the representation of a given problem or a given physical model.

In solid-liquid extraction of sugar beet or fruit those methods seem not to be recognized. If mathematical models are used at all, they are not analyzed in terms of dimensionless variables. This is true for the dissertation of Brüniche-Olsen (1962) as well as for the earlier attempts to make models of continuous sugar beet extraction (van Ginneken, 1913, 1915; Silin, 1937). An exception seems to be the model of Oplatka & Tegze (1952). It seems to be that the only authors to have made reference to mathematical models for apple juice extraction are Dousse & Ugstad (1975) who presented a formula normally used for absorption towers.

We wanted to test the models proposed under realistic production conditions and with the kind permission of A/S Rynkeby Mosteri, Denmark, measurements of concentration profiles of soluble matter in the apple juice and in the apple slices along the diffuser were made under operating conditions kept as stable as possible. Temperature profiles were also determined by means of thermistors.

The qualitative findings deserve to be mentioned in the introduction. At lower operation temperatures (45–55°C) a discontinuity in the derivatives of the concentration profiles with respect to position in the diffuser was clearly

observed. The time elapsed from the introduction of the apple slices into the lower end of the diffuser to the arrival of the slices to the point of discontinuity is interpreted as the time required for plasmolysis, *i.e.* break down of the lipid bilayer cell membranes. An unpublished investigation of Dousse cited by Lüthi & Glunk (1975) shows that the time required for plasmolysis increases rapidly with decreasing temperature for temperatures less than 60°C. At 60°C the time required for plasmolysis is around 10 min whereas the time required at 50°C is more than ten times that value. At 80°C the time is reported to be around 1 min, but at that temperature a rapid destruction of the *cell wall* sets in leading to 'cooking-out' of the apple slices and liberation of pectin. Plasmolysis leads to favourable increase of the mass transfer coefficient in the diffuser, whereas cell wall destruction is disastrous for drainage and for recovery of soluble matter.

To construct a model for the dependence of recovery on temperature it is therefore necessary to assume one mass transfer coefficient *before* the point of discontinuity is reached by a 'mean apple slice' and a greater mass transfer coefficient *after* that point. The reported plasmolysis times have been calculated as for Jonathan apples (slices of 3 mm thickness) although one might expect some dependence on the type of apple, the state of maturity and the slice thickness. Assuming the same dependence in our case, however, we find a marked increase in recovery of the soluble matter in the apples in going from 53 to 57°C (for any fixed water/apple feed ratio). From 57 to 65°C the increase in recovery is insignificant, and for mature or overmature apples it is advisable to stay below an operating temperature of 60°C in the diffuser to avoid any local 'cooking-out' due to overheating.

By the models given here only a part of the optimization problem has been solved. The recovery of soluble matter in the apples will be dependent on many other factors such as the degree of filling of the diffuser with apple slices and water, the tilting angle of the diffuser and the possible recirculation of the juice from the pressing of the processed apple pulp. A pertinent discussion of those factors appears in the recent review of Lüthi & Glunk (1979). Moreover, limits will be set by economic considerations. For example, in principle the loss of soluble matter in the extracted pomace can be decreased considerably by increasing the water feed : apple feed ratio, but capital and energy costs of the concentration process of the dilute juice following the extraction process will determine the limit of water use. Juice quality requirements may also be determining factors. We hope to come closer to such a total optimization in future publications.

Model development and solution

The most elaborate model to-date has been proposed by Brüniche-Olsen (1962). Principle diagrams of the diffuser and the concentration profiles are given in Fig. 1. Under stationary conditions the differential equations for the

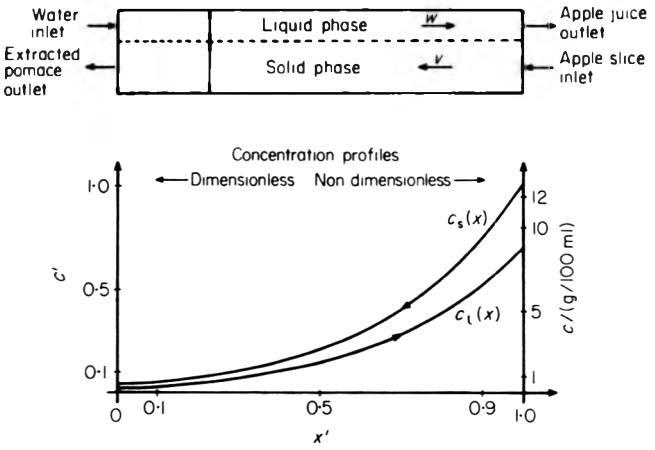

Figure 1. Concentration profiles and flow conditions in a diffuser.

concentration of soluble matter in the liquid phase or the juice (c_l) and in the solid phase, or more precisely, in the cell volume of the apple slices—or sugar beet cossettes—(c_s) are the following:

$$D_e^{(s)} \cdot \frac{d^2 c_s}{dx^2} + v \cdot \frac{dc_s}{dx} - k(c_s - c_l) = 0 \tag{1a}$$

$$D_e^{(l)} \cdot \frac{d^2 c_l}{dx^2} - w \cdot \frac{dc_l}{dx} + \frac{\mu\alpha}{1-\alpha} \cdot k(c_s - c_l) = 0 \tag{1b}$$

In those formulae v and w are the linear (mean) velocities of solid and liquid phase in the minus x- and x-direction, respectively. $D_e^{(s)}$ and $D_e^{(l)}$ are eddy diffusion coefficients (axial dispersion coefficients) for the apple slices and the soluble matter in the juice, respectively. Thus, $D_e^{(l)}$ is not to be understood in the usual sense as the eddy diffusion coefficient of turbulent flow. The effective mass transfer coefficient is called k. The fraction of the total volume used in the diffuser which is occupied by apple slices is called α, and μ is the volume fraction of water + soluble matter in the apple slices.

The philosophy behind eqns (1a) and (1b) is that mass transfer between the two phases is proportional to the difference in concentrations and is balanced by convection and eddy diffusion under stationary operation. Very similar equations occur in the theory of chromatography (Sørensen, 1974a), but in that case, the conditions are not stationary so the zero at the right hand side of eqns (1a) and (1b) has to be replaced by an accumulation term $\partial c/\partial t$. Also v and $D_e^{(s)}$ are of course zero in chromatography with a stationary and mobile phase. In recirculation experiments in a gelchromatographic column, the eddy diffusion coefficient $D_e^{(l)}$ has been measured for a number of substances and was found to increase quite strongly with elution rate w (Sørensen, 1974b). The same should be expected in the present case, so that both $D_e^{(l)}$ and $D^{(s)}$ will probably increase with w and v.

We shall now introduce dimensionless variables. The dimensionless distance is given by

$$x' \equiv \frac{x}{L} \; [x' = 0 \text{ water feed}, x' = 1 \text{ apple feed}] \tag{2}$$

where L is the length of the diffuser. The dimensionless concentrations are scaled by the concentration in the feed apples:

$$c_l' \equiv c_l/c_s \; (x = L); \; c_s' \equiv c_s/c_s \; (x = L) \tag{3}$$

Eliminating c_s from the coupled eqns (1a) and (1b) we obtain a 4th order equation. Introducing eqns (2) and (3) we obtain

$$\left[R\left(\frac{w}{v}\right)\cdot\hat{D}^2\cdot\frac{d^4}{dx'^4}+\left(1-R\frac{w}{v}\right)\hat{D}\cdot\frac{d^3}{dx'^3}\right.$$
$$-\left(1+\left\{\frac{\mu\alpha}{1-\alpha}\cdot R+1\right\}\hat{D}\tau\right)\frac{d^2}{dx'^2}$$
$$\left.+\tau\left\{1-\frac{\mu\alpha v}{(1-\alpha)w}\right\}\frac{d}{dx'}\right]c_l' = 0 \tag{4}$$

In eqn (4) we have defined the following dimensionless numbers:

$$R \equiv \frac{D_e^{(s)}}{D_e^{(l)}} \text{ (eddy diffusion ratio)} \tag{5}$$

$$\hat{D} \equiv \frac{D_e^{(l)}}{w \times L} \text{ (dimensionless eddy diffusion coefficient)} \tag{6}$$

$$\tau \equiv \frac{kL}{v} \text{ (dimensionless holding time)} \tag{7}$$

Equation (4) corresponds to Brüniche-Olsen's formula (XIII, 44) formulated in dimensionless variables. Brüniche-Olsen did not solve the complete equation, but neglected the eddy diffusion of the solid slices. In this case $R = 0$ and the equation reduces to a 3rd order equation. We shall show later, however, that there is a considerable axial dispersion of the apple slices in the Rynkeby diffuser, and probably $D_e^{(s)}$ and $D_e^{(l)}$ are of the same order of magnitude. It is therefore better to reason in a slightly different manner.

We shall show that $\hat{D}^{(s)} \equiv D_e^{(s)}/L \times v$ is of the order of magnitude 0.03 and that $\tau \sim 10$. Assuming that $\hat{D}$ also is of order of magnitude 0.03 we see that the coefficient to the 4th order term is of order of magnitude 0.001. $\hat{D}$ in the coefficient to the 3rd order term is about 0.03, but since R will probably tend

to be higher than one when w/v is less than one, it is most likely that the term $1-R\frac{w}{v}$ will almost cancel, so the third order coefficient is numerically less than 0.03. The $\hat{D}\tau$ term in the coefficient to the 2nd order term is seen to be of order of magnitude of 0.3, so that term is probably the only term of importance due to axial dispersion.

We shall therefore solve only the simplified eqn (4) with omission of the 4th and the 3rd order terms:

$$\left[1+p\hat{D}\tau\right]\frac{d^2c_1'}{dx'^2}-\tau\cdot q\,\frac{dc_1'}{dx'}\cong 0 \tag{8}$$

with definitions

$$p\equiv 1+\frac{\mu\alpha}{1-\alpha}\cdot R \tag{9}$$

$$q\equiv 1-\frac{\mu\alpha}{1-\alpha}\,\frac{v}{w} \tag{10}$$

The general solution to (8) is given by

$$c_1'=\frac{\mathrm{A}}{\tau' q}\cdot e^{\tau' q x'}+\mathrm{B} \tag{11}$$

Where A and B are two arbitrary constants and τ' is a modified dimensionless holding time:

$$\tau'\equiv\frac{\tau}{1+p\hat{D}\tau} \tag{12}$$

The arbitrary constants A and B in eqn (11) are to be determined from the boundary conditions in the diffuser. One boundary condition is

$$c_1'\ (x'=0)=0 \tag{13}$$

(pure water in the water inlet of the diffuser). From (11) and (13) we obtain

$$\mathrm{B}=-\mathrm{A}\cdot\frac{1}{\tau' q} \tag{14}$$

The concentration profile in the solid phase can be obtained by combination of eqn (1b) with eqns (11) and (14):

$$c_s'=\mathrm{A}\left\{\left[\frac{1}{\tau' q}+\frac{1-\alpha}{\mu\alpha\tau}\,\frac{w}{v}-\hat{D}\,\frac{1-\alpha}{\mu\alpha}\,\frac{w}{v}\cdot\frac{\tau'}{\tau}\cdot q\right]e^{\tau' q x'}-\frac{1}{\tau' q}\right\} \tag{15}$$

By use of the second boundary condition

$$c_s'\,(x'=1)=1 \tag{16}$$

A can be found and then B from (14). We obtain

$$A = \left\{\left[1+\frac{1-\alpha}{\mu\alpha}\frac{\tau'}{\tau}\cdot q\frac{w}{v} - \hat{D}\tau'\frac{1-\alpha}{\mu\alpha}\cdot\frac{w}{v}\cdot\frac{\tau'}{\tau}q^2\right]e^{\tau' q} - 1\right\}^{-1}\tau' q \tag{17}$$

Since the exponential function is very sensitive to changes in the dimensionless holding time, we shall for the sake of simplicity neglect the difference between τ and τ' except for the exponentials. Also, the $\hat{D}\tau'$ term in (17) will be neglected in comparison with unity. We then have as an approximation:

$$B \cong -\left\{\left[1+\frac{1-\alpha}{\mu\alpha}\cdot q\frac{w}{v}\right]e^{\tau' q} - 1\right\}^{-1};\quad A = \tau' q\,(-B) \tag{18}$$

$$c_s' \cong -B\left\{\left[1+\frac{1-\alpha}{\mu\alpha}\cdot q\frac{w}{v}\right]e^{\tau' q x'} - 1\right\} \tag{19}$$

and c_l' given by (11).

The *draft* in the diffuser is defined as the volume ratio of process water feed to the feed of water + soluble matter contained in the apple slices, i.e.

$$Dr \equiv \frac{w}{v}\cdot\frac{(1-\alpha)}{\alpha}\cdot\frac{1}{\mu} = \frac{\text{volume of extract water/hr}}{\text{volume of juice in apples/hr}} \tag{20}$$

From the definition in eqn (10) we obtain

$$q = \frac{Dr-1}{Dr} \tag{21}$$

and with the final approximations made in eqns (18) and (19) we obtain for the two profiles:

$$c_l'(x') \cong \frac{exp\left\{\frac{Dr-1}{Dr}\cdot\tau'\cdot x'\right\}-1}{Dr\cdot exp\left\{\frac{Dr-1}{Dr}\cdot\tau'\right\}-1} \tag{22}$$

$$c_s'(x') \cong \frac{Dr\cdot exp\left\{\frac{Dr-1}{Dr}\cdot\tau'\cdot x'\right\}-1}{Dr\cdot exp\left\{\frac{Dr-1}{Dr}\cdot\tau'\right\}-1} \tag{23}$$

It is seen that the draft and the dimensionless holding time suffice to describe the profiles. If eqn (1a-b) had been solved without eddy diffusion coefficients at all, the solution would have the same form as eqn (22) and eqn (23), but with τ instead of τ'. Therefore, to a first approximation, the effect of the two eddy diffusion coefficients is just to diminish the effective holding time of the apple slices, cf. eqns (9) and (12). Hereby, the recovery of soluble material is of course diminished. This is immediately seen from eqn (23). The *loss fraction*

of soluble material is the ratio of the concentration in the extracted pomace to the concentration in the feed apples, i.e.

$$\text{Loss fraction} = c_s'(x' = 0) = \frac{Dr - 1}{Dr \cdot exp\left\{\frac{Dr - 1}{Dr} \cdot \tau'\right\} - 1} \tag{24}$$

Since $\tau' < \tau$, the loss is greater with axial mixing than without.

From eqn (24) it also appears that the limiting loss for very high drafts and fixed holding time is given by

$$\lim_{Dr \to \infty} (\text{Loss fraction}) = \exp(-\tau') \tag{25}$$

For $Dr = 1.0$ a limiting expansion of eqns (22), (23) and (24) should be used.

The dimensionless holding time corrected for axial dispersion should therefore be well above unity to obtain a proper yield from the diffuser, and the draft should be as high as permissible by the hydraulic resistance. Figure 2

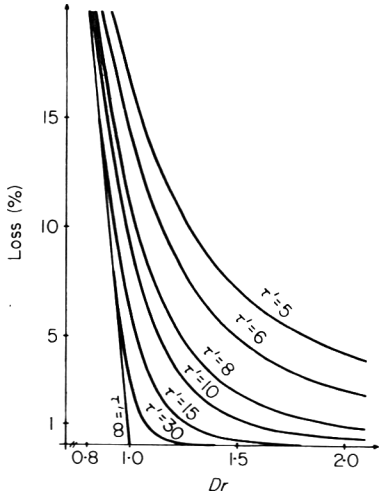

Figure 2. Calculated percentage loss as a function of draft and dimensionless holding time for the extraction. Model with only one mass transfer number.

shows the loss as a function of Dr and τ'. It appears that Dr should also be well above unity. The loss may be reduced to almost zero for Dr slightly greater than 1, but τ' has then to be very large. Since a τ' equal to say, 100, means very high capital costs (very large diffusers), this will, however, not be feasible. The advantage of working with dimensionless variables in diffuser optimizations is here made evident.

In the case of lower processing temperatures (sometimes made necessary for over-mature apples), the above model is insufficient, since there is a discontinuity in the experimental concentration gradients. This is shown in Fig. 3. Introducing the time of plasmolysis t_p as the time necessary for the

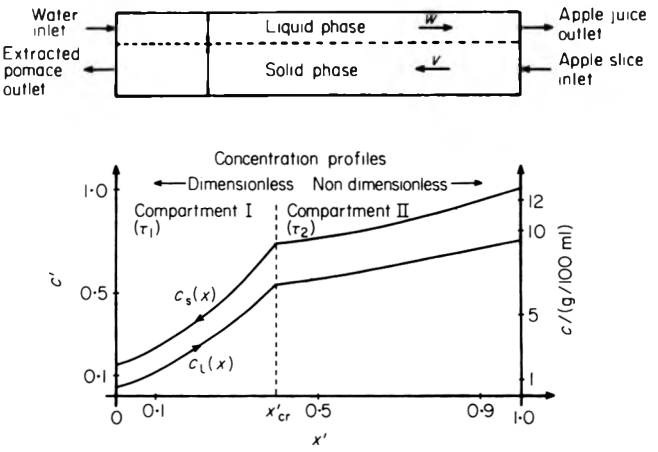

Figure 3. Concentration profiles and flow conditions in a diffuser operating at a too low temperature where plasmolysis time has to be taken into account.

breakdown of the cell plasma membrane (but *not* the cell walls) for an apple slice of a given thickness in contact with water at a given temperature, the position of the 'point of plasmolysis' in the diffuser will be given by

$$L - x_{cr} = v \cdot t_p \text{ or } x'_{cr} = 1 - \frac{v \cdot t_p}{L} \equiv 1 - \tau_2 \tag{26}$$

The mass transfer number k_1 after the plasmolysis in the section $)0 \leq x < x_{cr})$ of the diffuser will now be higher than k_2 before plasmolysis $(x_{cr} \leq x < L)$. The simultaneous eqns (1a-b) should now be solved in each section of the diffuser. The scheme of solution for each section is the same as in the simple model with only one mass transfer number. Four arbitrary constants appear, which can be eliminated using the boundary conditions in eqns (13) and (16) and in addition the conditions of continuity

$$c'_{cr} \equiv c'_l[0 \mid x'_{cr}] = c'_l[x'_{cr} \mid 1] \qquad x' = x'_{cr} \tag{27}$$

$$c'_s[0 \mid x'_{cr}] = c'_s[x'_{cr} \mid 1] \qquad x' = x'_{cr} \tag{28}$$

Calculations are elementary, but lengthy, and in case of no axial dispersion the final results are:

Section $0 \leq x' \leq x'_{cr}$:

$$c'_l\ (x') = c'_{cr} \cdot \frac{1 - exp\left\{\frac{Dr-1}{Dr} \cdot \tau_1 \cdot x'\right\}}{1 - exp\left\{\frac{Dr-1}{Dr} \cdot \tau_1 \cdot x'_{cr}\right\}} \tag{29}$$

$$c'_s(x') = c'_{cr} \cdot \frac{1 - Dr \cdot exp \cdot \left\{ \frac{Dr-1}{Dr} \cdot \tau_1 \cdot x' \right\}}{1 - exp \left\{ \frac{Dr-1}{Dr} \cdot \tau_1 \cdot x'_{cr} \right\}} \equiv c'_{cr} \cdot K_1(x') \tag{30}$$

Section $x'_{cr} \leq x' \leq 1$:

$$1 - c'_i(x') = (1 - c'_{cr}) \cdot \frac{exp \left\{ \frac{Dr-1}{Dr} \cdot \tau_2 (x' - 1) \right\} - Dr}{exp \left\{ \frac{Dr-1}{Dr} \cdot \tau_2 (x'_{cr} - 1) \right\} - Dr} \tag{31}$$

$$1 - c'_s(x') = (1 - c'_{cr}) \cdot \frac{exp \left\{ \frac{Dr-1}{Dr} \cdot \tau_2 (x' - 1) \right\} - 1}{\frac{1}{Dr} exp \left\{ \frac{Dr-1}{Dr} \cdot \tau_2 (x'_{cr} - 1) \right\} - 1}$$

$$\equiv (1 - c'_{cr}) \cdot K_2(x') \tag{32}$$

The two dimensionless holding times are given by

$$\tau_i \equiv \frac{k_i \cdot L}{v} \quad (i = 1, 2) \tag{33}$$

x'_{cr} is given by eqn (26) and the concentration in the juice in the point of plasmolysis c'_{cr} is given by

$$c'_{cr} = \frac{1 - K_2(x'_{cr})}{K_1(x'_{cr}) - K_2(x'_{cr})} \tag{34}$$

where the functions $K_1(x')$ and $K_2(x')$ were defined in eqns (30) and (32).

The loss fraction can be calculated from eqn (32) with $x' = 0$. Figure 4 shows a family of curves for the loss fraction as a function of the draft and of x'_{cr} for two dimensionless holding times τ_1 and τ_2 realizable in the Rynkeby diffuser (the holding times may be considered as corrected for axial dispersion in the manner of eqn (12)). The x'_{cr}-values may be translated to extraction temperatures by means of eqn (26) and knowledge of the relation between plasmolysis time and temperature. The temperatures in Fig. 4 were calculated using the curve given in the paper of Lüthi & Glunk (1975) for 3 mm slices of Jonathan apples (see Fig. 12). The slice thickness in the present experiments is a little bit more than 3 mm, and also an uncontrolled mixture of various apple sorts were used, so the temperatures in Fig. 4 should be taken only as a crude approximation.

Again, for $Dr = 1.0$ limiting expansions should be used.

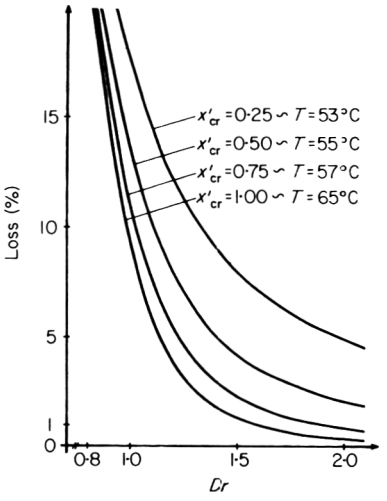

Figure 4. Calculated percentage loss as a function of draft and x'_{cr} (temp.) for constant $\tau_1 = 3.0$ and $\tau_2 = 10.0$. The connection between x'_{cr} and temperature has been calculated by using Fig. 12 and a total holding time of apple slices in the diffuser $t_{retention} = 100$ min.

Materials and methods

Diffuser

The diffuser used in the experiments, working at A/S Rynkeby Mosteri, Funen, Denmark, is shown on Fig. 5. It is operating according to the DdS-diffuser principle (De danske Sukkerfabrikker). It has a capacity of 4–5000 kg apple slices/hr which are transported through the diffuser by a double helix. Process temperature control is by a surrounding steam jacket.

The diffuser is divided into eight sections for constructional convenience, the six middle sections being 1 m long and the first and the last being 0.75 and 0.70 m long, respectively. Apple slices enter the diffuser in the middle of the first section and leave just after the seventh section while water enters between the seventh and eighth section. The extract leaves from the bottom of section one. The effective length of the diffuser (i.e. the distance over which the two phases are in contact) is $L = 6.5$ m. The depth of the diffuser is about 1.3 m and the cross sectional area about 2.2 m^2.

The slice thickness of the feed is between 3 and 5 mm depending on the physical condition of the apples used. The softer the apples, the thicker are the slices.

In the experiments reported here, the helix speed was set to give a mean holding time of around 120 min for the apple slices.

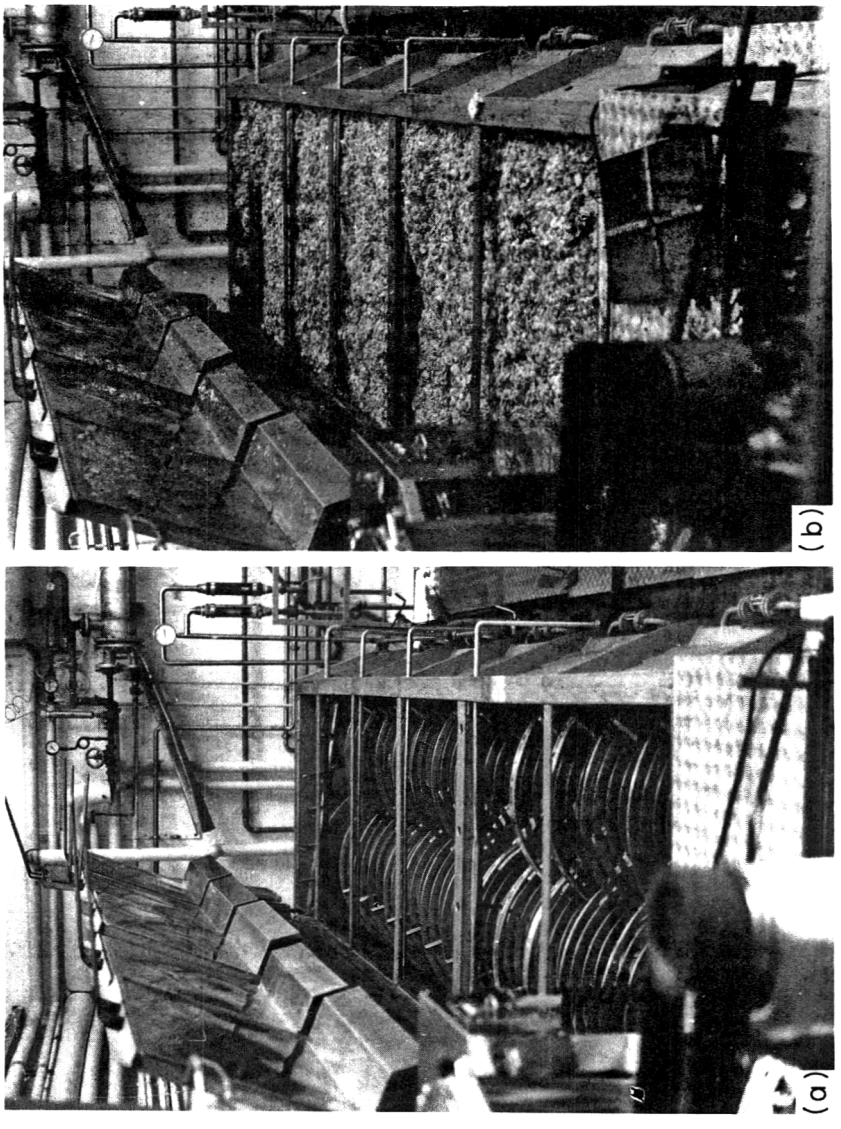

Figure 5. The Rynkeby diffuser. (a), Empty diffuser; (b), diffuser filled with apple slices and juice. The covers are normally closed during operation.

Apple varieties

The experiments reported here were carried out during the whole 1978 season (from October to December) with mixed, unspecified apples of various degrees of ripeness.

Measurement of temperature

The temperature at the bottom of each section of the diffuser was measured by a fixed thermistor (accuracy $\pm 3°C$) checked during operation by a moveable, polythene sheathed thermistor calibrated over the temperature range 10–80°C. It should be noted that, especially near the apple slice inlet, there is a temperature difference between the top layer and the bottom layer near the heating-jacket where the stationary thermistors are placed, because the apples are cold when fed into the diffuser. All temperatures reported here are measured with the stationary thermistors.

Measurements of concentration profiles through the diffuser

In order to measure the concentration profiles through the diffuser, samples of both the solid and liquid phase were drawn from the middle of each of the six middle sections (see Fig. 5) together with a sample from the first section where the apple slices have just turned once with the transport helix. Also, samples were taken from the extracted pomace outlet, from the fresh apple slices and from the juice produced.

The solid and liquid phases were first separated by means of a colander. Then, the liquid phase inside the apple slices was squeezed out by means of a cheesecloth bag. The quantities used in these samples were about 0.5 dm^3. The concentrations of soluble material in the samples were measured as described below.

Measurements of juice concentration and density

All concentration measurements of juice both in the liquid and in the solid phase were measured by means of a refractometer (Abbé '60' Refractometer (Standard) 60/95, Bellingham and Stanley Ltd) with two measurement scales. One shows the refractive index, the other *%Rt* which is g sugar/100 g pure sucrose solution. The refractometer was calibrated with pure water and with a sugar solution (10 g sugar/100 g solution).

In the sugar industry the concentration of a sugar solution is measured in degrees Brix (°Brix)—originally defined as g sucrose in 100 g sugar solution. Nowadays °Brix is used as a measure of solute matter in 100 g solution. It

Table 1. Comparison between measured density of three different fruit juice solutions and density of pure sugar solutions calculated from eqn (35) at the same concentration (°Brix)

Juice	°Brix measured (g/100 g)	20°C ρ (measured) (g/cm^3)	20°C ρ (pure sugar) (g/cm^3)
Thermally clarified apple juice	9.5	1.0373	1.0361
Diffuser juice (not clarified)	9.4	1.0384	1.0357
Cherry juice	27.0	1.1146	1.1124

might be questioned whether the directly measured $\%Rt$ is representative as °Brix (i.e. g soluble matter/100 g solution) in apple juice and other fruit juices, since the refractometer is calibrated only with pure sugar solutions. Some evidence for the approximate validity of the assumption is given in Table 1, where the measured density of three fruit juice solutions (all A/S Rynkeby Mosteri products) is compared to the calculated density of a sucrose solution with the same °Brix ($=\%Rt$). The calculated density is obtained from the formula

$$\rho^{20}\,(\text{sucrose solution}) = 0.99827 + 0.003848\,(°\text{Brix}) + 1.4072\,10^{-5}\,(°\text{Brix})^2 \quad (35)$$

which is based on data taken from *Handbook of Chemistry and Physics* (1973). The densities of the fruit juices were measured with a pycnometer at 20°C.

Measurements of density of apple slices

To convert the solid-phase flux based on weight to a flux based on volume and for calculating μ (the volume fraction of water + soluble matter in the apple slices) it is necessary to measure the density of the apple slices. This is done by weighing a given amount of apple slices and by measuring the volume of water displaced.

The density of the apple slices have been measured by this method on seven different samples with the result $\rho_{as} = 0.913 \pm 0.006$ kg/dm^3 ($_{as}$ ~ apple slices).

Content of soluble and total dry matter in apple slices

If we call the density of the apple slices ρ_{as}, one gram of apple slices takes up a volume equal to $1/\rho_{as}$ cm^3. With y being the number of grams of insoluble matter in 100 g apple slices we have that one gram of apple slices contains $(1 - y/100)$ g juice which again takes up a volume of $(1 - y/100)/\rho_{juice}$ cm^3. The volume fraction of juice in the apple slices is therefore given by

$$\mu = \frac{\rho_{as}}{\rho_{juice}}\,(1 - y/100) \quad (36)$$

In 100 g apple slices we have $(100-y)$ g juice and the amount of soluble matter in 100 g slices is (°Brix/100) · $(100-y)$ grammes. The total amount of dry matter in 100 g slices is therefore

$$DRY=\left(\frac{\text{°Brix}}{100}\right)(100-y)+y$$

The weight percent of insoluble dry matter in 100 g slices may thus be calculated as

$$y=\frac{DRY-\text{°Brix}}{1-\text{°Brix}/100} \tag{37}$$

For a given sample of apple slices it is possible to measure ρ_{AS}, ρ_{ju} and °Brix as described above and by drying a known amount of apple slices in an oven (105°C) to constant weight it is possible to determine *DRY*.

These measurements have been carried out on samples of apple slices taken from the apple inlet and it is found that μ is about 0.85 for the fresh apple slices. Further, μ has been determined through the diffuser as a function of x'. The results are given in Table 2 where it is seen that μ is increasing for decreasing x' and not strictly constant as assumed in the developed models.

Table 2. Measured composition of apple slices

Secton number	x'	ρ (apple) measured (g/cm³)	°Brix measured (g/100 g)	ρ (juice) (g/cm³)	Total dry matter g/100 g apple	Unsoluble dry matter g/100 g apple	μ (cm³/cm³)
Apple feed	1.00	0.911	10.77	1.0412	12.26	1.67	0.860
2	0.88	0.941	9.01	1.0341	11.23	2.44	0.888
4	0.57	0.954	8.66	1.0316	10.19	1.68	0.909
6	0.26	0.957	6.02	1.0219	8.89	3.05	0.908
8	0.00	0.968	1.39	1.0036	3.80	2.44	0.941

Measurements of apple slice retention time and axial dispersion of apple slices in diffuser

The apple slice retention time has been measured by means of packing apple slices into tightly fitting, coloured plastic bags and feeding the diffuser with such marker apple slices at $x'=1.0$ (section one) at the time $t=0$. The plastic bags are then caught when they are leaving the diffuser ($x'=0.0$) at the time $t=t_{retention}$. A retention time distribution is hereby produced. In these experiments about 100 marker apple slices with different surface areas were

used. A typical result is shown in Fig. 6. The approximate surface areas of the plastic bags were the following:

Bag number	½ (surface area)
1–30	6.3 cm²
31–60	12.3 cm²
61–90	25.0 cm²

As seen from Fig. 6, forty-six bags have been caught at $x' = 0$. Seventeen bags were picked up in the diffuser and the remaining twenty-seven bags were lost (not caught). There seems not to be any significant difference in holding time for the different sizes of bags.

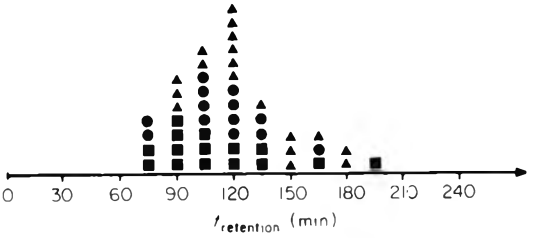

Figure 6. Experimental distribution of retention times. ■, Small bags; ●, medium bags; ▲, large bags. See text for further details.

The retention time distribution shown in Fig. 6 is not strictly Gaussian, but seems to have a 'long time tail'. It should be noted, that this should also be the case, even when the momentary position distribution in the diffuser can be assumed Gaussian, since the bags coming out last are sampled from a broader position distribution than the bags coming out first. For simplicity we shall ignore this effect, however. Then we have the following connection between the standard deviation of the retention time distribution (σ_t) and the standard deviation of the 'mean' position distribution (σ_x):

$$\sigma_t \cong \frac{\sigma_x}{v} = \frac{\sqrt{2 \cdot D_e^{(s)} \cdot t_{retention}^{mean}}}{v} \tag{38}$$

From the retention time distribution (Fig. 6) we have $\sigma_t = 30$ min. The last eqn (38) is obtained by means of Einstein's formula for random walk. The mean velocity of the apple slices (v) is equal to $L/t_{retention}^{mean}$. Using this together with eqn (38) and the definition of $\hat{D}^{(s)}$ we obtain

$$\hat{D}^{(s)} = \frac{D_e^{(s)}}{L\,v} = \frac{\sigma_t^2}{2 \cdot (t_{retention}^{mean})^2} = \frac{30^2}{2.120^2} = 0.031 \tag{39}$$

corresponding to a $D_e^{(s)} \sim 0.01\ m^2/min$. ($L_{eff} = 6.5$ m).

Results and discussion

One mass transfer number (high process temperature)

In Fig. 7 the theoretically calculated dimensionless concentration profiles are shown for fixed dimensionless holding time of apple slices in the diffuser (τ') and varying drafts when the process temperature is high, i.e. about 58°C or more. It is seen that the concentration profiles are strongly dependent on the draft and therefore also the loss of soluble material i.e. c_s' ($x = 0$)—see also Fig. 2. It should be noted that linear concentration profiles as reported by Bingley & Wiley (1978) in their experiments only occur for $Dr = 1.0$ and that this is not the optimal draft, i.e. the loss is too big (see Fig. 2).

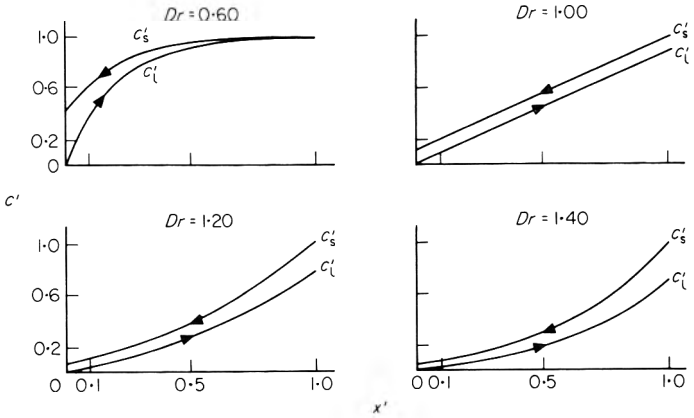

Figure 7. Four calculated sets of dimensionless concentration profiles calculated from the model with only one mass transfer number. The concentration profiles are shown as a function of Dr for constant dimensionless holding time $\tau' = 7.5$.

In Fig. 8 one set of dimensionless concentration profiles based on experimental values measured at high process temperatures are given together with calculated dimensionless concentration profiles. The experimental values are mean values of measurements taken with 2 hours' interval. The dimensionless concentration profiles based on experimental values are obtained by division of all the measured concentrations in both the liquid and solid phase by the measured concentration of the juice in the fresh apple slices ($c_s'(x' = 1.0)$) when this is not much greater than the measured value of the concentration of the juice in the solid phase in section 1.

Because of the structure of the solid phase in the apples, the cut apple slices can be thought of as being like sponges. When the apple slices are cut some pure apple juice is squeezed out from the solid phase. Because of the sponge-like structure of the apple slices this squeezed, pure apple juice will be replaced by the liquid contained in section 1 (where the apple slices are fed into the diffuser) when they are immersed in this liquid i.e. when they are fed

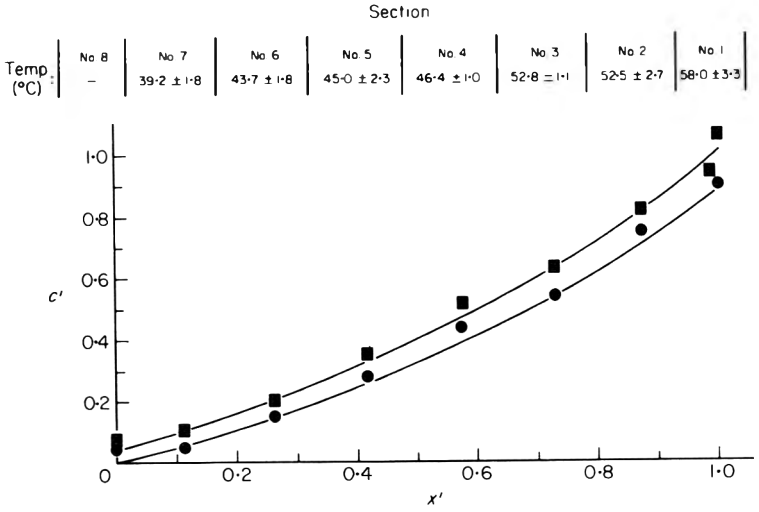

Figure 8. Measured and calculated concentration profiles for diffuser operating at a sufficiently high temperature. ■, Measured concentration of the juice contained in the solid phase; ●, measured concentration of the juice in the liquid phase. The theoretical concentration profiles are calculated for $\tau' = 12.0$ and $Dr = 1.10$. At the top of the figure the eight different sections are indicated together with the measured temperatures.

into the diffuser. The sponge-like structure of the apple slices will result in a considerable difference between the concentration of the liquid phase contained in the solid phase of the fresh apple slices just before they are fed into the diffuser and the concentration of the liquid phase contained in the solid phase of the apple slices when they are immersed in the free juice in section 1. This difference in concentration in the solid phase depends therefore on the physical structure of the apple slices (variety, ripeness, thickness, etc.) and on the knives in the cutter, and the concentration of the juice leaving the diffuser.

Because of the above mentioned effect, it is necessary to make the concentration profiles dimensionless with a value of c_s lying between $c_s(x' = 0)$ and c_s (section 1) because neither of the two models takes into account this juice dilution effect in the apple slices. Note that by means of this procedure c'_s $(x = 0)$ becomes greater than one.

In Fig. 8 the sections are indicated together with the mean values and standard deviations (s.d.) of the measured temperatures in the different sections. It is seen that the s.d. is somewhat higher in the two first sections and the reason is that the cold apples are fed in here.

In Fig. 8 it should be noticed, that the experimental value of $c'_l(x' = 0) \neq 0$ whereas the theoretical value of $c'_l(x' = 0) = 0$ according to the boundary conditions given in eqn (13). Of course $c'_l(x' = 0) = 0$ is an approximation implying that at $x = 0$ (the water inlet) the liquid phase consists of pure water. This is not so because of the eddy diffusion in the liquid phase which has been discussed earlier.

Taking into account the assumptions made and the experimental uncertainties (mixed apple varieties etc.) there seems to be an acceptable agreement between the experimental concentration profiles and the theoretical calculated profiles for high process temperature.

Two mass transfer numbers (low process temperature)

If the process temperature is low, i.e. less than about 58°C, the concentration profiles through the diffuser break into two parts (see Fig. 4) and the model with only one mass transfer number is insufficient to describe the extraction since it does not take into account the time of plasmolysis. For a low process temperature it is therefore necessary to use the model with two mass transfer numbers. In Fig. 9 the concentration profiles have been calculated for constant draft, mass transfer numbers and total holding time but for varying x'_{cr} which is a function of the process temperature. $x'_{cr} \rightarrow 0$ corresponds to no plasmolysis in the diffuser (very low process temperature) whereas $x'_{cr} \rightarrow 1$ corresponds to immediate plasmolysis (high process temperature).

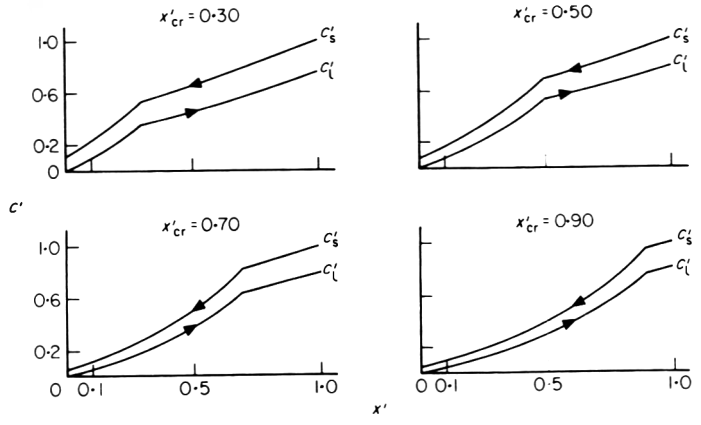

Figure 9. Four calculated sets of dimensionless concentration profiles as a function of x'_{cr} at low process temperature. The calculations have been carried out by the model with two mass transfer numbers setting $\tau_1 = 10.0$, $\tau_2 = 3.0$ and $Dr = 1.20$.

It is seen from Fig. 9 that the concentration profiles are strongly dependent on the temperature (x'_{cr}) which is also the case for the loss i.e. $c'_s(x' = 0)$. Note also that for $x'_{cr} \rightarrow 1$ the effect of the break point vanishes and we are back in the model with only one mass transfer number.

In Fig. 10 calculated concentration profiles are shown for constant x'_{cr}, mass transfer numbers and total holding time but for varying drafts. Note that both the shape of the profiles, the loss and the concentration of the produced juice is strongly dependent on the draft. Also note that it is only for $Dr = 1$ that the concentration profiles are linear just as in the previous model.

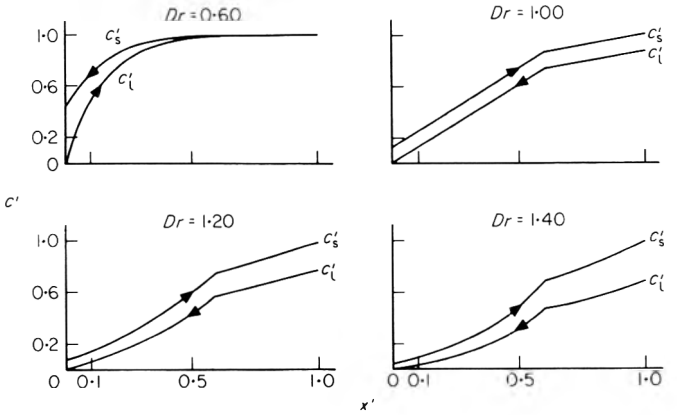

Figure 10. Four calculated sets of dimensionless concentration profiles as a function of Dr for constant $x_{cr} = 0.60$, $\tau_1 = 10.0$ and $\tau_2 = 3.0$. The calculations are based on the model with two mass transfer numbers.

In Fig. 11 dimensionless concentration profiles based on experimental values measured at low process temperatures are given together with calculated theoretical dimensionless concentration profiles. The experimental dimensionless concentration profiles are constructed as described above for the measurements at a high process temperature and are also based on mean values of two sets of measurements taken with 2 hours' interval. The profiles are linear, since the draft is equal to 1. The loss is large, since we have both that $Dr = 1.0$ and that the process temperature is low. Note again the

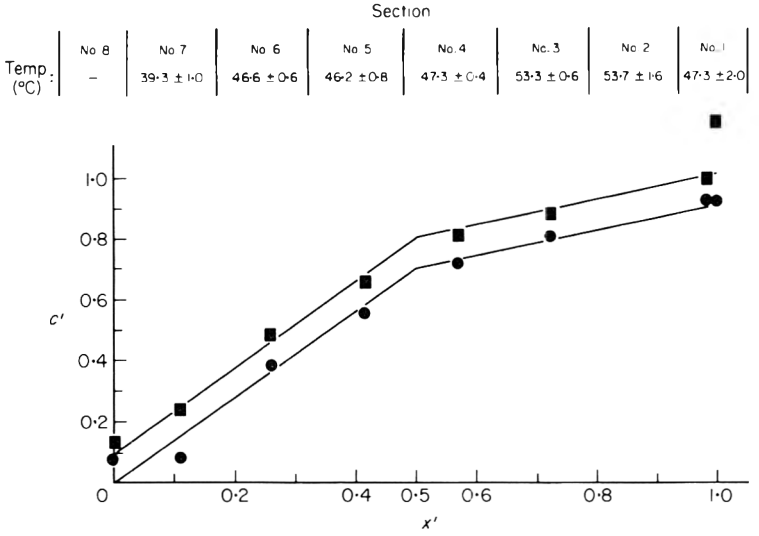

Figure 11. Measured and calculated concentration profiles for diffuser operating at low process temperature. ■ , Measured concentration of juice contained in the solid phase; ●, measured concentration of juice in the liquid phase. The theoretical concentration profiles are calculated for $x'_{cr} = 0.50$, $\tau_1 = 14.0$, $\tau_2 = 4.0$ and $Dr = 1.0$. At the top of the figure the eight different sections are indicated together with the measured temperatures.

difference between $c_s(x' = 1.0)$ and c_s (section 1) due to the 'sponge dilution' process in the apple slices as described above. Also note the difference between the experimental and theoretical value of $c_1(x' = 0)$ due to eddy diffusion—cf. Fig. 8.

In Fig. 11 the eight different sections are indicated and the mean value of the measured temperatures together with the standard deviations (s.d.) are also given. As in Fig. 8 the s.d. is also somewhat higher for the two first sections, since the cold apples are fed for $x' = 1.0$. In this case there seems to be good agreement between experimental and theoretical concentration profiles in Fig. 11. However, the process temperature is not optimal in the case with a break point and the process temperature should be higher to displace x'_{cr} toward unity as in Fig. 8. The draft should also be greater than unity, see Fig. 4.

How to run a diffuser

From the above results it is seen that the process temperature is very important in running a diffuser. Figure 4 indicates that the optimum process temperature is above 57°C and that a rise from this temperature will not increase greatly the yield from the extraction process. On the other hand a reduction of the process temperature below 57°C will increase the loss considerably. This is also in agreement with the experimental measurements given in Figs 8 and 11.

The connection between x'_{cr} and the process temperature indicated in Fig. 4 has been calculated by means of Fig. 12 where the time of plasmolysis is shown as a function of temperature for 3-mm thick Jonathan apple slices measured by Dousse (see Lüthi & Glunk (1975)). If we use Fig. 12 to estimate the time of plasmolysis for the process conditions given in Fig. 8, it can be seen that the time of plasmolysis is just below 15 min (58°C) which should be sufficient to describe the measured concentration profiles by the model with only one mass

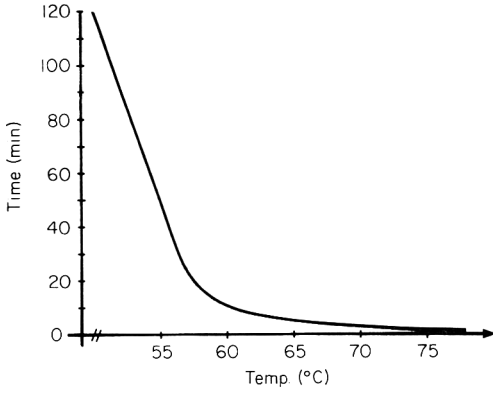

Figure 12. Experimental determination of the plasmolysis time as a function of temperature for 3-mm thick Jonathan apple slices (Lüthi & Glunk, 1975).

transfer number which was also the case in Fig. 8. The mean holding time for apples in the first section is around 15 min.

For the process condition given in Fig. 11 it is seen from Fig. 12, that the time of plasmolysis is about 60 min (53–54°C). From Fig. 6 the mean value of the total holding time is 120 min, so a time of plasmolysis at 60 min corresponds to $x'_{cr} = 0.50$ which is in excellent agreement with the x'_{cr} value used in calculating the theoretical concentration profiles shown in Fig 11, i.e. exactly $x'_{cr} = 0.50$. Therefore, it seems that Fig. 12 can also describe the time of plasmolysis as a function of temperature for the mixed apple varieties used in our measurements.

It is seen from Figs 2 and 4 that next to the process temperature the draft is very important for the yield. For *Dr* less than unity, the loss is very high, but as soon as *Dr* is just a little higher than unity, the loss decreases remarkably.

At the Rynkeby plant, the apple juice produced is clarified in a tank immediately after leaving the diffuser and the clarified juice is then concentrated in a steam evaporator. Because of the capacities of these two operations it has not been possible to make an intensive experimental study of the influence of the draft on the extraction process. In Table 3 the results are given for the comparison of fifteen sets of experimental concentration profiles and theoretically calculated ones. The fifteen experiments were spread over five days which were spread over a month. Table 3 shows that in thirteen of these experiments draft is between 1.00 and 1.10 which is somewhat below the optimal draft when compared with Fig. 2.

As seen from Fig. 2 the holding time of the apple slices is important for the extraction, but not as much as are the process temperature and the draft and we have, therefore, in all the experiments used the same transport helix speed

Table 3. Results of comparison between experimental, measured and theoretically calculated concentration profiles

Trial nr	Number of mass transfer numbers	Draft	x'_{cr}	τ_1	τ_2
12	1	1.20	—	8.0	—
15	1	1.10	—	12.0	—
16	1	1.10	—	12.0	—
29	1	1.00	—	10.0	—
23	2	1.10	0.3	12.0	3.0
27	2	1.05	0.6	12.0	4.0
28	2	1.05	0.7	12.0	4.0
21	2	1.00	0.5	14.0	4.0
18	2	1.00	0.3	13.0	5.0
20	2	1.00	0.4	12.0	4.0
22	2	1.00	0.5	12.0	4.0
17	2	1.00	0.6	12.0	4.0
25	2	1.00	0.5	12.0	3.0
26	2	1.00	0.6	12.0	2.0
24	2	0.90	0.6	9.0	2.0

corresponding to a mean holding time of the solid phase at 120 min (cf. Fig. 6). From the results given in Table 3 together with $t_{\text{retention}}^{\text{mean}} = 120$ min it is possible to estimate the effective mass transfer coefficients k_1 and k_2. From eqn (7) we have

$$k_i = \tau_i / t_{\text{retention}} \qquad i = 1, 2 \tag{40}$$

The effective mass transfer number *before* plasmolysis is about $k_2 = 0.03$ min^{-1} and the effective mass transfer number after plasmolysis is about $k_1 = 0.10$ min^{-1}.

Østerberg & Sørensen (1980) have carried out an energy-economy optimization taking into account that the apple juice produced is concentrated in steam evaporator on leaving the diffuser to around 70°Brix before it is stored in tanks. The result was a determination of an optimal draft of around 1.5 due to the increase in steam costs with increasing draft. It should be mentioned, that with a draft of around 1.5 and a temperature of around 60°C, the recovery of soluble matter from uncut apples to final juice is now (1980) as high as 90% at Rynkeby after an extension of evaporator capacity. However, a more detailed analysis should be made taking into consideration also capital costs and alternative methods of juice concentration, such as reverse osmosis.

Acknowledgments

We wish to thank A/S Rynkeby Mosteri, Denmark for permission to experiment with their industrial diffuser, Dagmar Andreasen for her great hospitality and Bent Bach Sørensen and Ole Møller for useful discussions.

List of symbols

v	linear (mean) velocity of apple slices
w	linear (mean) velocity of liquid phase
c_l	concentration of soluble dry matter in the liquid phase
c_s	concentration of soluble dry matter in the solid phase
x	distance
$D_e^{(l)}$	eddy diffusion coefficient (coefficient of axial mixing) for the soluble dry matter in the liquid phase
$D_e^{(s)}$	eddy diffusion coefficient for the apple slices
k	effective mass transfer coefficient
x'	dimensionless position in diffuser defined by eqn (2)
L	effective length of diffuser
c'	dimensionless concentration defined by eqn (3)
R	ratio of eddy diffusion coefficients defined by eqn (5)
$\hat{D}$	dimensionless eddy diffusion coefficient defined by eqn (6)

p	dimensionless quantity defined by eqn (9)
q	dimensionless quantity defined by eqn (10)
A	integration constant
B	integration constant
Dr	draft defined in eqn (20)
t_p	time of plasmolysis at given process temperature
x_{cr}	position of 'point of plasmolysis' in diffuser
$K_1(x')$	function defined by eqn (30)
$K_2(x')$	function defined by eqn (32)
y	percentage of insoluble dry matter in the solid phase
α	fraction of the total volume used in the diffuser which is occupied by apple slices
μ	volume fraction of water + soluble dry matter in the apple slices
τ	dimensionless holding time of apple slices in diffuser defined by eqn (7)
τ'	dimensionless holding time corrected for eddy diffusion (axial mixing) defined by eqn (12)
ρ	density
σ_t	standard deviation of retention time distribution of apple slices
σ_x	standard deviation of position distribution of apple slices
°Brix	grams of soluble dry matter in 100 g solution
$\%Rt$	grams of sucrose in 100 g sucrose solution

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Swelling and solubility behaviour of parboiled rice flour

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Summary

Parboiled rice flour swelled and dissolved more than raw rice flour in water at temperatures below 70°C, but less than raw rice at higher temperatures. This difference between raw and parboiled rice increased with an increasing degree of parboiling. A sample of parboiled rice produced by dry-heating soaked paddy in hot sand behaved differently; but when it was wetted and tempered to favour reassociation of starch, its properties fell in line with normal steam-parboiled rice. The above behaviours of raw and parboiled rice flour were similar to those of corresponding whole-grain rice. They also reinforce the earlier suggestion of starch reassociation in conventional parboiled rice.

Introduction

In previous publications from this laboratory (Ali & Bhattacharya, 1972a,b, 1976a,b), we pointed out a number of differences between the properties of parboiled rice and those of raw rice. In particular it was shown that parboiled rice grains absorbed more water and gave greater dissolved amylose than raw rice grains when soaked in water at low temperatures (below 75°C) but absorbed less water and gave lower dissolved amylose than raw rice at higher temperatures. Parboiled rice flour also gave a slightly higher sedimentation volume and cold-slurry viscosity than raw rice flour when suspended in water. Evidence was brought forward to suggest that these differences were related to some kind of reassociation or retrogradation of starch in parboiled rice, occurring after its gelatinization during the relatively slow drying of the rice after steaming. When soaked paddy was, on the other hand, parboiled by

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dry-heating in hot sand (instead of by steaming), gelatinization and dehydration took place simultaneously, resulting in reduced retrogradation.

In continuation of the above studies, it was thought of interest to investigate the comparative swelling and solubility behaviours of raw and parboiled rice flours—which are key measures of certain starch properties (Leach, 1965). Moreover it was pointed out in a recent study (Ali & Bhattacharya, 1980) that the characteristic differences in the viscograms between raw and parboiled rices strongly suggested that granule swelling was retarded after parboiling. This hypothesis too could be tested from the present studies.

Materials and methods

As in all the earlier studies of this series, Ratna Chudi (S 749) paddy, a local tall *indica* high-amylose variety, procured from the local market and stored in the laboratory for about a year, was used. The moisture content of the sample was approximately 12%. Parboiling was carried out by soaking paddy in warm water overnight (Bhattacharya & Indudhara Swamy, 1967) and then steaming it under different pressures for different times to produce rice parboiled to various degrees. To study the effect of dry-heating and of subsequent retrogradation, another sample of soaked paddy was heated in hot sand (Ali & Bhattacharya, 1976) to produce dry-heated parboiled rice, a portion of which was then allowed to retrograde by raising its moisture content and tempering. The precise conditions of preparation of the samples and their designation are listed in Table 1.

Table 1. Description of rice samples

Rice	Abbreviation	Treatment*
Raw	—	Nil
Mildly parboiled	L	Steamed at 0 psig 10 min
Severely parboiled	S	Steamed at 0 psig 60 min
Very severely parboiled	VS	Steamed at 15 psig 60 min
Dry-heated	D	Dry-heated in sand at 250C for 60 sec down to 18% moisture (wet basis)
Dry-heated and retrograded	D-R	Above rice raised to 27% moisture (wet basis) by mixing with water, held for 60 hr, then shade dried

*In each case (except raw), paddy is first hydrated in warm water overnight to about 30% moisture (wet basis), then steamed or roasted as described, shade dried to about 13% moisture, and milled. Raw (untreated) paddy was milled as such.

Paddy was milled in the laboratory using a McGill sample sheller and a McGill miller No. 1 to 7–9% degree of milling by weight. The milled rice was ground in a Buhler disc grinder (type MLI 204) followed by further grinding in a Micro-Wiley mill to pass through a 60-mesh screen.

Swelling power and solubility of the samples were determined essentially as prescribed by Schoch (1964). However, a smaller quantity of material (500 mg flour in 25 ml water) was used for each experiment and stirring was done intermittently with a thin glass rod kept immersed in the mixture. Dissolved amylose was determined in the supernatant liquid by the method described earlier (Ali & Bhattacharya, 1972a).

Results and discussion

The various results have been shown in Figs 1–3. Figure 1 shows that the swelling of rice flour in water is a two-stage process, as has been suggested by Elder & Schoch (1959) for cereal starches. But what is of greater interest for the present purpose is the clear gradation in the swelling power from raw rice to parboiled rices of increasing severity. In all cases parboiled rices swelled more readily than raw rice at temperatures below 70°C and less readily than raw rice at higher temperatures; this difference increased with an increasing degree of parboiling. It is to be noted that this is also precisely the result that was obtained with whole-grain raw and parboiled rices (Ali & Bhattacharya, 1972a). In other words, swelling of rice flour gives results identical in pattern to those given by whole-grain rice. It is of interest that dry-heated parboiled rice (D) gave a pattern which is slightly different from that of normal parboiled rices. It no doubt swelled very highly at low temperatures, simulating extremely heavily-parboiled rice, but its swelling at high temperatures was not

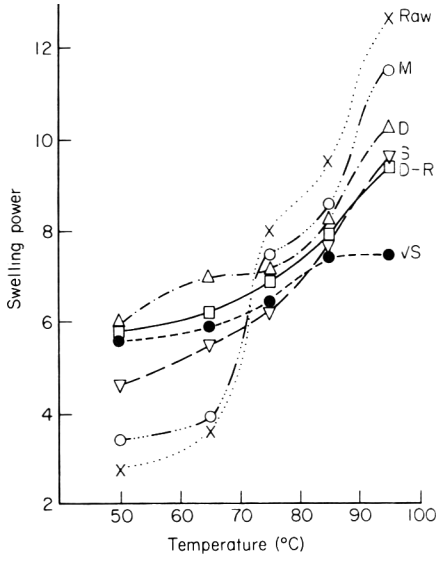

Figure 1. Swelling power of raw and variously parboiled rice flours in water at different temperatures. ×, Raw; O, mildly parboiled; ▽, severely parboiled; ●, very severely parboiled; △, dry-heated; □, dry-heated and retrograded.

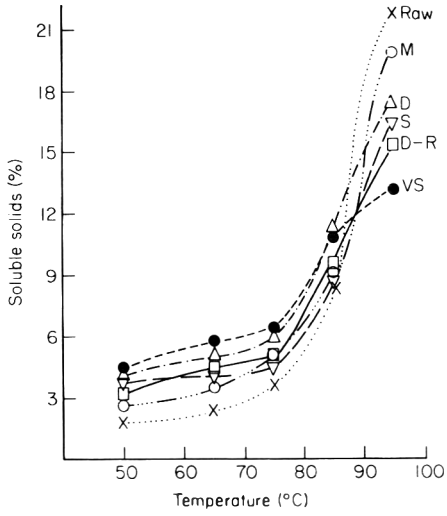

Figure 2. Solubility of raw and parboiled rice flours in water at different temperatures. For key, see Fig. 1.

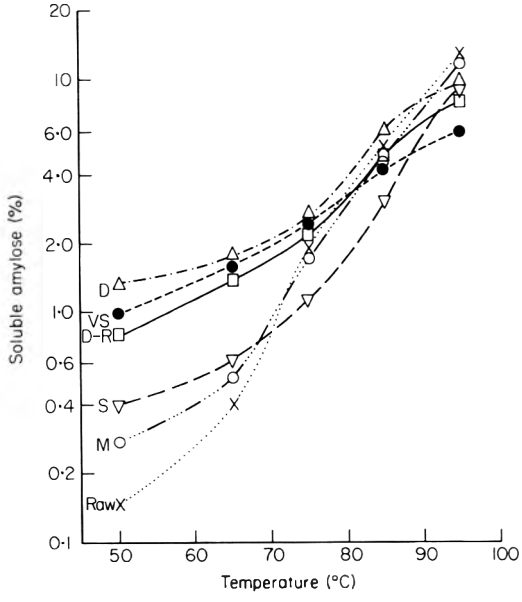

Figure 3. Amylose solubility of raw and parboiled rice flours in water at different temperatures. For key, see Fig. 1.

as low as could be expected therefrom. This is again very similar to the behaviour of whole-grain dry-heated parboiled rice (Ali & Bhattacharya, 1976a), which has been interpreted earlier from various data to be due to prevention of retrogradation of its starch on account of rapid dehydration during gelatinization by dry-heating (Ali & Bhattacharya, 1976b). The present results are thus in agreement with the hypothesis proposed earlier. In further agreement, it is found that dry-heated and retrograded rice, which was

obtained merely by moistening the roasted rice and tempering it to facilitate starch retrogradation, immediately showed a lower swelling power both at low and high temperatures, thus simulating normal severely parboiled rice.

Solubility of solids (Fig. 2) and of amylose (Fig. 3) results also were very similar to the above, giving essentially an identical pattern. The amylose-solubility data were very close to those observed earlier (Ali & Bhattacharya, 1972a) both with whole-grain and powdered rice. These results thus further confirm the trends revealed with respect to swelling power.

Conclusions

Three conclusions can be drawn from the above studies.

(1) While swelling and solubility behaviour of raw and parboiled rice flours reveal a characteristic and interesting pattern, it is clear from a comparative study of the present and the earlier results that nearly as much information is obtained by studying the whole-grain rices rather than rice flour. Water uptake by whole-grain rice being easier to study, it is to be preferred over the more sophisticated study of the swelling and solubility of rice flour.

(2) The present results support the earlier hypothesis that starch in parboiled rice is partially retrograded or reassociated and that this reassociation is prevented in dry-heated parboiled rice.

(3) The present results explain to a large extent the characteristic viscograms given by parboiled rice. We have recently shown (Ali & Bhattacharya, 1980) that parboiled rice viscograms show a higher 'gelatinization temperature' (i.e., the temperature of initial rise in viscosity), a lower peak viscosity and a lower breakdown and setback compared to raw rice when studied at identical slurry concentrations; when studied at identical peak viscosities, parboiled rice viscograms show a lower 'relative breakdown' and a higher viscosity value corresponding to the first perceptible breakdown. Moreover, these differences increase with an increasing degree of parboiling. These peculiarities were interpreted to suggest that swelling of rice starch granules probably was retarded by parboiling. It is interesting that the present results have clearly shown that parboiled rice flour has a reduced swelling power compared to raw rice flour at high temperatures and that the extent of this reduction is proportional to the degree of parboiling of the rice. The present results thus fully confirm earlier speculation.

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A comparative study of methods for prediction of water activity of multicomponent aqueous solutions

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Summary

Three methods of predicting the water activity of ternary and quaternary aqueous electrolyte and non-electrolyte solutions, with possible application to intermediate moisture foods, have been critically examined and their predictive accuracies compared. In general, the Ross equation was found to display poorer predictive accuracy when compared with the other two methods. The Zdanovskii–Stokes–Robinson and the Ferro Fontán–Benmergui–Chirife equations generally gave predictive water activity values in good agreement with measured values, but no consistent advantage of one over the other was observed.

Introduction

The basic principle underlying the intermediate moisture preservation of foods is the prevention or retardation of microbial (chiefly bacterial) growth and spoilage effected primarily through the judicious control of water activity (a_w). Other deteriorative processes are also profoundly affected by the activity of water. In general, IMF are known to be particularly susceptible to non-enzymic browning and oxidative rancidity (Karel, 1975; Labuza, 1975). Modern IMF production methods have been discussed by many workers (Brockmann, 1970; Kaplow, 1970; Potter, 1971; Heidelbaugh & Karel, 1975; Karel, 1973, 1976). These include infusion techniques involving the soaking and/or cooking of solid food pieces, either moist or previously dehydrated, in an equilibration solution containing the desired solutes or humectants (such as polyols, sugars and salts) to give the required a_w lowering effect. The blending of food components in the appropriate proportions, coupled with cooking or extrusion, can also be used to produce a finished product with the desired a_w.

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The ability to predict, with reasonable accuracy, the water activity of multicomponent food systems would obviously lead to easier and more efficient formulation and preparation of IMF. For example, the ability to estimate the a_w of complex infusion solutions from the composition would enable easy adjustments of solution composition to attain desired a_w levels. It is unlikely that a single solute would be able to depress a_w sufficiently without adversely affecting the organoleptic properties of the food as a result of the high concentration required. The limited range of suitable humectants available which are compatible with flavour at high concentrations is one of the major factors holding back the progress in the commercial production of new IMF for human consumption. Various combinations of electrolytes and non-electrolytes have been used in attempts to achieve the desired degree of a_w depression without impairing the flavour of the finished products. Certain combinations of solutes may also give rise to synergistic effects in a_w depression (Bone, 1973; Loncin, 1975). Examples include such combinations as sodium chloride–sodium lactate, sodium chloride–propylene glycol, and mannitol–sucrose. Sloan & Labuza (1975) and Flink (1977) have discussed the alternative humectants most commonly used in IMF.

Many mathematical models, ranging from the empirical to the completely theoretical and of varying complexity, accuracy and applicability, have been proposed to predict the water activity of multicomponent aqueous solutions (Zdanovskii, 1936; Robinson & Bower, 1965; Norrish, 1966; Stokes & Robinson, 1966; Reilly, Wood & Robinson, 1971; Meissner & Kusik, 1973; Pitzer & Kim, 1974; Ross, 1975; Ferro Fontán, Benmergui & Chirife, 1980). In most cases, the effects of specific solute-solute interactions are neglected, assumed to be either absent or mutually self-cancelling. Sangster & Lenzi (1974) have critically examined some of these methods and have made recommendations as to the best choice depending upon the data available, the accuracy required and the type of solute.

The present work deals with the comparative a_w predictive accuracies of three models which, from a search of the literature to date, appear to be suitable for use in estimating the a_w of complex aqueous solutions in connection with the fabrication of IMF. The predictive models to be discussed here are those of Zdanovskii–Stokes–Robinson (Chen *et al.*, 1973), Ross (1975), and Ferro Fontán *et al.* (1980). The first two named are also suitable for predicting a_w of non-electrolyte solutions whilst the last is strictly limited to electrolyte solutions, but may conceivably be extended to include systems containing non-electrolytes by treating non-electrolytes as 1–1 electrolytes.

The Zdanovskii–Stokes–Robinson (ZSR) relation

Zdanovskii (1936) found that linear or approximately linear relations exist between the molalities of many isopiestic aqueous solutions of electrolytes. Given a mixed solution of individual solutes at concentrations, m_i, in isopiestic

equilibrium with binary solutions of molality, m_{oi}, then:

$$\sum_i \frac{m_i}{m_{oi}} = 1, \text{ at constant } a_w$$

Stokes & Robinson (1966) independently derived the above equation for aqueous ternary non-electrolyte solutions, assuming semi-ideal behaviour of the mixed solutions. Chen *et al.* (1973) have applied this equation to predict a_ws of fifty-one representative ternary electrolyte/electrolyte, electrolyte/non-electrolyte and non-electrolyte/non-electrolyte systems and have obtained good agreement between calculated and experimental a_w values. However, as Sangster & Lenzi (1974) have pointed out, the predictive accuracy of this method, which neglects specific solute-solute interactions, will diminish at higher concentrations (usually above 12 m or below 0.85 a_w) due to increasing solute-solute interactions. The ZSR method requires the use of binary data of solutions at the same a_w in contrast to the Ferro Fontán–Benmergui–Chirife (1980) equation which uses binary data of solutions at the same total ionic strength as the mixture. A limitation encountered with the ZSR method is that, for reliability and feasibility of the calculation, the unknown a_w of the mixture must lie in the common region of the binary data available, i.e.

$$(a_w)_{\text{mixture}} > (a_w)^+$$

where $(a_w)^+$ is the highest of the limiting water activities of the binary data. Sangster & Lenzi (1974) observed that the more complicated equations of Reilly, Wood & Robinson (1971) and Meissner & Kusik (1973) have no consistent advantage in a_w predictive accuracy over the simpler ZSR model and the Robinson & Bower (1965) equation. They recommended that the ZSR method be used for solutions containing non-electrolytes.

The Ross equation

This equation, based on the Gibbs-Duhem relationship, has been vigorously tested by many workers and appears to provide a reasonably accurate and simple means of estimating a_w of multicomponent aqueous systems over the intermediate and high a_w ranges (Bone, Shannon & Ross, 1975; Chuang & Toledo, 1976; Sloan & Labuza, 1976; Sloan, Schlueter & Labuza, 1977; Chirife, 1978; Chirife, Ferro Fontán & Benmergui, 1980). The Ross equation can be conveniently expressed as:

$$a_w = \pi_i (a_w^o)_i$$

where $a_w^o)_i$ is the water activity of a binary solution of a given solute, i, at the same concentration and temperature as in the multicomponent system.

The Ferro Fontán–Benmergui–Chirife (FBC) model

Ferro Fontán *et al.* (1980) proposed a relation between a_w of a mixed solution and the a_ws of the related binary solutions at the same total ionic strength, thus:

$$a_w = \pi_i a_{wi}(I)^{I_i/I} = \pi_i a_{wi}(I)^{m_i/m_i(I)}$$

where $a_{wi}(I)$ = water activity of the binary solution, i, at the same total ionic strength, I, of the mixed solution
I_i/I = ionic strength fraction of solute i in the mixed solution
m_i = molality of solute i in the mixed solution
$m_i(I)$ = molality of solute i at the same total ionic strength as the mixed solution

The FBC method suffers from the same limitation evident also in the equations of Robinson & Bower (1965), Reilly *et al.* (1971), and Meissner & Kusik (1973) in that individual binary a_w data must be available at the same total ionic strength. Strictly, these equations can only be applied to solutions of electrolytes, since ionic strength is undefined for a non-electrolyte and there is some question as to the valency of polyelectrolytes (Ise & Okubo, 1967). Chen *et al.* (1973) have, however, shown that it is possible to extend the applicability of the Robinson & Bower (1965) equation to include systems containing non-electrolytes. Their adaptation method involves the assumption that unit molality is equivalent to one-half unit ionic strength, i.e. treating non-electrolytes as 1–1 electrolytes, an adaptation which was suggested earlier by Robinson & Stokes (1961). In the present study, the FBC equation has been similarly extended to include non-electrolyte systems using such a molality–ionic strength relation. The Ross and ZSR equations do not suffer from such a theoretical limitation, being applicable to solutions of electrolytes as well as non-electrolytes.

Results and discussion

Experimental data of a_w in multicomponent electrolyte and non-electrolyte solutions from many sources were utilized for the comparative testing of the three models. Most of the data were determined using very accurate isopiestic vapour pressure techniques. An excellent source of binary a_w data is Teng & Lenzi (1974) who calculated binary data representation to high solute concentrations by interpolation from the polynomial of a_w as a function of

molality in the form:

$$a_w = 1 + \sum_{i=1}^{k_{\sigma(\min)}} A_i m^i$$

where m = solute molality
k = degree of the polynomial
A_i = coefficient of the polynomial
σ = standard deviation

Varying k until a minimum σ of regression is obtained gives the optimum fit. Some of the solutes here examined are not likely to be used as a_w-lowering agents in IMF, but have been included for the sake of completeness.

Comparison of predicted and observed results

Table 1 shows the average percentage deviations between predicted and observed a_w values for the systems studied. The choice of systems and the experimental concentration ranges selected are arbitrary. A more detailed comparison of the results for some representative ternary and quaternary electrolyte systems of those studied are given in Tables 2 and 3 respectively. The detailed results of representative solutions containing non-electrolytes are summarized in Tables 4 and 5. All a_w values used are within the limit of applicability of the ZSR relation as discussed earlier. The predictive accuracy of the ZSR model falls off rapidly as the limit is exceeded (Chen *et al.*, 1973).

Percentage error in a_w prediction is expressed, in line with the practice of Sangster & Lenzi (1974) and Ferro Fontán *et al.* (1980), in the quantity $1 - (a_w)_{obs}$, thus:

$$\% \text{ error} = \frac{(a_w)_{pred} - (a_w)_{obs}}{1 - (a_w)_{obs}} \times 100$$

As those workers have pointed out, the error in the quantity $(1 - a_w)$ is more significant than the error in a_w itself for the following reasons:

(i) it represents directly the error in the vapour pressure lowering of the solution
(ii) it closely approximates the error in log (a_w) which is the most useful quantity in the calculation of other colligative properties of aqueous solutions
(iii) growth of most micro-organisms is inhibited within the $(1 - a_w)$ range of water activity in foods.

The results show that, with few exceptions (e.g. systems of NaCl–KNO_3, NaCl–sucrose, NaCl–urea, and sucrose–urea), the Ross equation displays

Table 1. Average percentage deviation between observed and predicted a_w values for various multicomponent aqueous solutions

System	Reference	Average deviation (%) (on $1-(a_w)_{obs}$)		
		Ross	ZSR	FBC
I. Electrolyte systems				
NaCl–LiCl	Kirgintsev & Luk'yanov (1963); Robinson *et al.* (1971)	10.13	0.83	0.19
NaCl–KCl	Kirgintsev & Luk'yanov (1963)	3.55	0.88	1.02
NaCl–KBr	Covington *et al.* (1968)	6.10	0.11	0.30
NaCl–$NaNO_3$	Kirgintsev & Luk'yanov (1964); Bezboruah *et al.* (1970)	2.80	1.28	0.61
NaCl–KNO_3	Bezboruah *et al.* (1970)	0.60	3.19	1.39
$NaNO_3$–NH_4NO_3	Kirgintsev & Luk'yanov (1965b)	4.87	0.77	0.44
$NaNO_3$–$Ca(NO_3)_2$	Kirgintsev & Luk'yanov (1965a)	1.97	0.94	1.73
$NaNO_3$–$La(NO_3)_3$	Kirgintsev & Luk'yanov (1965a)	2.42	1.12	5.61
KCl–NaBr	Covington *et al.* (1968)	3.94	1.62	1.64
KCl–$NaNO_3$	Bezboruah *et al.* (1970)	3.02	3.52	3.74
KCl–$MgCl_2$	Kirgintsev & Luk'yanov (1966)	7.14	2.10	0.73
LiCl–Na_2SO_4	Robinson (1972)	4.04	2.31	6.93
NH_4Cl–NH_4Br	Kirgintsev & Luk'yanov (1964)	2.96	0.28	0.35
NaCl–LiCl–KCl	Reilly *et al.* (1971)	10.54	0.69	1.85
NaCl–LiCl–$BaCl_2$	Reilly *et al.* (1971)	12.75	0.90	1.16
NaCl–LiCl–CsCl	Reilly *et al.* (1971)	9.93	4.77	7.10
NaCl–KCl–$BaCl_2$	Robinson & Bower (1965)	4.49	1.37	1.12
NaCl–KCl–$CaCl_2$	Kirgintsev & Luk'yanov (1967)	9.16	1.02	0.64
$LiClO_4$–$NaClO_4$–$HClO_4$	Briggs *et al.* (1973)	15.68	1.28	0.68
II. Systems containing non-electrolytes				
Sucrose–glucose	Stokes & Robinson (1966)	7.03	2.23	2.22
Sucrose–glycerol	Stokes & Robinson (1966)	4.26	1.00	1.51
Sucrose–urea	Ellerton & Dunlop (1966)	4.88	7.91	9.89
Sucrose–NaCl	Robinson *et al.* (1970)	1.16	2.99	2.62
Urea–NaCl	Bower & Robinson (1963)	0.21	1.52	5.89
Urea–$PhSO_3Na$*	Uedaira & Uedaira (1970)	5.36	2.46	2.64
Xylose–$PhSO_3Na$	Uedaira & Uedaira (1970)	1.26	0.57	1.50
Glycine–KCl	Bower & Robinson (1965)	1.79	1.48	1.34

* $PhSO_3Na$ = Sodium benzene sulphonate.

consistently lower predictive accuracy than the ZSR and FBC equations. Nevertheless, it succeeded in predicting a_w of most of the systems studied to within ± 0.01 a_w which can be considered as adequate in the application of IMF technology, as suggested by Ferro Fontán *et al.* (1980). Larger deviations (± 0.02 to 0.04) were, however, observed for some of the electrolyte systems, in agreement with the observations of Ferro Fontán *et al.* (1980). The ZSR and FBC methods are more consistent in predictive ability in this respect,

Table 2. Comparison of observed and predicted a_w for some representative ternary electrolyte systems (m : molality)

System	Reference	m_1	m_2	$(a_w)_{obs}$	Percentage deviation		
					Ross	ZSR	FBC
(a) NaCl(1)–KCl(2)	Kirgintsev & Luk'yanov (1963)	1.7959	0.2380	0.9312	0.93	−0.55	−0.30
		0.3819	1.7622	0.9312	1.53	−0.14	−0.55
		1.4973	0.7342	0.9225	6.04	2.58	2.84
		0.6743	1.7236	0.9225	2.36	−0.39	−1.10
		2.0285	0.5765	0.9110	2.72	−0.79	−0.45
		0.4903	2.2627	0.9110	2.26	−0.34	−0.75
		2.6332	0.3489	0.8956	1.84	−0.38	−0.39
		1.4731	1.6479	0.8956	5.06	−0.67	−1.17
		2.7839	0.7912	0.8740	4.04	−0.48	−0.83
		1.4046	2.3560	0.8740	5.88	−1.59	−1.45
		3.5966	0.4766	0.8516	2.60	−0.34	−0.65
		2.0403	2.2823	0.8516	7.39	−2.29	−1.80
(b) NaCl(1)–KBr(2)	Covington *et al.* (1968)	2.4391	0.5721	0.8945	3.66	0	0
		1.8907	1.1717	0.8945	5.59	−0.09	−0.21
		1.3032	1.8115	0.8945	5.80	−0.09	−0.29
		3.3748	0.7916	0.8479	5.10	−0.06	−0.16
		2.6312	1.6306	0.8479	8.05	−0.39	−0.47
		1.8262	2.5386	0.8479	8.42	0	−0.66
(c) NaCl(1)–KNO_3(2)	Bezboruah *et al.* (1970)	0.8703	0.2550	0.9639	−0.77	1.38	1.26
		0.2284	1.1020	0.9639	−0.88	2.77	0.65
		0.8822	0.8627	0.9483	−0.82	3.09	1.69
		0.5890	1.2977	0.9483	−0.79	5.80	1.37
		1.2431	1.3001	0.9265	0.02	2.04	1.84
		0.8421	1.9895	0.9265	0.30	4.08	1.55
(d) KCl(1)–$MgCl_2$(2)	Kirgintsev & Luk'yanov (1966)	1.7907	0.1243	0.9362	2.53	−0.78	0.16
		0.8830	0.6270	0.9362	6.10	−2.35	−0.33
		1.8934	0.2326	0.9262	4.64	−1.08	0.28
		1.2416	0.5835	0.9262	7.48	−2.17	−0.12
		2.4116	0.1674	0.9131	3.30	−1.04	−0.16
		1.4354	0.6746	0.9131	8.44	−2.53	−0.63
		2.6460	0.3250	0.8945	6.30	−1.52	−0.16
		1.3521	0.9599	0.8945	9.35	−2.94	−1.28
		3.5861	0.2489	0.8671	5.10	−1.28	−0.29
		2.0559	0.9661	0.8671	11.74	−3.39	−1.69
		3.6274	0.4456	0.8511	8.23	−2.22	−1.12
		2.2613	1.0627	0.8511	12.47	−3.96	−2.58

generally giving predictive a_w values in very good agreement with observed values. Where choice of method is concerned, however, the extreme simplicity in computation of the Ross equation may become the overriding factor. The results also show that the Ross equation does not always predict a_w values higher than measured and, as such, does not possess some sort of 'built-in' safety factor in connection with IMF as claimed by Ferro Fontán *et al.* (1980).

Table 3. Comparison of observed and predicted a_w for some representative quaternary electrolyte systems (m: molality)

System	Reference	m_1	m_2	m_3	$(a_w)_{obs}$	Percentage deviation		
						Ross	ZSR	FBC
(a) NaCl(1)–LiCl(2) –KCl(3)	Reilly *et al.* (1971)	0.9650	0.9650	0.9650	0.8958	9.66	−1.15	−2.27
		1.0000	1.0000	1.0000	0.8910	10.51	−0.73	−1.86
		1.0114	1.0114	1.0114	0.8895	10.72	−0.63	−1.81
		1.0321	1.0321	1.0321	0.8865	11.29	−0.26	−1.47
(b) NaCl(1)– KCl(2)–$BaCl_2$(3)	Robinson & Bower (1965)	0.4708	0.4708	0.3559	0.9524	3.22	−1.26	−0.74
		0.3110	0.3110	0.5654	0.9526	3.18	−1.48	−0.82
		0.1740	0.1740	0.7449	0.9526	2.29	−1.05	−0.65
		0.1127	0.1127	0.8250	0.9526	1.53	−0.84	−0.59
		1.1980	1.1980	0.2126	0.9093	6.02	−1.28	−1.28
		1.0281	1.0281	0.4292	0.9093	7.37	−1.65	−1.59
		0.5509	0.5509	1.0022	0.9106	7.11	−1.90	−1.77
		0.3521	0.3521	1.2443	0.9106	5.20	−1.50	−1.50
(c) NaCl(1)– KCl(2)–$CaCl_2$(3)	Kirgintsev & Luk'yanov (1967)	0.2130	0.7960	0.7067	0.9266	8.40	−0.82	1.04
		0.5931	1.2568	0.2361	0.9266	8.15	−1.23	−0.17
		0.7392	1.3454	0.0982	0.9266	4.45	−0.95	−0.45
		1.0481	0.3281	0.4649	0.9266	9.35	−0.41	0.85
		1.3211	0.6250	0.1451	0.9266	5.40	−0.54	−0.06
		0.2333	0.8718	0.7740	0.9184	9.23	−0.98	0.90
		0.6545	1.3870	0.2606	0.9184	6.96	−1.35	−0.28
		0.8174	1.4878	0.1086	0.9184	5.11	−0.98	−0.57
		1.1506	0.3601	0.5103	0.9184	10.29	−0.49	0.71
		1.4576	0.6896	0.1601	0.9184	6.07	−0.73	−0.21
		0.2873	1.0738	0.9534	0.8956	11.17	−1.44	0.62
		0.8205	1.7388	0.3267	0.8956	9.09	−1.63	−0.57
		1.0293	1.8736	0.1368	0.8956	6.88	−1.24	−0.83
		1.4198	0.4444	0.6297	0.8956	12.89	−0.57	0.61
		1.8215	0.8617	0.2001	0.8956	8.07	−0.86	−0.37
		0.3776	1.4110	1.2528	0.8543	13.85	−2.06	−0.55
		1.1054	2.3425	0.4401	0.8543	12.46	−1.99	−1.15
		1.3957	2.5404	0.1855	0.8543	9.76	−1.44	−1.23
		1.8679	0.5847	0.8285	0.8543	16.82	−0.69	0.40
		2.4501	1.1591	0.2691	0.8543	10.84	−1.37	−1.06

The results also demonstrate that the ZSR method has no consistent advantage over the FBC model where predictive accuracy is concerned. In some systems, the ZSR method is better than the FBC equation, whilst in others the reverse is true.

The suitability of any model for the prediction of a_w of complex solutions in connection with the production of IMF is subject to the following criteria:

(1) It must be based on sound theoretical principles
(2) It should be simple enough for easy computation
(3) It should exhibit reasonably good predictive accuracy in the concentrations likely to be used in IMF

(4) It should be applicable to both electrolytes and non-electrolytes and their mixtures. Systems of solutes of mixed types are more likely to be encountered in practice.

In order to satisfy the last condition, the FBC equation (which is strictly applicable only to electrolyte solutions) was adapted for predicting a_w of solutions containing non-electrolytes. Application of the adaptation method yielded good agreement between calculated and measured a_w values as shown in Tables 4 and 5. The limitation of binary solution data to solutions at or below saturation restricts the extended application to lower a_w values of predictive methods requiring the use of binary data. A subsequent paper will deal with the prediction of a_w of aqueous supersaturated binary solutions from ternary data using the reverse ZSR method.

Table 4. Comparison of observed and predicted a_w for some representative ternary non-electrolyte systems (m : molality)

System	Reference	m_1	m_2	$(a_w)_{obs}$	Percentage deviation		
					Ross	ZSR	FBC
(a) Sucrose(1)	Stokes & Robinson	2.8039	0.5542	0.9259	3.52	0	−0.63
–Glucose(2)	(1966)	2.3504	1.0996	0.9259	5.96	0.27	−1.01
		1.2166	2.4489	0.9259	7.08	−1.62	−1.01
		1.7391	1.8737	0.9250	7.49	−2.13	−1.25
		0.8286	2.5903	0.9250	15.02	8.67	9.07
		0.3648	3.4947	0.9250	3.11	−0.67	−0.34
(b) Sucrose (1)–	Stokes & Robinson	2.0405	1.3615	0.9302	4.74	−0.67	−2.17
Glycerol(2)	(1966)	1.0701	2.5640	0.9302	5.10	−1.57	−1.82
		0.3278	3.4537	0.9302	2.68	−0.29	−0.20
		2.4640	0.8524	0.9296	3.47	−0.57	−1.65
		1.5022	2.0650	0.9296	5.46	−2.13	−2.24
		0.6493	3.1014	0.9296	4.09	−0.85	−0.97
(c) Sucrose(1)–	Ellerton & Dunlop	0.6791	0.3386	0.9817	−1.99	−4.37	−3.69
Urea(2)	(1966)	0.9011	1.0149	0.9671	−4.44	−6.99	−8.18
		1.5838	1.7838	0.9429	−6.66	−10.51	−12.92
		2.0333	2.4134	0.9251	−7.30	−11.21	−14.92
		1.7273	3.8001	0.9136	−7.40	−11.57	−15.85
		0.3076	7.0566	0.8944	−1.50	−2.84	−3.77

Table 5. Comparison of observed and predicted a_w for some representative non-electrolyte–electrolyte systems (m : molality)

System	Reference	m_1	m_2	$(a_w)_{obs}$	Percentage deviation		
					Ross	ZSR	FBC
(a) Sucrose(1)–NaCl(2)	Robinson *et al.* (1970)	0.2209	0.2943	0.9862	1.40	2.17	0
		0.5082	1.2240	0.9495	1.32	−3.37	−2.84
		0.6830	1.4285	0.9389	1.57	−4.91	−4.00
		3.4190	0.2510	0.9132	1.01	−1.84	−2.09
		4.3200	0.2666	0.8881	0.78	−2.14	−2.33
		4.3570	0.2287	0.8881	0.85	−1.70	−1.82
		4.4670	0.6689	0.8715	1.19	−5.76	−5.90
		5.5290	0.2424	0.8550	0.58	−2.00	−2.00
(b) Glycine(1)–KCl(2)	Bower & Robinson (1965)	0.5007	0.2486	0.9837	−1.42	−1.23	−0.46
		0.7070	0.1430	0.9837	−1.38	−1.84	−0.46
		0.4470	0.6107	0.9734	−1.72	−0.75	−0.90
		0.8336	0.4164	0.9734	−2.21	−0.37	−1.25
		0.9100	0.7731	0.9614	−2.27	−0.52	−1.74
		1.4412	0.5116	0.9614	−2.30	−0.52	−1.74
		0.4851	1.2052	0.9543	−1.13	−0.22	−0.91
		1.3494	0.7870	0.9543	−1.95	−3.28	−1.90
		0.9836	1.1473	0.9487	−1.71	−0.19	−1.71
		2.0131	0.6521	0.9487	−1.82	5.85	−2.36
(c)Urea(1)–NaCl(2)	Bower & Robinson (1963)	0.9497	0.3437	0.9728	−0.52	−0.37	−1.36
		1.3933	0.1175	0.9728	0	0	−0.52
		0.6992	1.3657	0.9430	−0.35	−0.35	−2.54
		1.9058	0.8129	0.9430	−0.92	−0.53	−4.58
		1.6410	2.2042	0.8989	−0.12	−0.79	−5.62
		3.3504	1.5257	0.8989	−0.47	−1.38	−9.93
		1.1875	2.8232	0.8821	−0.06	−0.51	−5.29
		2.8039	2.2196	0.8821	−0.19	−1.53	−10.46
		1.3681	3.5868	0.8490	0.09	−0.66	−6.32
		2.5894	3.1775	0.8490	−0.03	−1.52	−11.03
		6.8252	2.5671	0.8201	−0.08	−4.28	—*
		8.2784	2.0828	0.8201	−0.12	−4.33	—
		1.3415	4.4082	0.8145	0.15	−0.65	−5.19
		4.4403	3.4657	0.8145	0.06	−3.23	—
		1.6963	5.1551	0.7767	0.21	−0.94	—
		5.6620	4.0765	0.7767	−0.04	−3.31	—

* Beyond the limit of applicability of the FBC equation.

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The stability and use of natural colours in foods: anthocyanin, β-carotene and riboflavin

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Summary

The stability of three natural colours—anthocyanin, β-carotene and riboflavin—under selected conditions was investigated. All were less stable than their synthetic counterparts—amaranth and tartrazine—especially to temperatures in excess of 50°C and exposure to light. Anthocyanin in particular was also sensitive to changes in pH levels. All colours were relatively stable to changes in water activity, however.

This inherent instability of natural colours does not preclude their use in foods since it has been shown that if certain processing modifications are made, i.e. addition of colour as a final action in a process, they can be used to colour certain foodstuffs, in particular boiled sweets.

Introduction

The inclusion of synthetic colours in food products is a practice which may be restricted or completely prohibited in the future and the food processor would therefore have to use no added colour in the product or use a natural colour to supplement the colour of the food. The colour of foods governs to a large extent their consumer acceptability and brightly coloured foods are generally eye-catching and appealing to prospective purchasers. Natural colours are unfortunately degraded, often severely, during processing whilst synthetic colours are, on the whole, more resistant to change. The stability and uses in foods of cochineal, copper chlorophyll and red beet colours have recently been described and their stability compared with comparable synthetic colours (Kearsley & Katsaboxakis, 1980).

The object of this study is to extend the techniques described in this previous study to assess the stability of anthocyanin, β-carotene and riboflavin.

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Materials

The three natural colours used in this study were: (1) Water soluble anthocyanin powder—a red colour; (2) water soluble β-carotene powder—a yellow colour; (3) water soluble riboflavin (vitamin B_2) powder—a yellow colour. All natural colours were supplied by Hoffman–La Roche and Co. Ltd, Dunstable, England and these kind gifts are gratefully acknowledged.

Two artificial colours were used in certain studies for comparison purposes: (1) Edicol amaranth (Red CL9); (2) Edicol tartrazine (Yellow CL4). These were obtained from I.C.I. Ltd, Blackley, Manchester, England and these kind gifts are gratefully acknowledged. All other reagents were obtained from BDH Ltd, Poole, Dorset, England.

Methods and results

Effect of temperature on colour stability

The stability of each colour—synthetic and natural—was determined over the temperature range 20–100°C. A known concentration of each colour was

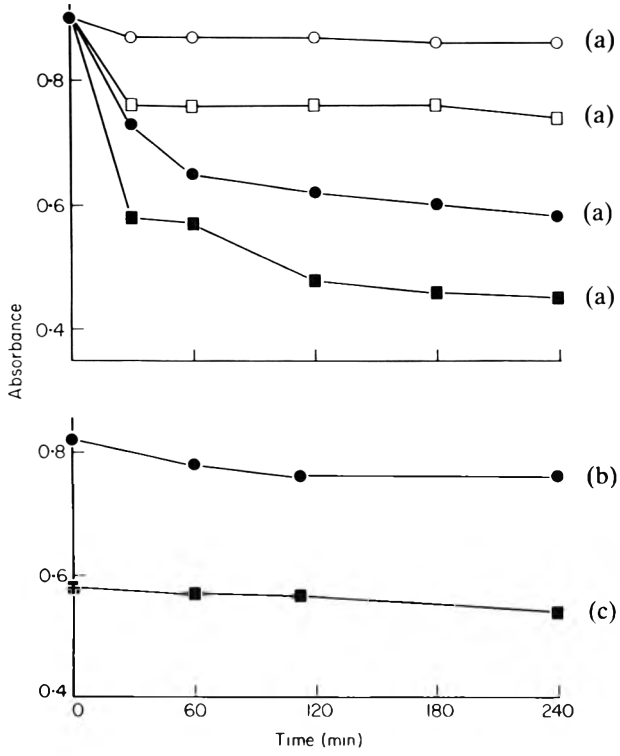

Figure 1. Effect of temperature on colour stability. (a) Anthocyanin, 100 mg/100 ml: O, 20°C; ●, 75°C; □, 50°C; ■, 100°C. (b) Tartrazine, 1.6 mg/100 ml, 100°C. (c) Amaranth, 1.6 mg/100 ml, 100°C.

prepared in distilled water and a known volume introduced into a sample tube. Glass condensers were placed in the neck of each tube to prevent evaporation and loss of liquid and the contents heated for 4 hr at each temperature. The absorbance of each solution was measured at the wavelength of maximum absorbtion (λ_{max}) at time zero and then at predetermined intervals throughout the test. The results are shown in Figs 1 and 2.

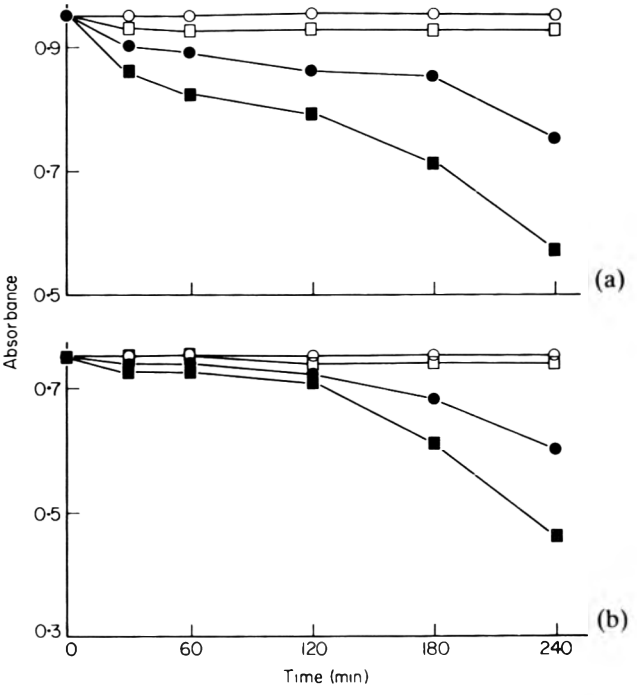

Figure 2. Effect of temperature on colour stability. O, 20°C; □, 50°C; ●, 75°C; ■, 100°C. (a) β-carotene, 40 mg/100 ml; (b) riboflavin, 2.5 mg/100 ml.

Effect of pH on colour stability

The stability of each natural colour was examined at different pH values by adding a known volume of an aqueous solution of each colour to citrate/phosphate buffers in the range pH 2.3–8.0. Solutions were placed in heat sealable ampoules and after sealing, the ampoules were stored at 7°C in the dark. Absorbance readings at λ_{max} were taken initially and for up to 96 hr.

To investigate the effect of temperature at each pH the colours were also stored at elevated temperatures in tubes fitted with condensers in the dark. The concentrations of the colours and storage data are included in the results in Tables 1 and 2.

Table 1. Effect of pH on colour stability (absorbance) at 7°C

	Colour and time of exposure (hr)								
	Anthocyanin			β-carotene			Riboflavin		
pH	0	24	96	0	24	96	0	24	96
2.3	1.50*	1.50	1.40	—	—	—	1.22	1.18	1.16
3.1	1.04*	0.98	0.96	0.94	0.85	0.84	1.22	1.15	1.14
3.6	0.69*	0.68	0.65	—	—	—	—	—	—
4.0	0.61*	0.57	0.53	0.94	0.87	0.86	1.20	1.12	1.10
5.0	0.48†	0.43	0.42	0.94	0.87	0.85	—	—	—
6.0	0.55‡	0.44	0.42	—	—	—	1.14	1.10	1.02
7.0	0.89§	0.73	0.70	0.94	0.85	0.84	0.89	0.84	0.80
8.0	—	—	—	—	—	—	1.08	1.00	0.90

Anthocyanin concentration = 450 mg/100 ml.
Riboflavin concentration = 20 mg/100 ml.
β-carotene concentration = 200 mg/100 ml.
* λ_{max} = 540 nm; † λ_{max} = 550 nm; ‡ λ_{max} = 570 nm; § λ_{max} = 610 nm.

Table 2. Effect of pH on colour stability (absorbance) at elevated temperature

	Colour and time of exposure (min)											
	Anthocyanin				β-carotene				Riboflavin			
pH	0	60	120	180	0	60	130	210	0	60	130	195
2.3	1.68*	1.60	1.59	1.58	—	—	—	—	1.40	1.40	1.28	1.24
3.1	1.10*	1.02	1.01	1.00	0.94	0.90	0.85	0.81	1.40	1.40	1.35	1.12
3.6	0.78*	0.73	0.72	0.70	—	—	—	—	—	—	—	—
4.0	0.62*	0.60	0.60	0.59	0.94	0.89	0.88	0.86	1.40	1.35	1.14	0.93
5.0	0.50†	0.49	0.47	0.45	0.94	0.87	0.86	0.82	—	—	—	—
6.0	0.58‡	0.47	0.46	0.42	—	—	—	—	1.28	1.27	1.21	0.94
7.0	0.92§	0.72	0.62	0.50	0.94	0.90	0.86	0.83	1.26	1.03	0.60	0.41
8.0	—	—	—	—	—	—	—	—	1.28	0.79	0.35	0.23

Anthocyanin concentration = 500 mg/100 ml (heated at 43°C).
β-carotene concentration = 200 mg/100 ml (heated at 55°C).
Riboflavin concentration = 25 mg/100 ml (heated at 75°C).
* λ_{max} = 540 nm; † λ_{max} = 550 nm; ‡ λ_{max} = 570 nm; § λ_{max} = 610 nm.

Effect of water activity (a_w) on colour stability

Model systems with a water activity from 1.0 to 0.37 were prepared using glycerol and water (Pasch & von Elbe 1975). Solutions of each colour were prepared at each water activity and incubated at elevated temperature in test tubes fitted with condensers. Absorbance readings at λ_{max} were taken initially and for up to 170 min thereafter. The results are given in Table 3.

Table 3. Effect of water activity (a_w) on colour stability (absorbance) at elevated temperature

	Colour and time of exposure (min)											
	Anthocyanin				β-carotene				Riboflavin			
a_w	0	60	90	160	0	50	100	150	0	60	120	170
1.00	0.84	0.78	0.76	0.74	1.10	0.95	0.92	0.90	1.45	1.38	1.30	1.22
0.95	0.85	0.82	0.81	0.76	1.00	0.89	0.88	0.85	1.50	1.45	1.30	1.24
0.87	0.86	0.82	0.81	0.78	0.92	0.83	0.77	0.75	1.50	1.48	1.28	1.22
0.74	0.91	0.88	0.85	0.84	0.95	0.84	0.80	0.77	1.45	1.35	1.24	1.18
0.63	0.92	0.88	0.86	0.85	0.85	0.80	0.76	0.74	1.35	1.33	1.22	1.15
0.47	0.96	0.89	0.87	0.86	0.79	0.78	0.69	0.65	1.25	1.20	1.15	1.02
0.37	1.03	0.90	0.89	0.87	0.70	0.65	0.56	0.50	1.20	1.16	1.10.	1.00

Anthocyanin concentration = 700 mg/100 ml (heated at 43°C).
β-carotene concentration = 300 mg/100 ml (heated at 65°C).
Riboflavin concentration = 30 mg/100 ml (heated at 75°C).

Stability of colours in model systems

Model food systems containing carbohydrate, organic acids, amino acids, ascorbic acid, preservative and anti-oxidant were prepared as in Table 4. The concentration of each colour in each system is given in Table 5 and storage temperatures were: anthocyanin 55°C; riboflavin 79°C; β-carotene 65°C. Absorbance at λ_{max} was determined at time zero and for up to 3 hr. The results are given in Table 5.

Table 4. Composition of model food systems (g/100 ml)

System code	Sucrose	Glucose	Fructose	Citric acid	Malic acid	Glycine	Trypto-phan	Cysteine	Ascorbic acid	Sodium metabi-sulphite
a	—	—	—	—	—	—	—	—	—	—
b	16	—	—	—	—	—	—	—	—	—
c	8	4	4	—	—	—	—	—	—	—
d	8	4	4	0.4	—	—	—	—	—	—
e	8	4	4	0.2	0.2	—	—	—	—	—
f	8	4	4	—	—	0.08	0.08	—	—	—
g	8	4	4	—	—	—	—	—	0.05	
h	8	4	4	—	—	—	—	0.1	0.05	—
j	8	4	4	—	—	—	—	—	—	0.05
k	8	4	4	—	—	0.1	—	—	—	0.05

Table 5. Stability (absorbance) of colours in model systems

	Storage time (min)											
	Anthocyanin				β-carotene				Riboflavin			
System	0	50	110	180	0	60	120	180	0	55	115	150
a	1.04	0.96	0.92	0.90	0.91	0.87	0.86	0.85	1.25	0.82	0.39	0.26
b	1.01	1.00	1.00	0.99	0.84	0.82	0.80	0.80	1.26	0.94	0.52	0.30
c	1.10	1.02	0.99	0.98	0.84	0.82	0.79	0.79	1.24	1.00	0.61	0.45
d	1.98	1.92	1.91	1.89	0.83	0.79	0.79	0.77	1.18	0.90	0.59	0.40
e	1.96	1.90	1.88	1.86	0.84	0.80	0.78	0.78	1.18	0.60	0.31	0.24
f	1.00	0.94	0.90	0.88	0.84	0.80	0.69	0.79	1.65	0.98	0.88	0.76
g	1.22	1.10	1.08	1.06	0.85	0.81	0.81	0.81	1.24	1.14	0.87.	0.86
h	1.24	1.11	1.10	1.08	0.82	0.80	0.80	0.80	1.25	0.90	0.57	0.37
j	0.45	0.44	0.43	0.41	0.82	0.80	0.79	0.79	1.16	0.92	0.65	0.58
k	0.44	0.43	0.42	0.41	0.87	0.86	0.86	0.86	1.45	1.26	1.09	1.06

Anthocyanin concentration = 120 mg/100 ml.
β-carotene concentration = 40 mg/100 ml.
Riboflavin concentration = 4 mg/100 ml.

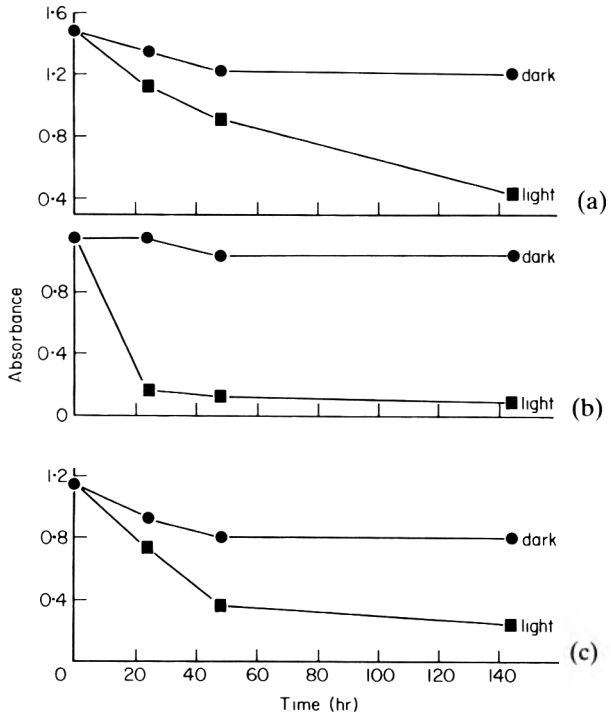

Figure 3. Effect of light on colour stability. ●, Dark; ■, light. (a) Anthocyanin, 400 mg/100 ml; (b) riboflavin, 20 mg/100 ml; (c) β-carotene, 200 mg/100 ml.

Effect of light on colour stability

This study was carried out at pH 2.3 and 25°C for anthocyanin and riboflavin and pH 4.0 and 25°C for β-carotene. The solutions were placed in heat sealable ampoules and half the ampoules exposed to light and half kept in dark storage. Absorbance readings at λ_{max} were taken intially and for up to 144 hr. The results are shown in Fig. 3.

Evaluation of natural colours as food colourants

The three natural colours were investigated as possible colourants in boiled sweets using amaranth and tartrazine for comparison purposes. The boiled sweets were prepared from the following components:

	parts by weight
sucrose	60
glucose syrup (36 D.E.)	25
citric acid	0.6
water	20

The method was as follows: The sugar was dissolved in the water, heated in a boiling pan, the glucose syrup added and the mixture boiled until a solids content of 96% was achieved. At this point acid, flavour and colour were added to produce an organoleptically acceptable product. After thorough mixing the mass was poured into a prepared mould and allowed to cool. The quantity of colour in each batch was determined by a trial mix. The intensity of colour produced using natural and synthetic colour was matched as far as possible and the concentration of each colour is shown in Table 6.

The colours were added after boiling to minimize heat damage to the colour. Samples were stored as shown in Table 6 and evaluated for colour acceptability by ten to twelve untrained panellists initially and after 3 and 6 weeks' storage.

A 9-point hedonic scale was used in each case where 9 was a highly acceptable colour and 1 a highly unacceptable colour. In the sensory assessment

Table 6. Boiled sweet specifications

Colour	Concentration in boiled sweet (mg/kg)	Storage temperature (°C)	Storage conditions
Anthocyanin	80	27 and 37	Dark
Amaranth	20	25 and 37	Dark
β-carotene	400	25	Light and dark
Riboflavin	40	25	Light and dark
Tartrazine	10	25	Light and dark

boiled sweets containing anthocyanin and amaranth were judged together and sweets containing β-carotene, riboflavin and tartrazine judged together. The results are shown in Tables 7 and 8.

Table 7. Organoleptic assessment of boiled sweets coloured with β-carotene, riboflavin and tartrazine

	Mean scores (± s.d.) after light storage		
Colour	0 days	21 days	42 days
β-carotene	6.8 (± 1.12)	6.9 (± 1.56)	6.5 (± 1.08)
Riboflavin	7.0 (± 1.05)	6.5 (± 1.35)	5.1 (± 1.45)
Tartrazine	6.4 (± 0.79)	7.0 (± 1.05)	6.8 (± 0.79)

NS: β-carotene vs Riboflavin (0 days); Riboflavin vs Tartrazine (0 days); β-carotene vs Tartrazine (0 days); β-carotene vs Riboflavin (21 days); Riboflavin vs Tartrazine (21 days); β-carotene vs Tartrazine (21 days); β-carotene vs Tartrazine (42 days)

$P \leq 0.05$: β-carotene vs Riboflavin (42 days)

$P \leq 0.01$: Riboflavin vs Tartrazine (42 days)

	Mean scores (± s.d.) after dark storage		
Colour	0 days	21 days	42 days
β-carotene	6.8 (± 1.12)	7.7 (± 0.95)	6.8 (± 0.42)
Riboflavin	7.0 (± 1.05)	6.6 (± 1.58)	5.8 (± 1.40)
Tartrazine	6.4 (± 0.79)	7.5 (± 1.08)	7.5 (± 1.18)

NS: β-carotene vs Riboflavin (0 days); Riboflavin vs Tartrazine (0 days); β-carotene vs Riboflavin (21 days); Riboflavin vs Tartrazine (21 days); β-carotene vs Tartrazine (42 days)

$P \leq 0.05$: β-carotene vs Riboflavin (42 days)

$P \leq 0.01$: Riboflavin vs Tartrazine (42 days)

NS = not significantly different.

Table 8. Organoleptic assessment of boiled sweets coloured with anthocyanin and amaranth

	Mean scores (± s.d.) at 25°C		
Colour	0 days	21 days	42 days
Anthocyanin	6.5 (± 1.00)	6.3 (± 1.06)	5.6 (± 1.71)
	NS	NS	NS
Amaranth	6.8 (± 1.36)	6.1 (± 1.91)	6.4 (± 1.35)

	Mean scores (± s.d.) at 37°C		
Colour	0 days	21 days	42 days
Anthocyanin	6.5 (± 1.00)	7.3 (± 0.82)	5.2 (± 1.61)
	NS	NS	$P \leq 0.01$
Amaranth	6.8 (± 1.36)	7.2 (± 1.40)	6.9 (± 0.92)

NS = not significantly different.

Table 9. Effect of storage conditions on colour stability (absorbance) of 20% solutions of boiled sweets

			Absorbance and time		
Colour	λ_{max}	Storage conditions	0 weeks	3 weeks	6 weeks
Anthocyanin	535	25°C dark	0.25	0.21	0.20
		37°C dark	0.25	0.16	0.12
Amaranth	530	25°C dark	0.24	0.24	0.23
		37°C dark	0.24	0.22	0.19
Riboflavin	450	25°C light	0.29	0.22	0.18
		25°C dark	0.29	0.24	0.22
β-carotene	475	25°C light	0.26	0.20	0.17
		25°C dark	0.26	0.22	0.20
Tartrazine	430	25°C light	0.34	0.30	0.26
		25°C dark	0.34	0.32	0.30

In addition to the sensory assessment an objective measurement of the colour was carried out. A 20% w/v solution of each sample was prepared and after filtering absorbance at λ_{max} determined. These results are shown in Table 9.

Discussion

Of the natural colours under investigation in this study none, as expected, was completely stable under all the conditions examined. Anthocyanin in particular was found to be heat labile which is in agreement with previous reports (Lukton, Chichester & Mackinnery, 1956; Markakis, 1974) whilst β-carotene and riboflavin were relatively stable, at least up to 50°C. Riboflavin was also stable to higher temperatures for periods of up to 2 hr. Similarly anthocyanin was sensitive to changes in pH with colour changes from red to pink to violet as pH increased although little change in intensity at individual pH values was apparent. Similar findings are well documented (Adams, 1973; Adams & Woodman, 1973; Shrikhande, 1976). β-carotene was stable to changes in pH and is reported to be stable in foods over the range pH 2–7 (Bunnell, Driscoll & Bavernfeind, 1958; Bavernfeind & Bunnell, 1962). Riboflavin was stable in acid but degraded quickly in alkaline environments.

Whilst water activity (a_w) played an important role in colour intensity for each colour the stability of each colour at a specific a_w was approximately the same. It has been reported that the water content of a food exerts a protective influence on carotenoid pigments (Ramakrishnan & Francis, 1980; Arya *et al.*, 1979; Kanner, Mendel & Budowski, 1978) and our results indicate a similar conclusion in that at higher levels of a_w the pigment was more intense.

All colours were light sensitive and in particular riboflavin suffered an 86% loss in intensity after 24 hr exposure. The light degradation of riboflavin in milk is of course well known (Peterson, Haig & Shaw, 1944; Ziegler, 1944).

The stability of natural colours under such conditions as exist in food products is the main consideration when selecting a colour for food use. Our studies indicate that only in the case of riboflavin do carbohydrates have any marked deleterious effect on colour stability. Addition of acid (systems d, e, g, h, j, k) produced the predictable effect in the majority of cases although some anomalous results were obtained. Sodium metabisulphite (sulphurous acid) conferred some stability on anthocyanin and β-carotene but not riboflavin. Ascorbic acid helped stabilize riboflavin but ascorbic acid and cysteine did not: possibly as a result of the reducing potential of the $-SH$ groups in cysteine playing a part. A similar effect was noted with citric acid and citric plus malic acids with riboflavin although not so pronounced. The presence of glycine and tryptophan appeared to stabilize riboflavin but had little effect on the other colours.

The use of the natural colours in food products indicated that boiled sweets could usefully be coloured with natural pigments. Detectable differences

between natural and synthetic colours were only established by the panellists after 22–42 days storage although spectrophotometry indicated degradation of all the colours after 21 days storage.

Conclusions

The natural colours under investigation are relatively stable under certain defined conditions and if foods are selected such that these conditions are met then the natural colour can be used with a reasonable degree of success. Certain processing modifications may be required. With boiled sweets for example, colour is added after boiling rather than before. Should present trends continue and synthetic colours are completely prohibited these changes may be tolerated.

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An assessment of the rapid batter expansion test for prediction of bread baking quality in New Zealand conditions

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Summary

The rapid batter expansion test has been compared with flour protein and with test baking by a mechanical dough development method for a range of thirty-five New Zealand wheats milled by Brabender Quadrumat Junior mill, and of fourteen commercial flours. We found no value in the test as a prediction of baking quality under our conditions.

Introduction

Khan & Elahi (1980) have recently published details of a test for prediction of bread baking quality of flours. It seemed that this test might have a use under our conditions for the checking of the qualities of flour shipments received at bakery plants. We have therefore examined the test for its ability to predict baking quality under our conditions. It must be emphasized that most New Zealand bread is produced by mechanical dough development (MDD) processes; to this extent our inquiry does not parallel that of Khan & Elahi.

Materials

We selected the following wheats and milled them by Brabender Quadrumat Junior laboratory mill:

Fourteen individual samples of cv. Karamu of 1980 season
Four bulks from many hundreds of samples of cv. Karamu of 1979 season
Seven bulks of main cultivars of 1980 season, excluding cv. Karamu
Ten commercial mill grists of 1980 season.

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We also selected:

fourteen commercial flours, representing the production from seven mills during 2 weeks in September 1980.

The main cultivars represented are Aotea, Arawa, Hilgendorf 61, Kopara, Oroua, Takahe, Tiritea. The cultivar Karamu is a selection from the Australian line WW15 which in turn was a selection from the Mexican dwarf line WW15, and is thus similar to Anza, Mexicali, T4 and others of other countries; it is grown particularly for its yield rather than for its baking quality.

Methods

The MDD baking test uses 125 g flour, about 3.75 g compressed moist yeast (depending on its gassing activity), 2.5 g salt, 1.88 g lard, 0.94 g sugar, 6.25 mg potassium bromate (50 ppm), 12.5 mg ascorbic acid (100 ppm), optimal water and optimal work input within a range 5 to 12 Wh/kg of dough during 1.5 to 2.5 min at 32°C controlled temperature. Doughs are given 10 min intermediate proof at 32°C, moulded through a Mono moulder, given 45 min final proof at 40°C and 85 to 90% relative humidity. They are then baked for 26 min in a travelling oven at 240°C with an oven atmosphere being 20% water vapour 80% air by volume. Loaves are cooled overnight and judged for volume (by rape seed displacement) and for crumb texture. The volume and texture scores are combined to give a total bake score in which volume generally makes up 40 to 50%. The mixers used have been designed and built in this Institute by T. A. Mitchell, and they have a facility for continuously recording the rate of work input. A preliminary mixing is made in the same equipment using fixed water absorption and work input; the optimal quantities of water and work input for a second mix for baking are then determined from the resulting graph of work input against time, analogous to a Brabender Do-corder record.

Protein contents of flours were determined with a Technicon InfraAlyzer, model 2.5A. This has been calibrated by Kjeldahl analyses on flours similar to those considered here.

The expansion test was performed exactly as published.

Results

The relationships between the batter expansion volume and the flour protein, loaf volume, or total bake score are shown in Figs 1 to 3. The wide scatter was so obvious that we have not performed statistical calculations.

Discussion

There is no obvious correlation between the batter expansion volume and any of the three other quality measures we have used. Indeed, of the four Karamu

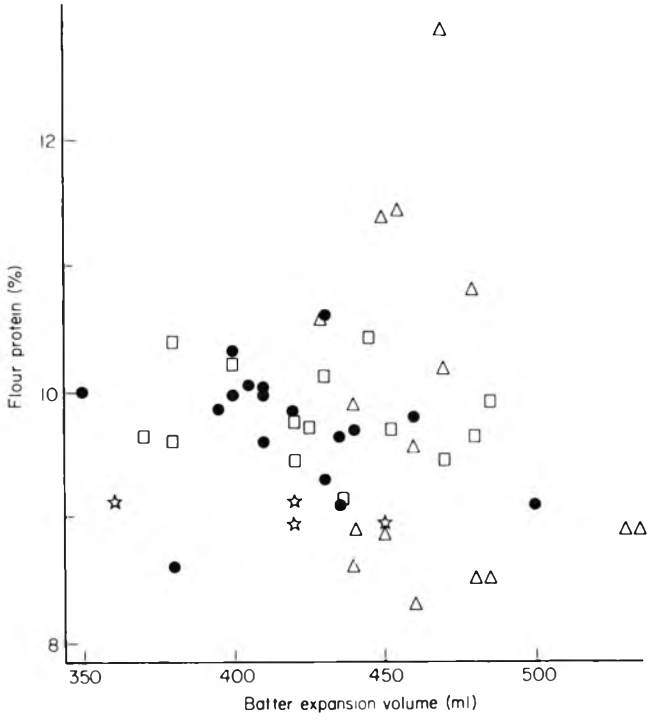

Figure 1. Relationship between batter expansion volume and flour protein. ★, Bulks of cv. Karamu of 1979; ▲, cv. Karamu of 1980; ●, other wheat cultivars; □, commercial flours.

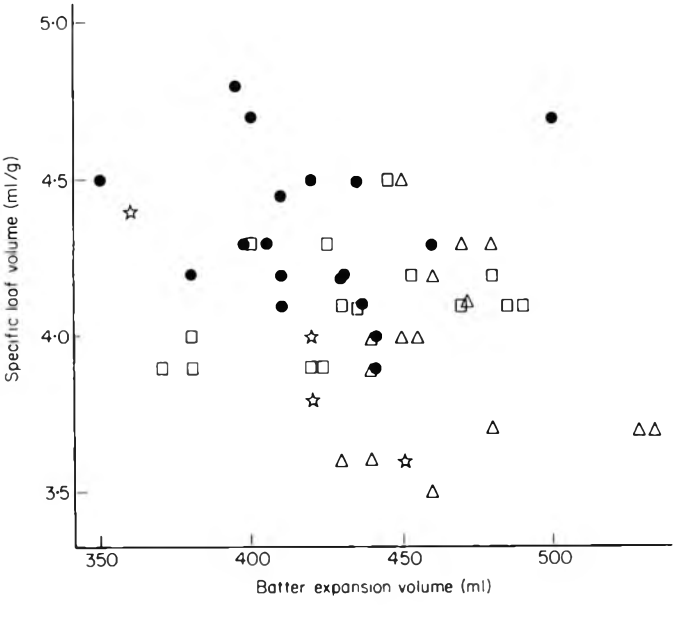

Figure 2. Relationship between batter expansion volume and specific loaf volume. Key to symbols as Fig. 1.

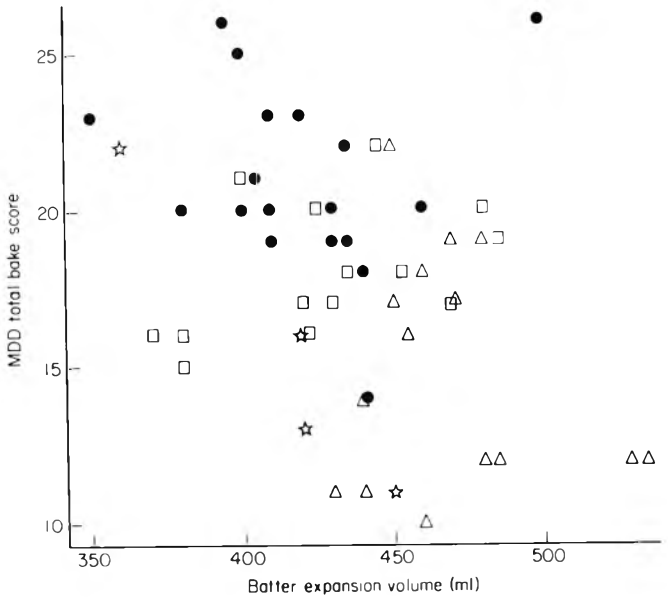

Figure 3. Relationship between batter expansion volume and total bake score. Key to symbols as Fig. 1.

bulks of the 1979 season, those having lowest bake scores and protein contents gave the highest expansion volumes.

We conclude from this trial that the rapid batter expansion test is of no obvious use for prediction of either good or poor baking quality for bread-making in our conditions.

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Major volatiles in Sri Lankan arrack, a palm wine distillate

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Summary

Arrack is a potable spirit produced in Sri Lanka by fermenting sap usually obtained from the coconut palm. In this study the major volatiles present in several commercial samples of arrack are found to be similar to those in other potable spirits although their individual concentrations differ. The variation between brands is attributed to differences in the quality of the fermented toddy used. This is thought to result from the uncontrolled and mixed nature of the fermentation. Ethyl lactate was found in all samples analysed and it is thought that production of this ester is a characteristic feature of mixed lactic-alcoholic fermentations.

Introduction

The sap obtained from different types of palm is the basis of fermented beverages in many tropical countries (Okafor, 1978). In Sri Lanka, where the inflorescence of the coconut palm is usually tapped (Nathanael, 1966), it is used to produce the popular local drinks sweet toddy, toddy (palm wine) and arrack.

The saccharine sap is collected in earthenware pots where it undergoes a spontaneous two-stage fermentation. During the first 12 hr a rapid lactic fermentation takes place to give the product known as sweet toddy. This is subsequently fermented by yeasts to produce toddy, a solution containing 50–60 g litre^{-1} ethanol (J. D. Atputharajah, personal communication). Toddy may be consumed directly or distilled to produce arrack. Before it is blended and bottled, arrack is matured in 'Halmilla' wood *(Berrya ammonilla)* casks for periods normally of 3–5 years, though some 10-year-old blends are produced.

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0022–1163/81/0800–0437 $02.00

Arrack is produced on both the large commercial scale and by small (illegal) stills. Individual products are of consistent quality but there are considerable variations between brands. The available analytical data are limited to chemical estimations of esters, acidity and furfural content of samples from the largest distilleries (Child, 1974). This work was undertaken as part of a project to identify some of the main differences between various types of arrack, to attribute reasons for any observed differences and to indicate how a more uniform quality product could be obtained.

Materials and methods

Samples

Arrack samples were obtained from commercial distilleries in Sri Lanka and by laboratory fermentation and distillation of sweet toddy at the Coconut Research Institute, Sri Lanka. Distillate samples (20 ml) were sealed in glass vials while sweet toddy samples (500 ml) were pasteurized at 90°C for 20 min and stored in sterile bottles. This procedure produced a slight caramelization in the medium but did not change the sugar, alcohol or acid content as measured. Samples remained unchanged for at least 6 months. All samples were air-freighted to TPI, London for analysis.

Laboratory fermentation and distillation

Yeast cultures, isolated from fermenting toddy and identified according to the scheme of Lodder (1970), and wine yeast, isolated from a commercial wine yeast preparation, were maintained on sweet toddy agar (sweet toddy + 2% agar) and prepared for fermentation by subculturing into 100 ml of sterile sweet toddy.

Sweet toddy was collected in polythene bags attached to the cut inflorescence. It was combined in 1-litre-conical flasks and steam sterilized at atmospheric pressure for 30 min. Sterile sweet toddy (3 litres) was transferred to a sterile 10-litre glass bottle, fitted with a fermentation lock, and inoculated with yeast. After fermentation was complete (about 3 days at 30°C), the fermented toddy was transferred to a 5-litre round-bottomed flask carrying a short fractionating column and condenser. The flask was heated by an electric mantle at a constant rate and two fractions of distillate collected in line with the practice used in distilleries. The first 25 ml (foreshots) were discarded and the next 500 ml collected for analysis.

Naturally fermented toddy was distilled under the same conditions.

Gas chromatographic analysis

Qualitative analysis. Analyses were performed on a Perkin-Elmer F-33 gas chromatograph fitted with flame ionization detector. Packed glass columns (6′ 6″ × ¼ o.d.) were used under the following conditions:

Packing material	*Operating conditions*	*Carrier gas flow*
Chromosorb 101 (80–100)	140°C (isothermal)	N_2, 50 ml min^{-1}
8% Carbowax 1540 on Chromosorb W AW–DMCS (80–100)	60°C (isothermal)	N_2, 18 ml min^{-1}

Fusel alcohols were identified by comparison of retention times with authentic samples in 40% aqueous ethanol and later confirmed by combined gas chromatography-mass spectrometry (GC–MS). Other compounds were first identified by GC–MS and confirmed by co-chromatography with standards.

Gas chromatography linked mass spectrometry was performed with a VG micromass 7080F using 70 eV electron impact ionization and linked with a VG data system. Samples were introduced from a Pye 104 gas chromatograph fitted with a 12′ × ⅛″ o.d. column, containing 15% Carbowax 20M. Helium carrier gas at a flow rate of 45 ml min^{-1} was used and the column was operated isothermally at 60°C.

Ethanol and methanol were obtained as Analar grade reagents from James Burrough Ltd, 60 Montford Place, London SE11; 1.1 Diethoxypropane was obtained from Fluka AG, CH9470, Buchs, Switzerland; acetaldehyde from BDH Chemicals Ltd, Poole, Dorset BH12 4NN; 3-ethoxypropanol and ethyl formate from Aldrich Chemical Co, New Road, Gillingham, Dorset SP8 4JL. 3-Ethoxypropanal was synthesized from the alcohol by the method of Corey (1975). 2-Methylpropyl and 3-methylbutyl acetates were prepared from the corresponding alcohols with acetic anhydride in pyridine. All other standards were obtained from Hopkin and Williams Ltd, Chadwell Heath, Essex, as analar grade reagents.

Quantitative determinations. Ethanol concentration was determined on Chromosorb 101 (see above) with 2-propanol as internal standard (Venturella, Graves & Lang, 1974). Fusel alcohols were determined as recommended by the Research Committee on the Analysis of Potable Spirits (1970). Methanol, acetaldehyde, ethyl acetate and ethyl lactate concentrations were calculated from standard curves under the same conditions with the same internal standard, 1-butanol. The other components occasionally present were estimated by comparison of the peak heights with those of standard solutions in 40% ethanol.

Lactic acid in the sweet toddy was determined after extraction as its methyl ester (Bricknell *et al.*, 1979). Succinic acid, internal standard, was added to the toddy and treated similarly. The small quantity of succinic acid naturally

present is sweet toddy, less than 0.6% the amount added as internal standard, did not interfere with the analysis. The methyl esters were separated on 8% Carbowax 1540 maintained isothermally at 120°C with a nitrogen carrier gas flow rate of 23 ml min^{-1}. A standard curve was prepared using lactic acid from which internal anhydrides has been removed by refluxing a 1.7% w/v aqueous solution for 5 hr.

Results and discussion

The concentrations of the major volatiles, including ethanol, in six commercially available brands of arrack are presented in Table 1. The data illustrate the considerable variation in character that is known to exist between brands. Sample 5, from an illicit still, is exceptional in that it has a far lower ethanol content than the other samples and a much higher level of the less volatile ethyl lactate. This suggests that the toddy used was either markedly different from that used by other distilleries or, which is more likely, a far larger fraction of distillate was collected as product.

There is considerable variation among the commercial brands in both the concentration of ethanol and the concentrations of other volatiles based on ethanol. The latter are generally lower than those reported for more well

Table 1. Major volatiles in arrack

	Congeners (mg kg^{-1} of ethanol) and brand no.					
	1	2	3	4	5	6
Ethanol (g $litre^{-1}$)	310	320	400	290	180	350
Methanol	9	16	14	26	8	22
1-Propanol	90	120	106	2	43	623
2-Methylpropanol	260	397	336	20	282	331
Amyl alcohol (principally 3-methylbutanol)	1080	1470	1350	60	1350	1310
Acetaldehyde	28	10	79	38	194	95
Ethyl acetate	208	70	570	105	568	1482
Ethyl lactate	706	49	108	43	1066	320
1-Butanol	trace < 30	trace < 30	trace < 30	trace < 30	trace < 30	trace < 30
2-Butanol	ND < 5	ND < 5	ND < 5	ND < 5	ND < 5	500
3-Ethoxypropanol	ND < 5	ND < 5	tr. < 10	ND < 5	ND < 5	ND < 5
3-Ethoxypropanal	ND < 5	ND < 5	ND < 5	tr. < 10	tr. < 10	700
Ethyl formate	tr. < 25	tr. < 25	tr. < 25	tr. < 25	tr. < 25	tr. < 25
Isobutyl acetate	ND < 4	ND < 4	tr. < 10	ND < 4	ND < 4	ND < 4
Isoamyl acetate	ND < 4	ND < 4	ND < 4	ND < 4	ND < 4	40
Acetic acid	ND < 12	ND < 12	tr. < 15	ND < 12	ND < 12	tr. < 15
1.1-Diethoxypropane	ND < 2	ND < 2	ND < 2	ND < 2	tr. < 5	30

ND = not detected.
tr. = traces.

known distilled liquors (de Becze, Smith & Vaugn, 1967). This is particularly so for sample 4 which is a blend containing neutral spirit.

The percentage composition of the fusel alcohols in arrack is close to that reported for cognac and American whiskey (Singer, 1966; Lehtonen & Suomalainen, 1979). Ethyl lactate and acetate were found in all samples while one, No. 6, contained a greater quantity and variety of congeners.

Apart from small quantities of double distilled arrack, the commercially produced brands are blends of the product from a single pot distillation with that from a patent still. Samples of these distillates before maturation and blending were obtained from the 5 major distilleries in Sri Lanka which use similar equipment and distillation practices (Table 2). The pot distillates had ethanol concentrations ranging from 390 to 660 g litre^{-1}. The levels of 2-methylpropanol and 3-methylbutanol were variable but showed a lower percentage variation than other volatiles which differed by as much as 1000% in some cases. A patent still distillate from Seeduwa showed a higher concentration of ethanol but similar levels of other volatiles, based on ethanol.

From the results presented above it is apparent that variation between arrack brands is not the result of different blending practices but may be due to differences in the toddy used or the maturation period employed. Support for the former hypothesis can be found in the results obtained with distillates from

Table 2. Major volatiles in palm wine distillates

	Congeners (mg kg^{-1} of ethanol), type of distillate and locality					
	Single distilled in pot still					French patent still
	Seeduwa	Wadduwa	Kalutara	Jaffna	Dankotuwa	Seeduwa
Ethanol (g litre^{-1})	390	580	610	660	390	730
Methanol	7	9	13	31	6	20
1-Propanol	80	885	339	1181	56	
2-Methylpropanol	273	270	346	325	286	303
Amyl alcohol (principally 3-methylbutanol)	1180	1260	1340	1460	1220	1130
Acetaldehyde	15	111	73	40	11	40
Ethyl acetate	35	2820	1800	5080	69	719
Ethyl lactate	1160	270	487	512	1220	545
1-Butanol	tr.<30	tr.<30	tr.<30	tr.<30	tr.<30	tr.<30
2-Butanol	ND<5	ND<5	ND<5	ND<5	ND<5	ND<5
3-Ethoxypropanol	ND<5	ND<5	ND<5	ND<5	tr.<10	ND<5
3-Ethoxypropanal	ND<5	ND<5	ND<5	900	tr.5	ND<5
Ethyl formate	tr.<25	80	tr.<25	tr.<25	tr.<25	tr.<25
Isobutyl acetate	ND<4	ND<4	ND<4	ND<4	ND<4	ND<4
Isoamyl acetate	ND<4	ND<4	ND<4	450	ND<4	ND<4
Acetic acid	120	ND<12	tr.<15	ND<12	200	35
1.1-Diethoxypropane	ND<2	tr.<5	tr.<5	250	tr.<5	ND<2

Jaffna where brand 6 in Table 1 is produced. In this region, which is remote from other arrack producing areas and is climatologically different, being arid, the toddy is obtained from a different palm, palmyrah *(Borassus flabellifer)*. Distillate from Jaffna showed a higher level of fusel alcohol than other samples and compounds only occasionally found in other cases, 3-ethoxypropanal, 1-1-diethoxypropane, isoamyl acetate and 2-butanol, were all detected in substantial amounts. These compounds are not unique to arrack, they have been found in whiskey and rum (Dubois, Parfait & Dekimpe, 1973; Kahn, Larce & Conneu, 1968) and biochemical pathways from sugar to ethoxypropane derivatives involving the action of both yeasts and bacteria have been proposed (Dubois *et al.*, 1973). Differences in the microflora responsible for the fermentation in the Jaffna region could account for this.

The important role of the mixed microflora in the quality of arrack was illustrated by analyses of laboratory distillates of fermented toddy produced by the natural fermentation and by pure yeast cultures.

The fermentation medium was sweet toddy collected over a period of 4 hr. During this time the pH level of the liquid dropped from around 7 to 4, at which stage the total acidity consisted almost entirely of lactic acid (2.6 g litre^{-1}). Some sweet toddy was left to continue its natural fermentation while other samples were taken, sterilized and inoculated with yeast.

The most striking feature of the results presented in Table 3 is the considerably higher concentration of the esters ethyl lactate and ethyl acetate in the naturally fermented samples. Ethyl lactate was found in most of the samples of commercial arrack in concentrations above its flavour threshold of 14 mg litre^{-1} (Salo, 1970). It has been reported in other spirits (Kahn, 1969; Maarse & ten Noever de Brauw, 1966; Otsuka, Iki & Yamashita, 1979; Schaeffer & Timmer, 1970), and concentrations similar to those in arrack have been found in samples of American brandy, Bourbon whiskey and Nubian gin

Table 3. Variations in major volatiles with respect to type of micro-organisms used in laboratory fermentation and distillation of coconut sap

		Congeners (mg^{-1} of ethanol)						
Micro-organism	Ethanol (g litre^{-1})	Methanol	1-Propanol	2-Methyl-propanol	Amyl alcohol	Acetaldehyde	Ethyl acetate	Ethyl lactate
Saccharomyces cerevisiae A	410	9	86	463	2450	237	259	34
Saccharomyces cerevisiae B	420	11	164	562	2180	169	307	30
Toddy yeast *Saccharomyces chevalieri* y11	380	11	50	366	2340	161	198	26
Saccharomyces chevalieri y 193	410	20	169	498	2680	85	329	37
Natural fermentation by wild yeasts	340	17	120	474	1560	123	3190	365

(Nout, 1979). It was not, however, found in all of the samples of Bourbon analysed.

The origin of the esters is almost certainly microbiological. Incubation of the respective acids and ethanol at the same conditions of concentration, temperature and pH level did not give appreciable quantities of ester on distillation.

Other workers have shown that the production of 3-methylbutyl acetate and other esters varies considerably with different species of yeast (Suomalainen & Lehtonen, 1979) and a mechanism of ethyl acetate formation in *Saccharomyers cerevisiae* has been shown to involve the activation of the free acid by reaction with coenzyme A (Nordstrom, 1962). This is a non-specific reaction as substitution of acetic acid by another carboxylic acid leads to the formation of the corresponding ethyl ester. Such a mechanism would account for the presence of both ethyl lactate and ethyl acetate in arrack and Nubian gin, both products of a mixed lactic-alcoholic fermentation.

Analysis of samples of both pot and patent still distillates produced at Seeduwa showed that over a five-year maturation period changes take place in the composition of the major volatiles. The fusel alcohols increase by between 12 and 15%, the ethyl acetate concentration is halved and there are slight increases in the acetic acid and methanol levels. However, as most arracks are matured for the same period, between 3 and 5 years, the greatest changes were found to occur during the first 3 years of maturation, it would seem that the most important source of variation between brands is the composition of the fermented toddy used.

Acknowledgments

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Technical note: Limitations in the use of an enzymic test for monitoring the thermal pasteurization of dried chicken egg-white

J. B. MONSEY AND J. M. JONES

Introduction

The object of pasteurization is primarily to destroy salmonellae. The heat resistance of salmonellae in egg-white pasteurized in the liquid state is described by Kline *et al.* (1965) and by Corry & Barnes (1968) and in egg-white pasteurized in the dry state by Northolt, Wiegersma & Van Schothorst (1978) using a different serotype of Salmonella.

Two methods of producing pasteurized dried egg-white are currently in use commercially. The first is to pasteurize (in the U.K. at 57.2°C for 2.5 min) the homogenized liquid egg-white in a conventional continuous-flow heat-exchanger prior to drying. The second method, which is extensively used, is to place the unpasteurized dried egg-white in a hot room at 55°C for up to 3 weeks. A test for the adequate pasteurization of liquid egg-white was developed by Monsey & Jones (1979) based on the deactivation by heat of a starch-degrading enzyme, naturally present in egg-white. The applicability of this test to dried egg-white pasteurized by both methods has been investigated.

Results and discussion

Because the pH of solutions of commercial egg-white powders was found to vary, in some cases enough to overcome the buffering capacity of the phosphate buffer used in the test, it was found necessary to adjust the pH of the solutions to 8.5 (with strong HCl or NaOH and with vigorous magnetic stirring). Otherwise, the test was used unchanged from that described by Monsey & Jones (1979).

At an egg-breaking plant, a batch of liquid egg-white was divided into two parts, one being pasteurized conventionally, before both were spray-dried. Solutions (13% w/v) of these powders were made in distilled water and the test applied. The solution containing pasteurized egg-white passed the test

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0022–1163/81/0800–0445 $02.00

(absorbance at 585 nm of the final solution, 0.638) and the solution containing unpasteurized egg-white failed (absorbance 0.068). This experiment also demonstrated that the enzyme activity was not noticeably affected by spray-drying.

Of five samples of imported dried egg-white labelled 'pasteurized' (method unknown), only two samples passed the test. A further twenty from Holland which had been taken from different areas of a commercial hot room held at 55°C for 3 weeks, all failed the test. When samples of unpasteurized dried egg-white were placed in a laboratory hot-air oven at 55°C for 3 weeks, they failed the test; in addition, further samples treated at 80°C also failed. The enzyme, therefore, is apparently more stable to heat in dried egg-white than in the liquid form.

The existing test (Monsey & Jones, 1979) can thus be used to detect the thermal pasteurization of dried egg-white only if the pasteurization occurred while the egg-white was in liquid state. However, even in this case, it is not known whether the sensitivity of the test (for example, the effect of any possible reduction in the temperature/time of pasteurization or of the effect of a subsequent contamination with unpasteurized egg-white) is affected by drying.

It is concluded therefore that the enzymic test is not readily applicable to the assessment of the thermal pasteurization of dried chicken egg-white.

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Technical note: Predicting quality of cassava products with the aid of chemical and rheological properties

A. O. OLORUNDA, O. C. AWORH, AND F. A. NUMFOR

Introduction

There are over 100 cultivars of cassava (*Manihot esculenta*, Crantz syn *M. utilissima*, Pohl) with a large degree of variation in foliage, stem and tuber characteristics (Kay, 1973).

While some research efforts have been directed towards evaluating the chemical composition of cassava roots and the changes that occur in these chemical constituents during processing (Ketiku & Oyenuga, 1972; Ketiku *et al.*, 1978; Ogunsua & Adedeji, 1979), there is little information as to specific composition and the quality requirements for the various processed products (Booth *et al.*, 1976). It has been observed that chemical and textural changes in stored cassava roots affected their acceptability when cooked as a fresh vegetable for human consumption, but had no noticeable effect on the eating quality of cassava meal (Booth *et al.*, 1976).

With the advent of large-scale production of 'gari' and starch from cassava, processors are faced with the problem of selecting cultivars that will yield the most acceptable products to an increasingly discriminating consumer.

This paper reports a study of some chemical and rheological properties of the tubers of five selected cassava cultivars and their relationship to the quality of the processed product, gari.

Materials and methods

Ten-month-old roots of five cassava cultivars, 'Isunikakiyan'—a local cultivar, 30555, 30375, 30395 and 60444 were harvested from 'Uniform Trial' plots at the International Institute of Tropical Agriculture, Ibadan, Nigeria. The roots from each cultivar were divided into three groups for fresh analysis, starch extraction and processing into gari.

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0022–1163/81/0800–0447 $02.00

Tubers for fresh analysis were peeled, cut into 1 cm thick slices, dried for 24 hr at 40°C in a forced draught oven, milled into flour and stored in glass jars for subsequent analysis. For starch extraction, the roots were peeled, grated and the resultant mash washed with distilled water. The liquor was then filtered through cheese cloth. After standing for 1 hr, the starch sedimented and the supernatant was decanted. The starch was then dried at 40°C for 24 hr in a forced draught oven. The traditional method was used for gari processing. Fresh tubers were peeled, washed with tap water, grated into a mash which was put in cloth sacks and tied firmly with ropes to sticks. After fermentation for five days at room temperature, the hardened mass was removed from the sacks, scattered in open pans, sieved and then fried in an open iron pan at a temperature of about 95°C.

Chemical analysis

Starch content of cassava tubers was determined by the AOAC method (1965) and amylose as described by McCready & Hassid (1943).

Amylograph test

Pasting curves for cassava starch were determined with a Brabender amylograph. Starch of particle size less than 250 μm, was suspended in distilled water, heated from 30 to 95°C at the rate of 1.5°C per min, held at 95°C for 20 min, and then cooled for 45 min.

Sensory evaluation

Sensory tests were carried out on gari prepared from the five cassava cultivars by a nine-member panel using a multiple comparison difference analysis (Larmond, 1977). The panelists rated the gari samples for colour, flavour and texture on a nine-point scale where 9 and 1 represented 'like extremely' and 'dislike extremely' respectively.

The texture of the cassava tubers was assessed by four trained panelists. Freshly harvested roots were peeled, washed, cut into 2-cm slices and boiled for 15 min. The panelists were asked to describe the texture of the cooked samples since it was difficult to score.

Results and discussion

Little difference was observed in the starch content of the tubers of the five cassava cultivars used in this study (Table 1). On the other hand, significant varietal differences in amylose content and pasting characteristics of the starch

were noted. Cultivars with higher amylose content, Isunikakiyan, 30555 and 30375, tended to have slightly higher amylograph peak viscosity levels (Table 1). Gelation time of the starch also varied between cultivars but bore no relationship to the maximum viscosity or amylose content.

Table 1. Starch and amylose content of fresh tubers and amylograph data of the starch of five cassava cultivars

Cultivar	Starch* (percentage dry wt)	Amylose* (percentage dry wt)	Amylograph peak viscosity (B.U.)	Gelation time (min)
Isunikakiyan	80.7±2.3	23.3±0.2	785	16
30555	79.3±2.8	19.4±0.0	780	8
30375	80.2±3.1	20.7±0.6	775	15
30395	80.5±2.9	17.7±0.3	750	5.5
60444	78.7±1.0	13.6±0.5	760	10

* Values shown are the mean and standard deviation of duplicate samples.

Table 2. Sensory quality attributes of cooked tubers and gari made from five cassava cultivars

Cultivar	Texture	Gari		
		Colour*	Flavour*	Texture†
Isunikakiyan	Soft, dry and mealy	6.2a	7.4a	5.2
30555	Slightly hard, dry and mealy	5.0ab	5.1b	5.0
30375	Soft, mashy, slightly mealy	3.3bc	5.4b	3.7
30395	Hard, dry and gummy	4.1bc	4.2b	4.1
60444	Hard, dry and waxy	3.1c	1.8c	3.0

* Means followed by the same letter are not significantly different at 5% level of probability by Tukey's test.

†No significant difference between cultivars.

The results of the sensory evaluation of cooked tubers and gari made from the five cassava cultivars are presented in Table 2. In general, there seems to be a relationship between mealiness of the cooked tubers on the one hand, and amylose content and amylograph peak viscosity of the starch on the other. Panelists described the cultivars 30395 and 60444 with lower amylose content and amylograph peak viscosity (Table 1) as being gummy and waxy while Isunikakiyan and 30555 were rated as mealy. Earlier studies (Unrau & Nylund, 1957; Kuhn, Desrosier & Ammerman, 1959) have also suggested a relationship between mealiness in potato varieties and pasting characteristics of the starch.

Sensory evaluation of gari prepared from the cassava cultivars showed a definite preference for the local cultivar Insunikakiyan in terms of colour and flavour over the other cultivars (Table 2). Panelists found only minor

differences in texture of gari prepared from the different cultivars (Table 2) presumably because sieving reduces the fibrousness of gari. In general, apart from Isunikakiyan, 30555—another mealy cultivar—was rated slightly better than the other cultivars in terms of colour, flavour and texture of the gari. The waxy cultivar 60444 with the lowest amylose content (Table 1) produced the least acceptable gari (Table 2).

In conclusion, amylose content and amylograph peak viscosity of cassava starch were related to subjectively evaluated mealiness. Mealier cassava cultivars had higher amylose content and slightly higher amylograph peak viscosity and produced the most acceptable gari.

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Book reviews

Basic Food Microbiology. By George J. Banwart.
Westport, Connecticut: Avi, 1979. Pp. vii + 781. ISBN 0 87005 322 4. Paper $22.00.

The reader of this book is assumed to have a background knowledge of chemistry, biology and general microbiology. The basics of food microbiology are emphasized, in order that a better understanding of microbiological aspects of processing and compounding of foods may help the reader to comprehend the microbiological implications involved in a new or modified process, additive or product. The author's intention was to develop a text that did not merely describe the microbiology of a few simple foods.

There are thirteen chapters. After an introductory chapter of 16 pages on General Aspects of Food, there follow chapters on Estimating the Number of Micro-organisms; Micro-organisms Associated with Food; Conditions that Influence Microbial Growth; Sources of Micro-organisms; Foodborne Illness; Indicator Organisms; Food Spoilage; Useful Micro-organisms; The Control of Micro-organisms; Control of Micro-organisms by Retarding Growth; Control of Micro-organisms by Destruction; and finally Regulations and Standards (mainly relating to the United States).

There are numerous references to relevant literature, the list of references being given at the end of each chapter, but there is no author index.

The literature is well surveyed up to 1977, with occasional references to 1978 and 1979 publications. One or two very recent events are not found recorded here. For example, the change of name of *Microbacterium thermosphactum* to *Brochothrix thermosphacta*; and, more importantly, the recent implications of *Campylobacter coli/jejuni* in the occurrence of human gastro-enteritis. Sometimes critical discussion is sacrificed inexcusably in the cause of simplicity. For example on page 668 the important literature relating to non-linearity of thermal destruction curves and 'multiple hit theory' is barely given a passing reference.

As can be seen from the chapter titles, the author has tried to avoid the oversimplified commodity approach. However, in a number of the chapters, subheadings do relate to the major commodity groups, so that the author has not been entirely successful in his aim of tackling food microbiology in the ecological context of the microbial reactions to the main physical and chemical parameters of the food environment.

Graphs, line diagrams and tables are, for the most part, well chosen. The

book suffers slightly from the typical Avi fault of consistently poor reproduction of sometimes inconsequential photographs, but luckily they do not form an important feature of the text.

Overall, an easily readable and very informative textbook, which this reviewer much prefers to that of Frazier and Westhof, and which forms a useful complementary text to that of Jay.

W. F. Harrigan

Nutrition and Food Processing. By H. G. Muller and G. Tobin. London: Croom Helm, 1980. Pp. 302. ISBN 0 85664 540 0. £15.95.

The initial reaction to this book is 'Haven't I heard of this somewhere before?' Indeed, the titles 'Nutrition and Food Processing' by the present authors and 'Food Processing and Nutrition' by Professor A. E. Bender are alike. The similarity, however, ends there. Whereas the latter can be regarded as a reference book as well as a summary of the scientific literature, the present book is predominantly an introductory text which will be of particular use to undergraduate students of food science, nutrition, dietetics, catering, etc.

Undoubtedly this book can fill this necessary gap in the currently available sources of information on food production, its processing and its relation to nutrition, health and disease. It is infinitely more readable than Harris and Karmas' 'Nutritional Evaluation of Food Processing', but it does not provide extensive coverage of any one topic, and most students would have to obtain more detailed information from elsewhere. The bibliography does, however, provide key references for further reading.

The book is composed of 14 chapters. The first four chapters include introductory accounts of the historical development of nutrition research, the chemistry of nutrition, nutritional requirements and the evaluation of nutrients. The chapter on food chemistry is very limited in extent, and refers to a number of vitamins by their less well-known names, e.g. vitamin M for folic acid and vitamin H for biotin. Pantothenic acid, which is generally regarded as a vitamin with no numerical designation, is referred to as vitamin B_5 and sometimes as B_3. Chapters 5 to 10 deal successfully with a description of the major food groups from animal and vegetable sources. Chapter 6 on cereals and legumes and Chapter 10 on foods of animal origin are particularly well written. Chapters 11 to 13 discuss the use of food additives in food processing, specific refining operations, refrigeration, heat treatments and dehydration. The reference to food preservation and the lack of nutritional effect of salt other than to improve flavour may cause consternation among nutritionists and protagonists of nutrition guidelines. The majority of nutritionists associate an excessive intake of salt with the development of hypertension, at least in some individuals.

Furthermore, most recommendations for a healthier diet include advice to reduce salt intake.

Reference to hypertension, a major health problem and a nutrition-related disease, is also omitted from the final chapter on 'Food and Disease'. This chapter, on the whole, avoids the more controversial issues of the nutritional factors associated with many of the diseases of over abundance, such as heart disease and some forms of cancer, and presents the information as objectively as possible. On page 283, the nutritional factors associated with ischaemic heart disease include water softeners. This is probably a misprint for water softness. The epidemiological evidence is that there is a negative association between the hardness of the domestic water supply and the local death rate from cardiovascular disease – that is to say, the softer the water the higher the death rate from this disease.

The book is elementary, interesting and readable, and as such should appeal to students wishing for an introductory overview of the topic. The cost, however, is prohibitive, and is substantially more than the price of each of the other two reference texts mentioned in this review.

David P. Richardson

The Technology of Wine Making, 4th ed. By M. A. Amerine et al. Westport, Connecticut: Avi, 1980. Pp. xi + 794. ISBN 0 87055 333 X. $42.50.

Whether the authors who launched the first edition of this book in 1960 anticipated the benefit that it would bring to students of wine is a moot point, but the fourth edition must surely establish it as an outstanding contribution to the literature. Thus, it has grown from a book of essentially American orientation into one that deals with wine-making as an international practice, and this improved balance has enhanced the stature of the book immensely. It is, of course, a trend that can be discerned in the intermediate editions, but the latest edition has, perhaps as a result of the expanded 'band' of authors, provided a world-wide coverage that is as extensive as it is informative.

The text begins with a resumé of the types of wine produced in different parts of the world, but there is nothing superficial about the subsequent chapter devoted to the composition of grapes. Indeed it is chapters like this latter one, and a subsequent one on the chemistry of fermentation, that represent the most dramatic improvement with time, for the review of each subject area is now truly comprehensive. The chapter on winery design, operation and sanitation represents a valuable 'up-date' from previous editions, as does the section on the 'laboratory' evaluation of wines and brandies. The production of wines ranging from red and white table wines through to the various fortified wines like sherry, port and vermouth, is, as in earlier versions, described in a clear and authoritative manner, and the only criticism of these chapters is that, on

occasions, too much is left to the imagination of the reader. In describing the 'dosage' of champagne, for example, the reader is merely told that 'a measured amount (of sugar syrup) is added, and if the bottle is then not sufficiently full, sufficient champagne from another bottle is added'. How the operative achieves these additions accurately and without loss of in-bottle pressure is not revealed, even though a few extra words would have made the operation much more intelligible.

Inevitably in any book of some 800 pages, there are bound to be statements that are erroneous, ambiguous, or even seemingly irrelevant, but such blemishes will not prevent this book from becoming a 'classic' among the texts on food and beverage science. What these problems do suggest, however, is that the book has probably reached its maximum useful length, and that future revisions should concentrate on excellence rather than expansion. The poor quality of some of the photographs, for example, raises the question of whether, in the absence of printing techniques of better resolution, a rather tougher editorial policy might not be in order. A similar comment could be made about occasional paragraphs in the text as well. Thus, the admonition that handlers of sparkling wines should ensure that their faces are protected by masks, is placed adjacent to a photograph of one such worker who does not appear to have read the author's advice. Obviously this particular association is just unfortunate, but if a more appropriate and informative legend had been placed under the photograph, then there would be no cause for comment.

Perhaps it is unfair to record such trivial irritations, and certainly they do not detract from the overall value of the text. Nevertheless, this book has, over the years, carved a niche for itself that is almost unique, and this privileged position demands a standard of editorial proficiency that should not be sacrificed.

R. K. Robinson

Food Sanitation, 2nd Edition. By Rufus K. Guthrie.
Westport, Connecticut: Avi, 1980. Pp. xii + 326. ISBN 0 0 97055 361 5. $24.00.

Professor Guthrie's *Food Sanitation* was first published in 1972 following requests from his catering students. This second edition was undertaken in light of recent developments in food science and food microbiology and also because of the growth of federal, state and municipal legislation. As Professor Guthrie states: 'the science of sanitation has changed little', so that much of what is new in the book relates to matters in the U.S.A. and may seem of little immediate interest to the British reader. However, details of municipal legislation such as the Dallas City Code show that hygiene codes of practice are more detailed and specific in the States than the Food Hygiene Regulations in this country and point the way forward for us.

The early chapters of the book set out the elementary principles of microbiology and the micro-organisms important in food sanitation. Treatment of topics is wide rather than deep. Micro-organisms mentioned range from the usual food poisoning bacteria to those which cause whooping cough and diphtheria, but the list does not include *Campylobacter coli/jejuni*, an organism of growing significance in food hygiene, details of which should be found in an updated text.

In the second section chapters deal with chemicals and food, water supplies and sewage disposal; the text is somewhat verbose and facts given could have been expressed more succinctly. There are some useful details on the nature and quantities of food processing wastes.

Chapters in the third section of the book are concerned with sanitation in food manufacturing and processing, the dairy industry and catering. The most pleasing of these is the chapter 'Sanitation in Dairy Food Plant' by W. J. Harper; it is very detailed and precise and makes very rewarding reading. There are more figures and tables in this chapter than in any other in the book. The chapter on sanitation in the canning industry is very broad and superficial in approach and there is little which is specific to the industry. The closing chapters are devoted to sanitation in catering. These are less informative than that on the dairy industry and lack specificity.

There is an index which contains a glossary of terms and some details of food hygiene legislation. In Appendix III there are twenty-eight pages of selected laws, codes and ordinances, which provide the most interesting feature of the book and to those unfamiliar with U.S.A. hygiene legislation it is a valuable introduction to affairs on the other side of the Atlantic.

There are useful tables and figures throughout the book and some photographs which are used to break up the text rather than to illustrate some important principle.

The book would make a suitable addition to the reading lists of students of Food Technology at Higher Diploma or first year undergraduate level and provides a good overview of food sanitation practice. The broad general nature of the text makes it less satisfactory for catering students who require a more definite treatment of the problems and principles of food service hygiene.

R. G. Cockett

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

SI UNITS

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

NON SI UNITS

inch	in	= 25·4 mm
foot	ft	= 0·3048 m
square inch	in^2	= 645·16 mm^2
square foot	ft^2	= 0·092903 m^2
cubic inch	in^3	= 1·63871 × 10^4 mm^2
cubic foot	ft^3	= 0·028317 m^3
gallon	gal	= 4·54611
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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