ISSN 0022-1163

Volume 20 Number 5 October 1985

IFST

Journal of Food Technology

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

JOURNAL OF FOOD TECHNOLOGY

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

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The Journal of Food Technology is published bimonthly, six issues form one volume. The subscription price for 1985 is £79.00 (U.K.), £95.00 (Overseas), $195.00 (N. America, including cost of airfreight). Current issues for the Indian Sub-Continent, Australasia and the Far East are sent by air to regional distribution points from where they are forwarded to subscribers by surface mail. Any back numbers are normally despatched by surface to all regions, except North America, where they are sent by a.s.p., and India, where they are sent by air freight. Back volumes are still available. This journal is covered by *Current Contents*, *ASCA* and *Science Citation Index*.

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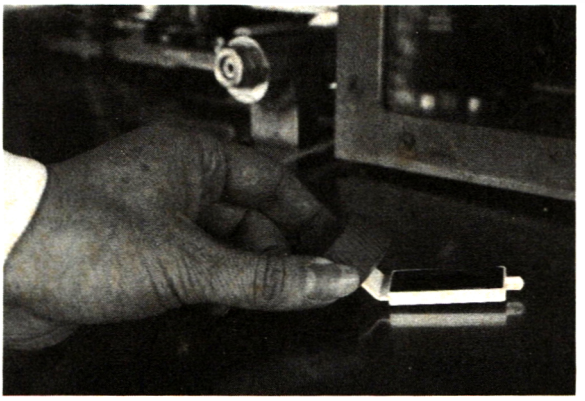

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Journal of Food Technology (1985) **20**, 541–551

Note on the economics of batch freeze dryers

M. J. MILLMAN*, A. I. LIAPIS† AND J. M. MARCHELLO

Summary

An economic analysis of various operational policies associated with a batch freeze dryer is presented. Skim milk is used as a model material. It is shown that the lowest cost of production corresponds to the highest production rate. This occurs when large sample thicknesses are used, the chamber pressure is at its lowest value and the upper and lower heating plates are independently controlled such that the melting and scorch constraints are both encountered and held during the free water removal phase. Therefore, the operational regime that provides the shortest drying times during the free water removal phase (Millman, Liapis & Marchello, 1984, 1985) will also provide the smallest production costs and highest capacities.

Introduction

Freeze drying is being applied to an increasing number of biological, pharmaceutical and food products (Goldblith, Rey & Rothmayr, 1975; Mellor, 1978; Hill & Hirtenstein, 1983). Dehydration and/or solvent removal by freeze drying results in dried products of quality unsurpassed by any other drying process (Mellor, 1978; Hill & Hirtenstein, 1983).

The dried products of the freeze drying operation have high structural rigidity, low density, high resistance to bacterial degradation, are easily rehydrated and retain more of the original aroma and taste than the products of conventional drying methods. The major disadvantage of freeze drying is that the drying rates are low since the drying is done at relatively low temperatures and very low pressures, less than 2.0 mm Hg. This makes freeze drying a time consuming and energy intensive process.

Various mathematical models have been developed to describe the freeze drying process (King, 1971; Greenfield, 1974; Mellor, 1978; Liapis & Litchfield, 1979a; Pikal *et al.*, 1983; Pikal, Roy & Shah, 1984). These models account for the removal of frozen water only, and the models of Pikal *et al.* (1983; 1984) involve a large number of empirical constants whose values may change as the types of material to be freeze dried and the design and operating conditions in the freeze dryer are varied. A number of investigators (King, 1971; Greenfield, 1974; Mellor, 1978; Liapis & Litchfield, 1979a; Litchfield & Liapis, 1982; Millman *et al.*, 1984) used these models to determine operating policies which would reduce or minimize the time required for freeze drying a sample of certain thickness. In these studies it is assumed that the operational policy that minimizes the time for the removal of free water, would also provide the optimum cost of operation.

Sandall, King & Wilke (1967), King (1971), Meo (1972), Aguilera & Flink (1974) and Mellor (1978) have shown with experimental data that the removal of sorbed or bound water may require as much as or more drying time than that necessary for the

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removal of frozen or free water. This has also been shown theoretically through the use of a sorption sublimation model (Litchfield & Liapis, 1979; Liapis & Marchello, 1983, 1984; Millman *et al.*, 1985; Millman, 1984) which accounts for the removal of frozen and bound water. The sorption sublimation model does not involve any empirical parameters and has been found to predict accurately the experimental drying rates and times of the freeze drying process (Litchfield & Liapis, 1979; Liapis, 1980a, 1980b; Liapis & Marchello, 1984).

Millman *et al.* (1985) and Millman (1984) used the sorption sublimation model and various operational policies whose heat transfer mechanisms and plate conditions are given in Table 1, in order to analyse the freeze drying process and establish the regime that would provide the shortest drying times. They used skim milk as model material and found that the best operational regime was to keep the chamber pressure at its lowest value and the upper and lower heating plates were independently controlled such that the melting and scorch constraints are both encountered and kept during the free water removal phase, while during the terminal drying phase the scorch constraint is kept. This regime uses the heat transfer mechanisms and plate conditions of case C, Table 1. Millman *et al.* (1985) define the free water removal phase as the drying time required for the sublimation interface to reach the lower surface of the sample being freeze dried. The terminal drying phase starts at the end of the free water removal phase and ends when a certain criterion with respect to the amount of sorbed water in the dried layer is satisfied.

Table 1. Heat transfer mechanisms and plate conditions

Case	Heat transfer mechanisms	Plate conditions
A	Radiation only to both upper and bottom surfaces	Upper and lower plates at the same temperature, $T_{UP} = T_{LP}$, with a maximum permissible value of 30°C
B	Radiation to upper dried surface; conduction through a film layer at $x = L$	Upper and lower plates are at the same temperature, $T_{UP} = T_{LP}$, and the plate temperatures are controlled subject to the constraints that $T_I \leq T_s$ for $o \leq x < X$, and $T_{II} \leq T_m$ for $X \leq x \leq L$
C	Same as in B	Upper and lower plates operate at different temperatures, and the plate temperatures are controlled subject to the constraints that $T_I \leq T_s$ for $o \leq x < X$, and $T_{II} \leq T_m$ for $X \leq x \leq L$
D	No radiation to upper dried surface. Otherwise as B.	Lower plate temperature, T_{LP}, is controlled subject to the constraint that $T_{II} \leq T_m$ for $X \leq x \leq L$

Frozen water and some sorbed water is removed during the free water removal phase while only bound water is removed during the terminal drying phase. In this work, the sorption sublimation model of the freeze drying process is used to study the effects of the operational conditions of Table 1 and of the sample thickness on the daily capacity and drying cost of batch freeze dryers. Skim milk is used as a model material (Aguilera & Flink, 1974; Millman *et al.*, 1985).

Economic parameters and functions

The economic study in this work considers only the costs directly related to the drying stage of freeze drying, and the quality–cost relationship has not been explicitly studied in the present paper. The pre-drying stage where the material is either made or obtained and frozen, and the post-drying stage of packaging, storage, sales and distribution are not considered. The costs associated with the drying stage are (i) capital equipment costs, (ii) labour and energy costs of preparing the drying chamber for use, and (iii) labour and energy costs associated with the actual water removal.

The following assumptions are used in the development of the cost functions and in the economic calculations of this study:

(a) Only dryer preparation, drying, and equipment costs are considered.

(b) The capital cost of the dryer is independent of the operating regimes.

(c) The variation in the free volume of the dryer associated with the different sample thicknesses used, is negligible.

(d) Outgassing effects on the chamber pressure are not considered.

(e) The heating and condenser plates are considered to be 100% efficient.

(f) The time required to prepare the dryer for the drying stage, is dependent on the number of trays and on the operating chamber pressure.

(g) The defrosting of the condenser plates is considered to be rapid, requiring less time than the loading and unloading of the trays.

Jelen (1970) cites various depreciation techniques for tax purposes, and notes that straight line depreciation is the easiest and preferred method for internal cost analyses. Since the capital cost is assumed to be independent of the operational policies, the use of straight line depreciation allows the capital cost to be considered as a fixed daily operating cost. Accordingly, three capital costs were used to examine if a variation in the capital cost would have any effect on the choice of the best operational regime as determined from non-economic criteria in the work of Millman *et al.* (1984, 1985).

The time required to prepare the dryer for the next active drying period is termed the preparation time. During this period, the dried product of the previous drying stage is unloaded, new material to be dried is loaded, the condenser plate is defrosted, and the chamber must be evacuated to the desired operating pressure for the next drying period. The condenser plate is defrosted by melting the ice from its surface with heat supplied from condensing steam. Relatively low pressure steam (2311 mm Hg) is used, and it is assumed that the condenser can be defrosted while the loading and unloading of the dryer is being done. The time required by two unskilled labourers to load and unload a dryer of ten trays, is t_1, estimated to be 20 min.

The time required to evacuate the drying chamber is a function of the desired operating pressure. Since outgassing effects are not considered, the following expression from Mellor (1978) is used to estimate the time required to evacuate the chamber:

$$t_p = \frac{V_{ch}}{S_p} \ln \frac{P_{atm}}{P_{ch}} \tag{1}$$

The time t_p is added to the time t_1 to give the total preparation time.

The cost during the preparation time is given by the following expression:

$$C_{pt} = N_{lab} S_{lab} (t_p + t_1) + t_p C_{pump} + M_{stm} C_{stm} \tag{2}$$

The amount of steam required to defrost the condenser will vary as the thickness of the

material to be dried is varied. For a condenser temperature of 225 K, 436.1 kJ/kg ice of heat are required to raise the ice temperature to 273 K and melt the ice at 273 K.

The freeze dryer is taken to be an automated piece of equipment (Mellor, 1978; Stevanovic, 1982) which requires little monitoring. Therefore, it is assumed that one operator can monitor up to ten dryers simultaneously during the drying period, from a centralized location such as a control room. The energy requirement during the drying phase is a sum of the energy required to keep the vacuum in the drying chamber, the energy required to heat the plates that provide the heat for sublimation and desorption, and the refrigeration energy which keeps the condenser at a temperature such that the water vapour condenses in the form of frost or ice on the condenser plate. The energy used to keep the vacuum is taken to be directly proportional to the actual time the vacuum is held, and the refrigeration energy required is considered to be directly proportional to the amount of water removed from the dried material. The energy required to heat the plates must be summed over the entire drying period, and is dependent on the operational policy used.

The cost of the entire drying period is estimated by the following expression:

$$C_{dp} = N_{op}S_{op}t_d + M_{ice}C_{ref} + N_{tr}EC_{elec} + t_dC_{HP} \quad (3)$$

The overall cost function is then the sum of the daily operating costs. In the following section the operating costs are presented as a cost per kilogram of dried product. This is done by dividing the total daily cost by the daily capacity of the dryer corresponding to the operational regimes. The values of the economic parameters and those of the other variables in equations (1)–(3) are shown in Table 2.

Table 2. Values of the economic parameters and variables in equations (1)–(3)

C_{elec}	\$15.28/10^6 kJ
C_{HP}	\$0.0008/min
C_{pump}	\$0.0048/min
C_{ref}	\$8.21/10^6 kJ
C_{stm}	\$3.21/10^6 kJ
N_{lab}	2
N_{op}	1/10
N_{tr}	10
P_{atm}	760 mm Hg
S_{lab}	\$6.00/hr
S_{op}	\$6.00/hr
S_p	1 m^3/min
T_{cond}	225 K
V_{ch}	1 m^3
t_1	20 min

Results and discussion

Skim milk is used as a model material in this study, and the value of its physical parameters as well as the drying times resulting from the operational policies of cases A, B, C, and D, predicted by the sublimation model (Liapis & Litchfield, 1979a, 1979b; Millman *et al.*, 1984) and the sorption sublimation model, are given in Millman *et al.*

(1985). The sublimation model accounts only for the removal of frozen water while the sorption sublimation model accounts for the removal of frozen and bound water. In the calculations of this study, the condenser temperature is kept at its lowest value of 225 K, since Mellor (1978) and Millman *et al.* (1984) have shown that this is a desirable operational regime, since the lower the condenser temperature the higher the mass flux of water vapour and through that a shorter drying time is obtained.

In Fig. 1, the effect of sample thickness on the daily capacity of a freeze dryer is shown. The daily capacity of the dryer is based on the drying times predicted by (i) the sublimation and (ii) the sorption sublimation models. For the results of the sorption sublimation model, two different criteria were used to end the terminal drying phase. Criterion 1 requires that the final average water weight fraction in the dried product is 0.05 kg water/kg solid. Criterion 2 requires that the final maximum water weight fraction at any point in the dried material does not exceed 0.05 kg water/kg solid. Millman *et al.* (1985) have shown that criterion 2 would provide a better quality product

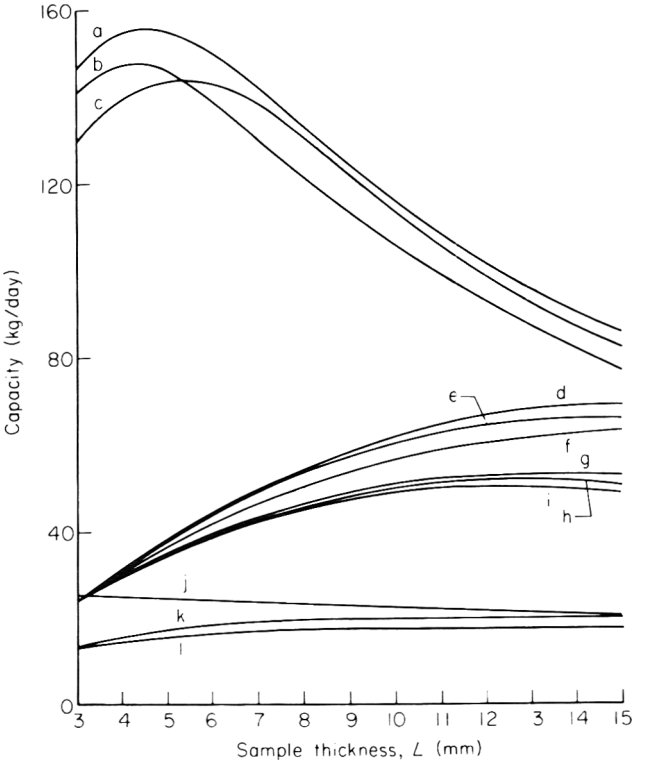

Figure 1. Effect of sample thickness on dryer capacity (chamber pressure of 0.1 mm Hg; condenser temperature of 225 K).

Sublimation model; curves a, b, c, and j: a: operating conditions of case C, b: operating conditions of case B, c: operating conditions of case D, j: operating conditions of case A.

Sorption sublimation model with criterion 1 used to terminate drying; curves d, e, f, and k, d: operating conditions of case C, e: operating conditions of case B, f: operating conditions of case D, k: operating conditions of case A.

Sorption sublimation model with criterion 2 used to terminate drying; curves g, h, i, and l, g: operating conditions of case C, h: operating conditions of case B, i: operating conditions of case D, l: operating conditions of case A.

since the moisture profiles in the dried material are such that degradative reactions may be significantly reduced (Aguilera & Flink, 1974; Mellor, 1978; Millman *et al.*, 1985; Millman, 1984).

It is clearly shown in Fig. 1 that the sublimation model erroneously predicts too large a daily capacity of the dryer and also predicts that maxima in the daily capacity occur at rather small sample thicknesses, 3.0–5.5 mm, depending on the operational regime. Also, criterion 1 used together with the sorption sublimation model predicts a daily capacity of as much as 50% higher than that predicted when criterion 2 is used.

The results of Fig. 1 show that significant and erroneous overestimation of dryer capacity may be introduced in any economic analysis that would employ models which can only account for the removal of frozen water. It is also shown that the sorption sublimation model predicts higher daily capacities with increasing sample thickness, and it is suggested that criterion 2 should be used in the economic analysis since a better quality dried product is obtained. Finally, the results in Fig. 1 show clearly that case C (Table 1) provides a higher daily capacity for a given sample thickness than any of the other operational policies.

In Table 3, the daily capacities predicted by the sorption sublimation model with criterion 2 for different chamber pressures and sample thicknesses are shown. The highest capacity is obtained with case C and when the chamber pressure is at its lowest value. This operational regime is also the one that provides the shortest drying times (Millman *et al.*, 1985).

Table 3. Daily capacity predicted by the sorption sublimation model with criterion 2

Sample (mm)	Chamber pressure (mm Hg)	Capacity (kg per day per dryer) by operating case			
		A	B	C	D
3	0.1	12.59	22.06	22.45	22.43
	1.0	12.78	22.39	22.44	22.24
	2.0	12.75	22.11	22.26	21.90
4	0.1	14.42	28.30	28.91	28.69
	1.0	14.48	28.42	28.56	28.13
	2.0	14.47	27.74	28.11	27.38
5	0.1	15.56	33.88	34.49	34.14
	1.0	15.58	33.52	33.74	32.87
	2.0	15.60	32.21	32.91	31.68
6	0.1	16.35	38.88	39.26	38.66
	1.0	16.38	37.47	38.05	36.68
	2.0	16.38	35.55	36.57	34.85
9	0.1	17.43	48.21	48.62	47.15
	1.0	17.51	44.07	45.53	42.73
	2.0	17.51	39.99	42.33	38.86
12	0.1	17.66	51.09	52.10	49.58
	1.0	17.76	44.80	47.04	43.21
	2.0	17.78	39.29	42.53	38.00
15	0.1	17.55	50.37	51.85	48.71
	1.0	17.67	42.84	45.59	41.20
	2.0	17.70	36.67	40.37	35.38

In Table 4, the costs per kilogram of dried product for the four operational regimes and for various sample thicknesses, chamber pressures, and capital costs are shown. Due to the lower heat input and longer drying time for case A, the cost of production is significantly higher than the costs obtained for the other cases under similar process conditions; Millman *et al.* (1984, 1985) have shown that the operating regime associated with case A, is inferior to cases B, C, and D with respect to drying time and sorbed water distribution. However, there may be systems in the production of pharmaceutical and biological products (Mellor, 1978; Liapis, 1985) which may require the operating regime of case A and then, operating at an elevated chamber pressure would decrease the production costs of the drying stage. The higher pressure will prolong the free water removal phase, but will shorten the terminal drying phase (Millman *et al.*, 1985) and the preparation period by decreasing the pump-down time; however, the lengthening of the free water removal phase is smaller than the aggregate reduction in the terminal drying phase and in the preparation phase. For case A, the maximum capacity is obtained when the largest sample size is used and the chamber is at 2.00 mm Hg (Table 3); at these conditions the lowest production cost is also obtained.

The production costs for cases B, C, and D are significantly lower than those of case A for all capital costs and conditions studied; case C provides the lowest costs. From Table 3, it is seen that cases B, C, and D give similar daily capacities at similar pressures and sample sizes, which are two to three times larger than the capacity given by case A under similar conditions. This is directly related to the decrease in cycle time as shown by Millman *et al.* (1985).

Figure 2 illustrates the predicted costs for the various operating regimes when the chamber pressure is 0.1 mm Hg and the capital cost of the dryer is $50 000. It is observed

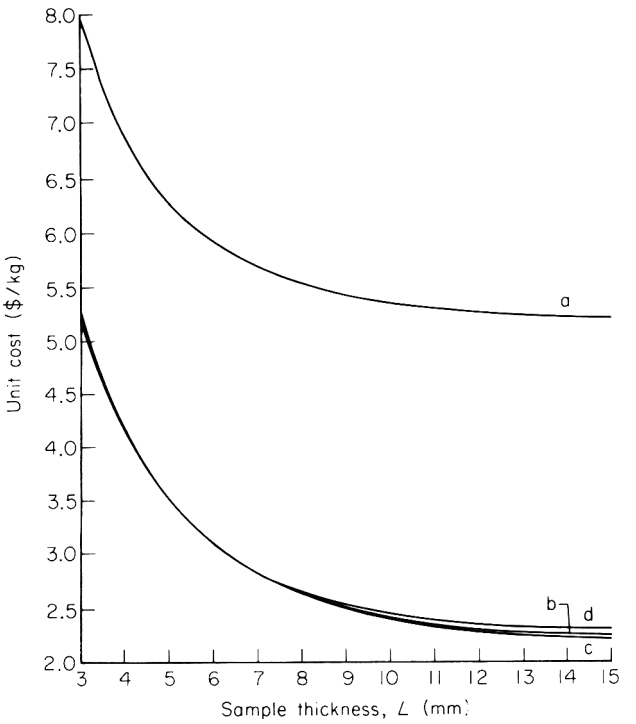

Figure 2. Effect of sample thickness on cost for various regimes (chamber pressure of 0.1 mmHg).

Table 4. Production cost per kg of dried product

Sample (mm)	Chamber pressure (mm Hg)	Unit cost (dollars)			
		A	B	C	D
3*	0.1	6.02	4.17	4.17	4.10
	1.0	5.86	4.04	4.09	4.03
	2.0	5.84	4.03	4.00	4.04
4	0.1	5.16	3.31	3.29	3.26
	1.0	5.07	3.24	3.25	3.23
	2.0	5.05	3.24	3.21	3.26
5	0.1	4.69	2.80	2.78	2.77
	1.0	4.63	2.77	2.77	2.79
	2.0	4.61	2.80	2.76	2.82
6	0.1	4.40	2.47	2.46	2.46
	1.0	4.35	2.48	2.46	2.50
	2.0	4.33	2.53	2.49	2.56
9	0.1	3.99	1.99	1.89	2.00
	1.0	3.95	2.07	2.03	2.10
	2.0	3.93	2.17	2.10	2.21
12	0.1	3.85	1.82	1.80	1.84
	1.0	3.81	1.95	1.89	1.99
	2.0	3.80	2.11	2.00	2.15
15	0.1	3.81	1.77	1.74	1.80
	1.0	3.77	1.95	1.87	1.99
	2.0	3.76	2.15	1.97	2.20
3†	0.1	8.00	5.30	5.28	5.21
	1.0	7.82	5.15	5.20	5.15
	2.0	7.80	5.16	5.12	5.18
4	0.1	6.89	4.19	4.15	4.14
	1.0	6.79	4.12	4.12	4.12
	2.0	6.78	4.14	4.10	4.17
5	0.1	6.30	3.54	3.51	3.50
	1.0	6.24	3.52	3.51	3.55
	2.0	6.21	3.58	3.52	3.61
6	0.1	5.93	3.11	3.10	3.11
	1.0	5.87	3.15	3.12	3.18
	2.0	5.86	3.23	3.17	3.27
9	0.1	5.43	2.51	2.49	2.53
	1.0	5.37	2.63	2.58	2.68
	2.0	5.36	2.80	2.69	2.85
12	0.1	5.27	2.31	2.28	2.35
	1.0	5.22	2.51	2.42	2.56
	2.0	5.20	2.74	2.59	2.81
15	0.1	5.23	2.27	2.23	2.32
	1.0	5.18	2.53	2.42	2.60
	2.0	5.17	2.83	2.59	2.91

3‡	0.1	11.98	7.57	7.51	7.44
	1.0	11.74	7.39	7.43	7.40
	2.0	11.72	7.42	7.37	7.47
4	0.1	10.36	5.96	5.88	5.88
	1.0	10.25	5.88	5.87	5.89
	2.0	10.23	5.95	5.88	6.00
5	0.1	9.51	5.01	4.96	4.97
	1.0	9.45	5.01	4.99	5.07
	2.0	9.41	5.13	5.04	5.19
6	0.1	8.99	4.40	4.37	4.40
	0.1	8.93	4.49	4.43	4.54
	2.0	8.91	4.64	4.54	4.71
9	0.1	8.30	3.54	3.52	3.59
	1.0	8.23	3.77	3.67	3.85
	2.0	8.22	4.05	3.87	4.14
12	0.1	8.10	3.29	3.24	3.36
	1.0	8.03	3.62	3.49	3.72
	2.0	8.02	4.01	3.77	4.12
15	0.1	8.08	3.26	3.19	3.34
	1.0	8.01	3.70	3.52	3.81
	2.0	7.99	4.19	3.83	4.32

Condenser temperature, 225 K.
Capital cost of dryer, *$25 000, †$50 000, ‡$100 000.

that the unit cost is approaching a limiting value which depends upon the operational regime. Since the unit cost approaches a limiting value, it seems proper to operate the dryer in a manner that maximizes capacity.

From the data of Tables 3 and 4, it is clear that case C with low chamber pressures and large sample sizes, produced higher capacities and lower costs of production.

Conclusions and comments

This study of the economics of batch freeze dryers has shown that the operational regime which provides the shortest drying times during the free water removal phase will also give the smallest production costs and highest capacities. This regime involves large sample sizes, the chamber pressure is kept at its lowest value, and the upper and lower heating plates are independently controlled so that the melting and scorch constraints are both encountered and held during the free water removal phase.

It is also shown that the capital cost does not alter the choice of operational regime. The capital cost increases the unit cost by an amount equal to the total capital cost divided by the capacity of the dryer. For case A, in Table 1, it was found that an elevated chamber pressure should be used since it decreases the unit cost by shortening the overall drying time.

Finally, it was shown that simple models that account only for the removal of frozen water and neglect the bound or sorbed water, can lead to erroneous results.

Acknowledgment

The authors gratefully acknowledge the financial support of the DuPont Corporation.

Notation

C_{dp} cost of entire drying period;
C_{elec} electricity cost;
C_{HP} operating cost of holding pump;
C_{pump} operating cost of pump;
C_{pt} cost of preparation period;
C_{ref} refrigeration cost at 225 K;
C_{stm} steam cost at 2311 mm Hg absolute;
E total energy added to material during drying per tray;
L sample thickness;
M_{ice} mass of ice deposited on condenser;
M_{stm} mass of steam needed to remove ice from condenser;
N_{lab} number of labourers;
N_{op} number of operators per dryer;
N_{tr} number of trays in dryer;
O.D. operating day (1440 min);
P_{atm} atmospheric pressure;
P_{ch} operating pressure in chamber;
S_{lab} salary of general labourer;
S_{op} salary of operator;
S_p vacuum pump capacity;
t_d time required for drying period;
t_l time required for loading the dryer.
T_{LP} lower plate temperature;
t_p pump down time;
T_{UP} upper plate temperature;
T_m melting temperature;
T_s scorch temperature;
T_I temperature in dried region:
T_{II} temperature in frozen region;
V_{ch} volume of chamber;
x space coordinate;
X position of interface.

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(Received 26 November 1984)

Journal of Food Technology (1985) **20**, 553–560

Quality changes in the fresh water prawn, *Macrobrachium rosenbergii* during storage on ice

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Summary

The effect of stratification in ice on the quality of fresh water prawns was monitored by microbiological, subjective and objective textural analyses. In general, prawns in the lower layers of ice exhibited more marked textural changes and greater numbers of potential spoilage bacteria were found on them. No connection was found between the number of proteolytic bacteria and textural changes in the prawn muscle.

Introduction

In a previous study with ice-stored prawns (Angel *et al.*, 1981), their shelf life was monitored by sensory, microbiological and chemical tests; the prawns' quality was found to be acceptable when kept for up to 10 days on ice. Several workers have reported that prawns kept on ice have a shelf life of only a few days due to breakdown of muscle tissue, referred to as mushiness (Nip & Moy, 1979; Rowland, Finne & Tillman, 1982). Unfrozen prawns are generally marketed packed in ice. Under these conditions a washing effect by the melting ice is expected. The purpose of this study was to follow textural and microbial changes of ice-stored prawns as a function of stratification on ice.

Materials and methods

Source of material

Live prawns were brought from monocultured and polycultured fish ponds, separately. In experiments 1 and 3 the prawns originated from monocultured ponds at a density of 15000 and 8000 prawns per 0.1 ha. The prawns in experiment 2 were taken from a polycultured pond kept at a density of 1500 prawns per 0.1 ha. Following rinsing and icing, the prawns were transferred to the laboratory.

Experimental procedure

The prawns were stored in columns of perforated pots, stacked three pots to a column. Holes in the bottom of the pots allowed the percolation of ice water from the upper to the lower layers. An extra pot at the bottom allowed drips to be collected in Petri dishes for microbiological tests (Fig. 1).

The prawns were placed in crushed ice in the pots following removal of appendages, thirty-five prawns to a pot at the onset of the storage period. Every 2 days ice was added to the pots to compensate for ice loss due to melting, and the prawns were rearranged to

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Contribution from The Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel, No. 1221-E, 1984 series.

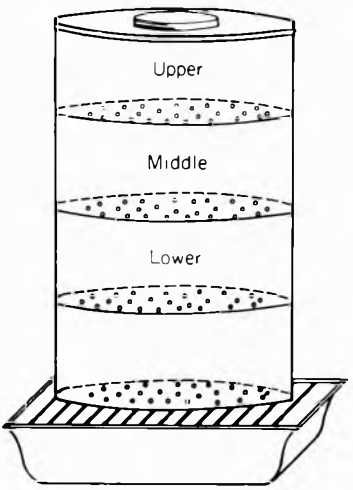

Figure 1. Experimental arrangement: stack arrangement of the pots in layers.

ensure that they were surrounded by ice. The pot columns were stored in a walk-in cooler kept at 1–2°C. Prawns were withdrawn periodically from each pot for sensory, instrumental and microbiological analyses.

To determine possible influences on the degree to which the ice melted in the columns, weights were recorded of the upper, middle and lower pots in the columns located in different places in the cold room. The ice was replenished twice in each pot and weights were recorded after three days.

Preparation of the prawns for quality evaluation

Whole prawns removed from the containers were boiled in tap water for 3 min, cooled immediately in iced water, drained and headed. The tails were peeled just prior to analysis.

Sensory evaluation

The peeled, cooked tails from the upper (U), middle (M) and lower (L) pots in the columns were presented to panellists after every 2 or 3 days of storage for the first 2 weeks of the storage period, and thereafter once a week for up to 30 days of storage. Textural changes were determined by placing the tails between the fingers and feeling the degree of firmness, mealiness-mushiness or disintegration, segment by segment. Each panellist examined two prawn tails from each treatment and gave a verbal description of the feel. The wording used was later converted into a scale, as follows: very mushy, 1.5; mushy or disintegrating, 1.0; slightly mushy, 0.5; firm or non-mushy, 0. When only part of the segment was mushy (top or bottom, or half-way along the segment only), the segment was assigned a mushiness score of 0.5.

Instrumental evaluation of the prawn texture

The cooked first and second segments closest to the cephalothorax were sliced separately from the tails with a sharp knife and each segment was subjected to a compression test in a compression cage of an Instron Model TM-1026. Segments were placed in the cage vertically, and compressed using a 50 kg load cell at a speed of 5 cm per minute to 50% of their original height.

Four tails from each treatment (U, M, L pot layers) were tested. Peak heights were converted to grams. The compression tests were carried out concurrently with the panel evaluations. In order to test the effect of segment size on compression forces, a preliminary experiment was conducted with frozen thawed prawns of various sizes, stored on ice for 1 and 3 days.

Statistical analysis

The panel scores and results from the Instron measurements were subjected to an analysis of variance and Duncan's multiple range test, using a GLM procedure with the SASS program on a 4143 IBM computer. The program also yielded correlations between forces and scores.

Microbiological sampling

Microbiological tests were carried out in conjunction with experiment 3. Prawn homogenates were prepared for plating by blending *circa* 20 g of deheaded prawns with nine times the quantity (in ml) of 0.5% peptone in 0.05 M phosphate buffer (pH 7.0) for 2 min in a blender. Both shelled and unshelled samples were used. Drip samples were taken from melted ice which accumulated during 1–2 hr before the selected sampling time. Three columns were tested in each experiment with two replicates analysed per sample.

Bacterial counts and isolates

Decimal dilutions of homogenates and drip samples were prepared in peptone diluent and surface-plated on both plate count agar (PCA) and milk nutrient agar (MNA) plates, which were then incubated for 3–5 days at 20°C. Total aerobic counts on PCA were considered as counts of potential spoilage organisms; colonies producing a clear zone of casein hydrolysis on MNA (confirmed by a negative reaction with trichlorocetic acid) were recorded as proteolytic. Results are given as logarithms of colony-forming units (CFU) per g or ml.

Potential spoilage bacteria were isolated from total aerobic count plates. The isolates were tested for proteolytic ability on milk nutrient agar (MNA), gelatin agar (GA) medium and prawn extract agar (PEA). To determine microbial types, all the colonies were picked from PCA plates which had fifteen to thirty-five colonies and identified to the genus, using a scheme which was basically that recommended by Vanderzant & Nickelson (1969). A total of about 100 isolates were tested.

Bacteriological media

Skim milk nutrient agar was prepared as follows: 30% of reconstituted skim milk (10% solids), prepared from skim milk powder (Oxoid) was incorporated into nutrient agar (Difco). Previously dried poured plates were surface-inoculated. The gelatin medium used was that described by Levin (1968), which consisted of a double layer with a soft agar-gelatin overlay. The procedure recommended here for the preparation of prawn extract agar (PEA) is based on the results obtained in numerous trials in which the variables tested were: possibility of filter sterilization, liability of the extract to heat treatments, and concentration of extract needed to produce an opaque agar medium (PEA). It was prepared as follows: one weight of prawn flesh, aseptically removed, was mixed with four weights of sterile distilled water and the mixture was homogenized for 2 min in a pre-sterilized blender. The homogenate was centrifuged at 27 000 × **g** for 20 min at *circa* 10°C. The supernatant was collected in sterile bottles and then heated at 65°C

for 15 min in a water bath. Nutrient agar (Difco), prepared in 70% of the final water amount, was heat sterilized and cooled to 65°C. Thirty millilitres of the pre-heated diluted extract was added per 70 ml of the nutrient agar preparation, mixed thoroughly, cooled at 45°C, and plates poured. Protein analyses of the prawn extract were performed using the method described by Lowry *et al.* (1951). A calibration curve was prepared using bovine serum albumin.

Confirmation of protein hydrolysis in agar medium was done by flooding the plates, after the incubation period, with a solution of 5% trichloroacetic acid; if a clear zone produced round a colony is not the result of partial or complete protein hydrolysis, a precipitate will be formed there.

Results

In the preliminary experiment to determine the degree of ice melt in the three layers of ice, the lowest pots exhibited the greatest amount of ice melted (averaging 66% ice melt in the lower pot as compared with 56% and 53% for the middle and upper pots, respectively.

Textural changes

Experiment 1. Figure 2a shows the compression forces for the first segments of the prawns throughout the storage period. The segments tended to become less firm in all three layers after 11 days of iced storge. The prawns in the upper layer of the pots were significantly firmer at the 0.05 level than those in the middle and lower layers, throughout the storage period. The compression forces for the second segment (Fig. 2b) tended to be higher than for the first segment, but there were no significant differences between the layers.

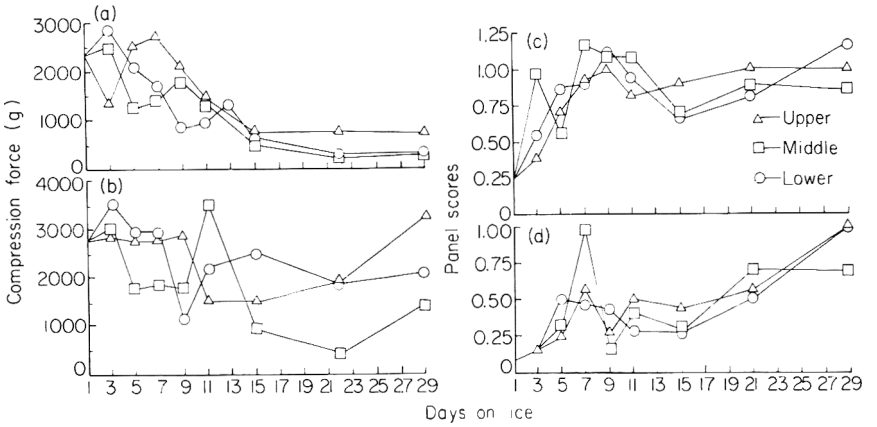

Figure 2. a–d from top to bottom. Compression forces (2a, 2b) and panel scores (2c, 2d) for the first and second segments (respectively), throughout the storage period of experiment 1.

Figures 2c and 2d show the panel scores for segments 1 and 2, respectively. For the first segment there was an increase in panel scores after 8 days of iced storage, denoting increasing mushiness, which levelled off after that until the end of the storage period. For the second segment the scores for mushiness rose gradually with storage, but were significantly lower (at $P = 0.05$) than for the first segment. The panel scores for the

upper layer for both the first and second segments tended to be lower, indicating less mushiness than in the lower layers.

Experiment 2 (Fig. 3). The compression forces for the first and second segments were similar to those of experiment 1, except that the initial forces were significantly lower (at $P = 0.05$). Panel scores for mushiness for segment 1 showed increases up to 15 days of storage, which then tended to level off. For the second segment mushiness scores rose gradually with storage, but were significantly lower (at $P = 0.05$) than for the first segment.

With regard to the different layers, the compression forces for the first segments in the upper layer were significantly higher (at $P = 0.05$) than for the lower layers. For the second segment, prawns tended to be firmer in the upper layer than in the lower layers. Similar differences between the layers for segments 1 and 2 were obtained with the panel scores for mushiness.

Experiment 3 (Fig. 4). Both first and second segments had lower compression forces than in the previous experiments. Therefore, the advance of mushiness from the start was not as pronounced. Panel scores for mushiness tended to follow the trend observed

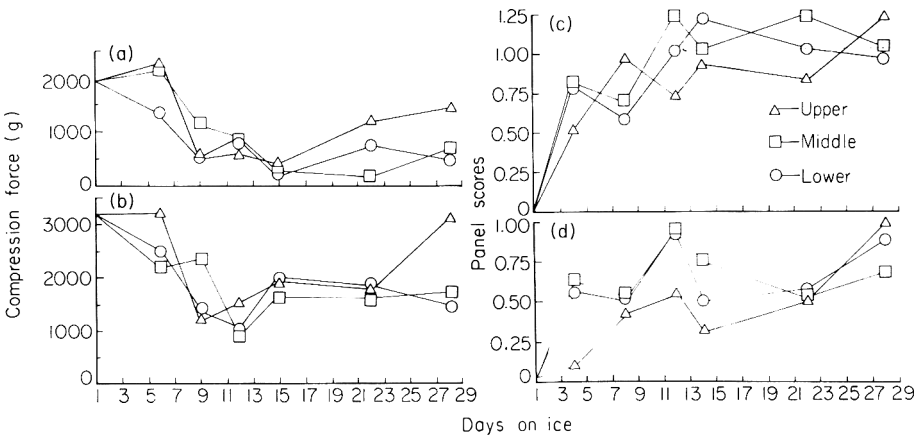

Figure 3. a–d from top to bottom. Compression forces (3a, 3b) and panel scores (3c, 3d) for the first and second segments (respectively), throughout the storage period of experiment 2.

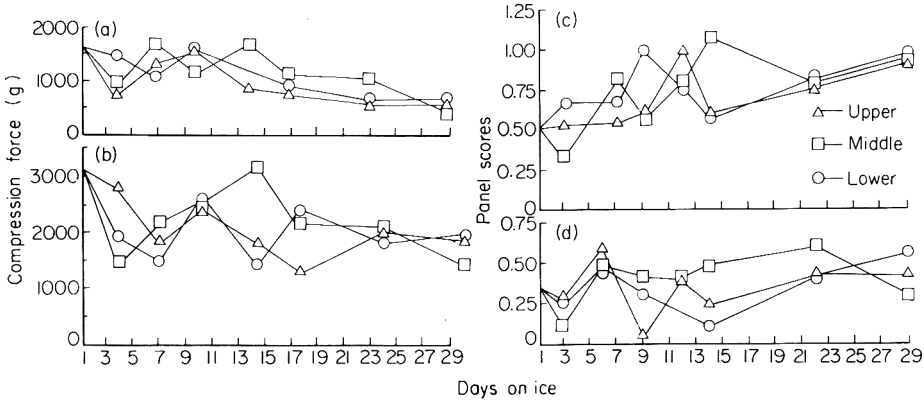

Figure 4. a–d from top to bottom. Compression forces (4a, 4b) and panel scores (4c, 4d) for the first and second segments (respectively), throughout the storage period of experiment 3.

with the compression forces. The differences between the layers were not significant for either compression forces or panel scores.

Microbiological changes

A continuous increase in total and proteolytic counts in prawns and drip samples was observed during 20 days of iced-storage. The changes in total counts and proteolytic bacterial counts are pesented in Figs 5 and 6, respectively. Total counts, as well as proteolytic counts, were 4–14% lower in shelled prawns than in unshelled prawns. The data presented here refer to samples of shelled prawns.

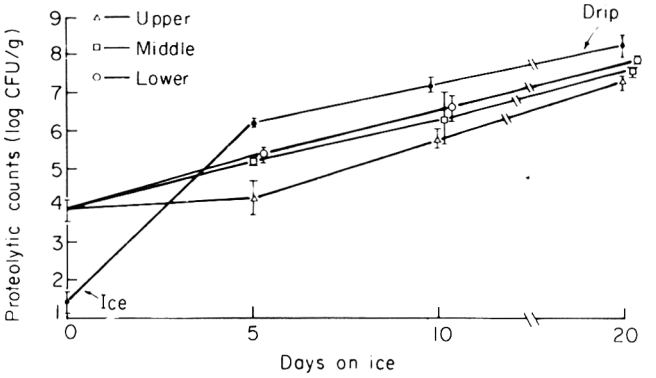

Figure 5. Changes in total aerobic bacterial counts in prawns and melting ice (drip) during iced storage. Data presented are average values and ranges.

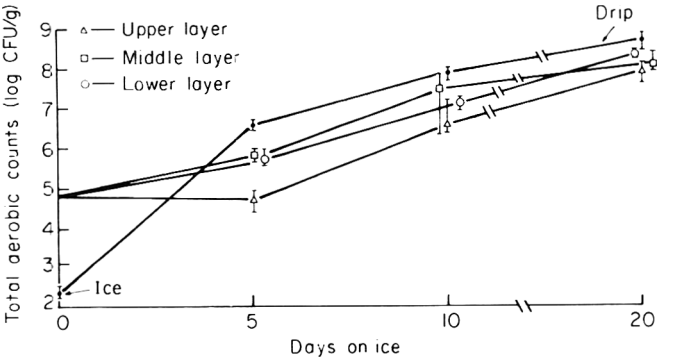

Figure 6. Changes in proteolytic bacterial counts in prawns and melting ice (drips) during iced storage. Data presented are average values and ranges.

Trends of changes in counts of proteolytic bacteria followed those of total counts. In prawns, proteolytic counts averaged 85% of total counts and ranged from 78 to 96% of the total counts. In drip samples, proteolytic counts represented an average of 95% of the total counts, and ranged from 92 to 97% of the total counts, whereas in ice they represented an average of 62% of the total counts. Loads of proteolytic bacteria in prawns were higher in the lower layer of the ice column and decreased towards the top layer.

Initial proteolytic counts in prawns ranged from 3.6 to 4.2 logs CFU/g. After 10 days storage on ice, an increase of approximately 2.0 (top) to 2.7 (lower) logs/g was observed; after 20 days of storage the increase observed was 3.5 (top) to

4.0 (lower) logs/g. The predominant proteolytic bacterial groups isolated from ice-stored prawns were pigmented *Pseudomonas* (84%) and *Flavobacterium* (11%). Counts of proteolytic bacteria in the drip increased from *circa* 1.5 (in ice) to 6.3 logs/ml after 5 days of iced storage, continued increasing with storage time, and reached 8.3 logs CFU/ml after 20 days.

Bacterial proteolytic activity

A full correlation was found between the ability of the bacteria isolated from prawns to hydrolyse prawn extract proteins and their ability to hydrolyse casein in MNA. More than 95% of the isolates found proteolytic on MNA were proteolytic also on GA and PEA. The clearing zone of proteolysis, which developed in MNA, was usually better defined than that produced by the same organism on PEA; therefore, MNA was preferred as an enumeration and screening plating medium. The PEA medium (pH *circa* 7.3) had about 180 mg protein over 100 ml medium, as compared with *circa* 105 mg/100 ml in MNA.

Discussion

The results showed that the development of mushiness differed according to the source of the raw material. It is possible that differences in the condition of the prawns, which might be related to aquacultural practices, could have induced stress during growth and at harvest.

The prawns in the upper layer of ice demonstrated better keeping quality than the lower layers. Results had shown that there was increased ice melt in the lower pot, probably from the water accumulating from the upper layers. Thus more drip passed through the lower layers which could carry down proteolytic enzymes responsible for textural changes.

Bacterial counts in the drip indicated the washing ability of melting ice: they were always higher than the counts obtained in any of the layers of prawns. These data indicate that the melting ice rinses bacteria from the top layers and causes an increase in the degree of bacterial contamination in the bottom layers, thereby increasing the chances of early spoilage in these lower layers.

Textural changes in a muscle food are likely to be connected with changes in proteins. However, artificial inoculation of sterile muscle with markedly proteolytic strains of *Pseudomonas* and *Flavobacterium* isolated from ice-stored prawns failed to induce textural changes (B.J. Juven, unpubl.). The above results, and the fact that mushiness developed primarily in the segments closest to the cephalothorax, leads to the assumption that bacteria were not responsible for the onset of mushiness in the prawn tail. In a preliminary biochemical study no proteolytic endogenous enzymatic activity was detected in the prawn tail. The possibility that the cephalothorax might be the source leading to the onset of mushiness in the tail is being investigated.

Correlations between compression force measurements and panel scores were found to be low. Peleg (1983) indicated that instrumental methods of measuring texture of solid foods might incorporate ambiguities due to dimensional effects, and panel scores might be ambiguous due to semantic problems. This might help partially to explain the low correlation obtained between panel scores and compression forces. However, both panel scores and compression forces yielded valuable information as to the onset of mushiness in the ice-stored prawns.

Conclusions

(i) Israeli pond raised prawns did not develop mushiness problems during the first 9 days of storage on ice.

(ii) Prawns in the bottom layer of ice tend to become mushy quicker, and to carry higher loads of spoilage organisms, than those in upper layers. It is therefore advisable to prevent prawns from soaking in lower layers of ice water.

(iii) Mushiness is assumed to be a result of enzymatic activity originating in the cephalothorax; inoculation of sterile muscle with markedly proteolytic strains of *Pseudomonas* and *Flavobacterium* failed to induce mushiness.

(iv) Both subjective valuation and a compression test were found to be valuable tools for evaluating textural changes in prawns stored on ice.

Acknowledgments

Contribution No. 1221-E, 1984 series from the Agricultural Research Organization, Bet Dagan, Israel. This research was suppored by a grant from the United States-Israel Binational Research and Development Fund (BARD). The authors wish to thank H. Weisslowicz for technical assistance.

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(Received 13 September 1984)

Journal of Food Technology (1985) **20**, 561–572

Silage from tropical fish 1. Proteolysis

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Summary

Acid silages were prepared from silverbelly (*Leiognathus* sp.) at 30°C with 3% (w/w) of 98% formic acid. After 14 days, total protein solubilization was 45–50%, of which 80% was TCA soluble and collagen solubilization was 42%. Centrifugation of 14 day old silage gave a cystine-rich undigested sediment and a liquor with a low tryptophan content. Gel chromatography of the liquor of a 14 day old silage showed it to be composed of at least two fractions: one consisting of protein fragments of molecular weight greater than 5000 daltons and the other of free amino acids and small peptides. Soluble collagen breakdown products were found predominantly (75%) in the high molecular weight fraction. A mechanism for the solubilization of collagen by the action of acid pH, elevated temperature and non-specific proteases is proposed. The limited autolysis in silverbelly silage is compared with that found in cold water fish silages and attributed principally to the resistance of warm water fish collagen to the effects of acid pH and temperature during ensilation.

Introduction

Fish silage can be defined as the liquid product formed by the action of naturally occurring proteolytic enzymes on whole fish or parts of fish preserved in acid conditions. Many aspects of silage production and use in animal feeds have been reviewed by Raa & Gildberg (1982). The characteristic process of ensilation is the self digestion (autolysis) of the fish, resulting in increased liquefaction which has been measured by a fall in viscosity (Tatterson & Windsor, 1974) or by an increase in the volume of the aqueous phase after centrifugation (Backhoff, 1976; Gildberg & Raa, 1977). Protein breakdown has been measured by determining total nitrogen or TCA soluble non-protein nitrogen (NPN) present in the aqueous phase after centrifugation (Tatterson & Windsor, 1974; Backhoff, 1976).

Centrifugation of a silage typically produces a lipid layer, a protein-rich lipid-free aqueous layer and a solid layer, or sediment, composed of undigested protein and the remainder of the lipid from the raw material (Gildberg & Raa, 1977). Reasons for the presence of undigested protein are not clear and may be related to the pH, temperature and duration of ensilation and the nature of the raw material. The amino acid composition of the solid layer differs from that of the aqueous layer in being enriched with tyrosine, phenylalanine and cystine. Raa & Gildberg (1976) suggested that sulphur–sulphur cross links may account for a structural resistance of the sediment proteins to enzymic attack. In addition, the denaturation of collagen may be important in that this may lead to other proteins being exposed to enzymic attack. Thus, Raa & Gildberg

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(1976) suggested that the rate of autolysis in cold water fish increased rapidly over a narrow temperature range, at about 20°C, thought to be that at which collagen is heat denatured and more susceptible to enzymic attack.

Information about the biochemistry of fish silage such as that discussed above has almost exclusively been gained using cold water fish. There is no certainty that such findings can be extrapolated to fish from warm waters, which may form an important source of raw material for silage manufacture. The present paper deals with the production and characteristics of silage made from silverbelly (*Leiognathus* sp.), a warm water species. Factors investigated were the extent of autolysis and physical breakdown of the tissues and the nature of the fractions produced by centrifugation of the silage.

Materials and methods

Raw material

The variety of fish which make up the by-catch of shrimp trawling operations in South East Asia and which could be used for silage production makes controlled investigations difficult. To overcome this problem, one species of fish was selected for investigation. Fish of the family *Leiognathidae* (silverbelly/slipmouth/ponyfish) were chosen as they are widely distributed in the Indo-Pacific region and make up a large proportion of the shrimp by-catch in Malaysia, Indonesia and Sri Lanka (Raa & Gildberg, 1982). The fish used were from the Jaffna/Mannar area of Sri Lanka and were mainly *L. splendens* (known locally as 'karalla'), The fish were kept deep frozen (−22°C) until required.

Silage production

A standard method was adopted and the incubation period varied as necessary. Frozen fish were thawed at room temperature, washed in cold running water, drained and minced through a plate with 4 mm holes. Formic acid (98%) was added at 3% (w/w) of the raw material and mixed in thoroughly. The mixture was incubated at 30°C in sealed jars. After 1 day, then on alternate days, the contents of the jars were mixed to prevent the formation of acid-free material.

Physical composition of silverbelly

Eighteen whole, undamaged fish, of average weight 6–8 g, were thawed at room temperature, washed in cold running water, dried by dabbing with filter paper then treated as follows: nine whole fish were minced into tared dishes, dried to determine the moisture content and the ground residue used for other proximate analyses and hydroxyproline determinations. Nine fish were dissected into muscle, skin, viscera and head, skeleton and fins. Each part was dried for the determination of moisture content and the ground residue used for proximate analyses and hydroxyproline determinations.

Characteristics of silage production

A silage was prepared as described above and divided into five portions. Three portions (A, B, C) were incubated for 28 days and sampled at 0, 1, 2, 7, 14, 21 and 28 days. In addition, silage A was sampled after 6 hr, 3 and 4 days; silage B after 6 hr, 3 and

6 days; and silage C after 5 and 6 days. The NPN of the silage liquor after centrifugation was determined and expressed as a percentage of the total silage nitrogen.

The fourth portion was incubated for 21 days and the viscosity and NPN of the whole silage (expressed as a percentage of total silage nitrogen) determined at 0, 2, 7, 14 and 21 days.

The fifth portion was incubated for 14 days and then separated by centrifugation into liquor and undigested sediment. These fractions were freeze dried and proximate analysis and amino acid compositions determined.

Fractionation of silages by gel chromatography

A silage was prepared as described above and sampled at 0, 7 and 14 days. Liquors produced by centrifugation of 0, 7 and 14 day old silages were fractionated according to molecular size by gel chromatography. A Wrights GA 22×450 mm column was packed with Sephadex G-25 (Medium grade) and eluted with 0.05 M tris buffer, containing 0.02% sodium azide, at a flow rate of 1 ml/min. The column was calibrated using compounds of known molecular weight. Samples of these compounds (1 ml) were loaded onto the column and detected by absorbance at 280 nm for those containing aromatic amino acids and by the ninhydrin reaction (detected at 600 nm) for the non-aromatic standards (Bailey, 1962). The void volume (V_0) of the column was determined with dextran blue and the elution volume (V_e) of each standard was expressed as its relative elution volume (V_e/V_0). A 1 ml sample of liquor was applied to the column and a fraction collector set to collect 2.6 ml of eluant per tube.

Fractionation of the liquors by this method gave four distinct fractions which were further characterized by determining their amino acid compositions.

Methods of analysis

Moisture content was determined by oven drying at 105°C for 16 hr and fat content by extracting the residue with 40°–60° BP petroleum ether for 8 hr. Ash content was determined by ignition at 550°C to constant weight. Nitrogen content was determined by a semi-micro Kjeldahl method and converted to crude protein by multiplying by 6.25. Silages were separated by centrifugation at 80000 **g** for 30 min. The viscosity of whole silages was determined by the HAAKE Viscotester. The NPN was determined by TCA precipitation according to Backhoff (1976). Amino acids, including methionine and cystine oxidized by performic acid, were determined, after acid hydrolysis, by a Locarte Mark 4A analyser. Hydroxyproline was determined by the method of Stegemann & Stalder (1967) and tryptophon by the method of Buttery & Soar (1975). Free amino nitrogen was determined in the fractionated liquors by a ninhydrin method (Bailey, 1962).

Results and discussion

Physical composition of silverbelly

The physical and proximate compositions of silverbelly are given in Table 1. As expected, the fat content is sufficiently low for silages made from silverbelly to be incorporated into animal diets without lipid extraction (Raa & Gildberg, 1982). The high ash content may include a variable amount of ingested sand (Jayawardena & Poulter, 1979). Hydroxyproline is concentrated in those tissues rich in the connective tissue protein, collagen. The hydroxyproline content of fish collagen varies widely,

being higher in warm water fish than in cold water fish (Gustavson, 1953) and for this reason conversion of hydroxyproline to collagen content was not attempted. The variable hydroxyproline content of fish collagens is of practical importance because the structural and chemical stability of collagen is due, in part, to the presence of hydroxyproline.

Table 1. Carcass composition (% wet weight), proximate composition (% wet weight) and hydroxyproline content (g/16 g N) of silverbelly (*Leiognathus splendens*)*

Sample	Carcass composition	Moisture	Protein (% N×6.25)	Fat	Ash	Hydroxyproline
Whole fish	100.0	72.5 (±2.7)	16.8 (±1.7)	1.9 (±0.5)	9.2 (±0.1)	1.8 (±0.2)
Flesh	41.4 (±1.1)	79.9 (±0.3)	18.5 (±0.2)	0.3 (±0.2)	0.8 (±0.1)	0.2 (±0.1)
Skin	4.5 (±0.3)	65.9 (±0.9)	23.2 (±0.1)	3.7 (±0.2)	0.5 (±0.1)	3.6 (±0.1)
Viscera	7.1 (±0.9)	67.0 (±1.8)	7.6 (±1.1)	2.4 (±0.6)	20.6 (±2.7)	0.9 (±0.1)
Head, frame and fins	47.1 (±1.4)	64.9 (±1.6)	17.9 (±0.7)	3.2 (±0.2)	14.9 (±1.1)	2.8 (±0.1)
Additive total†	100.1	71.3	17.3	2.0	8.8	1.6

*Results are quoted as mean (± standard error) for nine samples.
†Calculated using the means of carcass and proximate composition.

Time course of the silage process

The change in soluble NPN during ensilation is shown in Fig. 1 and demonstrates the typical pattern of nitrogen release which is rapid during the first few days then slower thereafter. Jayawardena & Poulter (1979) measured NPN in whole silverbelly silages, at 28–30°C, and found an increase from 14% (of total nitrogen) to 39% after 7 days, 46% after 14 days and 51% after 28 days. Tatterson & Windsor (1974) measured NPN in whole silages made from several cold water fish at 23°C. The results were similar for all

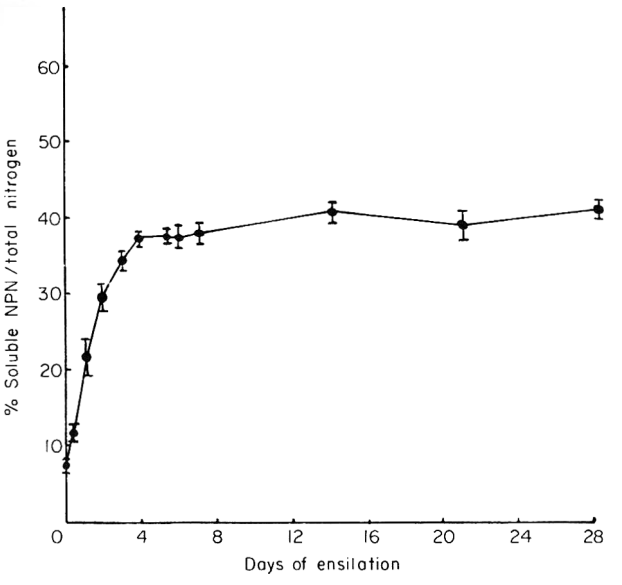

Figure 1. Change in soluble NPN (% of total N) during ensilation of silverbelly at 30°C. Each point is the mean of three silages except for 6 hr, 3 and 6 days (two silages) and 4 and 5 days (one silage). Bars are standard errors of the means.

species and sprats (*Sprattus sprattus*) were typical, showing an increase from 15% (of total nitrogen) to 68% after 7 days and over 89% after 14 days. Thus, it appears that autolysis is limited in silverbelly silages. The difference between the NPN found after 28 days in the present work (40%) and that found by Jayawardena & Poulter (1979) may be due to seasonal effects. However, seasonal effects are unlikely to explain the difference between the limited autolysis found in silverbelly and that found in sprats. It is unlikely that these differences are due to enzyme activation in cold water fish as the temperature of optimum activity for enzymes from cold and warm water fish is similar (Raa & Gildberg, 1982). Since the optimum is about 30°C, the silverbelly silages were at a more favourable temperature than the sprat silage held at 25°C. As mentioned earlier, the critical temperature for collagen denaturation in cold water fish is about 20°C and rapid autolysis occurs above this temperature. The stability of the collagen in silverbelly silages may serve to protect other proteins, limiting their susceptibility to enzymic attack.

The changes in viscosity and NPN of a whole silage are shown in Fig. 2. Measurement of NPN in a whole silage gave similar results to those found in the silage liquor, showing that practically all of the TCA soluble breakdown products are released into the liquor on centrifugation, where they constitute a large proportion of the soluble nitrogen. The rapid decrease in viscosity follows the course of autolysis closely and could be a useful indicator for process control in developing countries.

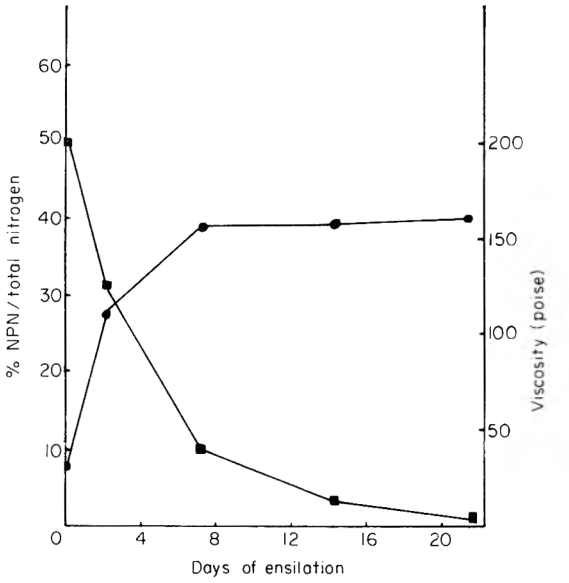

Figure 2. Change in total NPN (% of total N) (—●—●—) and viscosity (—■—■—) during ensilation of silverbelly at 30°C.

Composition of the silages

Centrifugation of a 14 day old silage gave separation into four phases: an upper dark red–brown fat layer, a yellowish aqueous layer (liquor) and two sediment layers. The upper sediment layer was off-white/yellow in colour and had a smooth texture whilst the lower layer was dark grey and contained pieces of bone. The two layers could not be separated and were collected together and are referred to as the undigested or sediment

fraction of silage. The ratio of liquor to sediment was 62:38 (wet weight basis). The upper fat layer represented 44% of the total fat content of the silage, the rest remaining in the sediment. The presence of lipid in the sediment has been suggested as a reason for its resistance to autolysis (Raa & Gildberg, 1976) but as outlined above, the part played by collagen and other resistant proteins may be of more importance.

The proximate compositions of whole fish (minced silverbelly prior to addition of formic acid) and whole silage, liquor and sediment after 14 days ensilation (Table 2) show no measurable loss of nitrogen through the formation of volatile bases and ammonia during ensilation.

Table 2. Proximate composition (% dry weight basis) of whole fish, whole silage and silage liquor and sediment*

	Whole fish	Whole silage	Liquor	Sediment	Additive total†
Fat	6.9	7.9	0.1	4.9	1.9‡
Protein (% N × 6.25)	61.1	64.6	66.7	53.2	61.5
Ash	33.5	29.9	16.9	34.8	23.8

*After centrifugation at 80 000 g for 30 min. Silages incubated at 30°C for 14 days.

†Calculated from a liquor : sediment ratio of 62 : 38 (% wet weight).

‡This value is low as the upper fat layer is not included in the calculation.

The amino acid compositions of whole fish, whole silage, liquor and sediment (Table 3) give an insight into the process of autolysis. The only significant difference between whole fish and whole silage is for tryptophan, which is known to be labile under acid conditions (Backhoff, 1976). Comparison of the liquor and sediment indicates that although most amino acids are equally distributed between the two phases, some interesting differences are apparent. Thus the more water-soluble acids, such as lysine, histidine and arginine, are more prevalent in the liquor whilst tyrosine and phenylalanine tend to remain in the sediment. The most obvious differences though are in the significantly higher tryptophan and cystine contents of the sediment.

Tryptophan is unstable in acid conditions, particularly in the free form (Kompiang, Arifudin & Raa, 1979) so the liquor would be expected to be more deficient than the sediment in this amino acid. This confirms the work of Raa & Gildberg (1976). The sediment is rich in cystine, which suggests that disulphide bonds may be responsible for the resistance of at least some of the sediment proteins to hydrolysis. Similar results were found with cod (*Gadus morhua*) viscera silages (Raa & Gildberg, 1976). Because collagen does not contain cystine and tryptophan, another protein must be responsible for their presence in the sediment.

The hydroxyproline contents of liquor and sediment are similar and calculation shows that about 42% of the hydroxyproline has been released into the liquor after 14 days ensilation. This release of collagenous material into the liquor is supported by the distribution of glycine, which makes up about one third of collagen amino acid residues and therefore reflects, less precisely than hydroxyproline, collagen breakdown. Subsequent hydroxyproline determinations in later studies showed that collagen breakdown typically reaches 40–50% after 14 days ensilation. Measurement of collagen break-

Table 3. Amino acid composition (g/16 g N) of whole fish, whole silage and silage liquor and sediment*

Amino acid	Whole fish	Whole silage	Liquor	Sediment	Additive total†
Lysine	7.94	7.42	8.47	6.14	7.21
Histidine	2.21	2.09	2.28	1.74	1.99
Arginine	6.57	6.28	7.15	6.04	6.55
Tryptophan	1.51	1.04	0.54	1.49	1.05
Aspartic acid	8.85	8.51	8.24	9.27	8.79
Threonine	3.85	3.69	3.78	3.65	3.71
Serine	3.81	3.62	3.69	3.69	3.69
Glutamic acid	14.04	13.19	14.30	12.82	13.49
Proline	4.58	4.07	4.69	4.18	4.41
Glycine	6.83	6.58	6.99	7.69	7.37
Alanine	6.22	5.90	6.66	5.60	6.09
½ Cystine	0.79	0.78	0.40	1.10	0.78
Valine	3.99	3.88	4.29	3.18	3.69
Methionine	2.53	2.48	1.92	2.80	2.39
Isoleucine	3.75	3.54	3.63	3.87	3.76
Leucine	6.82	6.61	6.55	6.12	6.32
Tyrosine	3.04	2.73	2.35	3.06	2.73
Phenylalanine	3.51	3.47	2.64	3.61	3.17
Hydroxyproline	1.83	1.46	1.43	1.75	1.60

*After centrifugation at 80 000 g for 30 min. Silage incubated at 30°C for 14 days.

† Calculated from a liquor nitrogen content : sediment nitrogen content ratio of 45.8 : 54.2 (% wet weight basis).

down in cold water fish suggests a difference in susceptibility to silage conditions between cold water fish and warm water fish collagens. In silages made from cod viscera (at 23°C for 8 days) hydroxyproline was detected only in the liquor and glycine was found predominantly in the liquor (Raa & Gildberg, 1976; Gildberg & Raa, 1977). Backhoff (1976) prepared silages from whole herring (*Clupea harengus*) and whole cod (*G. morhua*) and stored them at ambient temperatures in winter and summer. After 5 days, herring silages showed 83% and 72% (of total hydroxyproline) in solution and cod silages 72% and 60% in solution for winter and summer respectively, the winter silages being held at a higher temperature than the ambient summer temperature.

Plots of absorbance at 280 nm against elution volume for 0, 7 and 14 day old silage liquors (Fig. 3) show four distinct fractions (F1–F4) at each sampling. Similar relative elution volumes and thus molecular weights (Table 4) were seen for each sample. The relative elution volume of F1 is identical with the void volume of the column and so components of F1 have molecular weights greater than 5000 daltons. The relative elution volume for free amino acids was between 1.80 and 2.50, which agrees with the values of 1.87–2.62 found on Sephadex G-25 by Olley, Ford & Williams (1968). Both F2 and F3 fall within this region (Table 4), suggesting the components are free amino acids and possibly some dipeptides and tripeptides. The relative elution volume of F4 is greater than the predetermined limit for free amino acids.

The interpretation of absorbance measurements of protein solutions at 280 nm is complicated by the amino acid composition of the proteins. Absorbance is principally due to the presence of tyrosine and tryptophan and the change in the absorbance

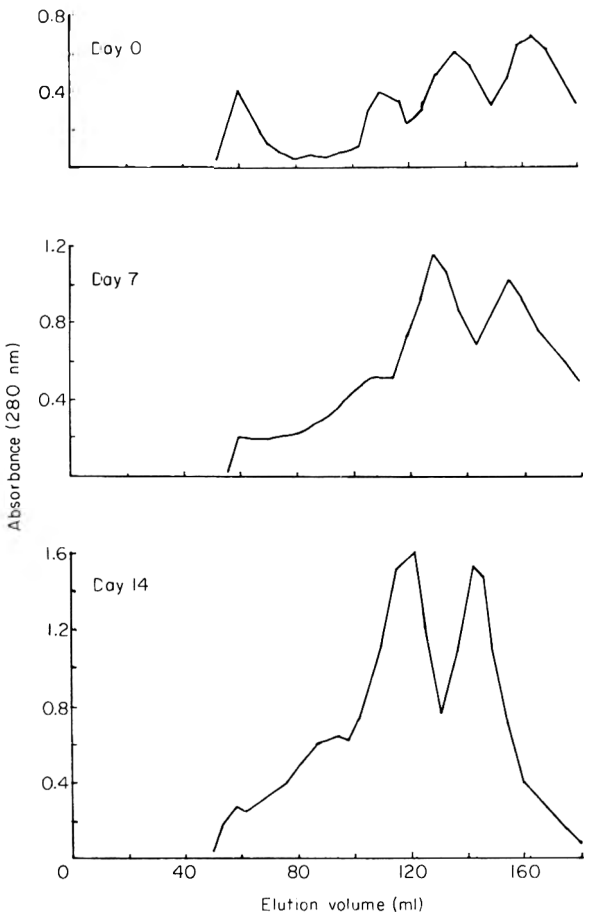

Figure 3. Fractionation of 0, 7 and 14 day old silage liquors on Sephadex G-25. V_0 was 60 ml.

characteristics of the fractions with time can be explained by the distribution of amino acids between them in the liquor (Table 5). At day 0 the amino acid composition of F1 was similar to that of whole fish and after 14 days, it resembled that of collagen, indicating that ensilation of collagen primarily yielded soluble fragments of high molecular weight ($\geq$ 5000 daltons). Hydroxyproline determinations showed that initially all was present in F1 and after 14 days, 75% still remained in this fraction. The composition of the lower molecular weight fractions changed to become more like that of whole fish during ensilation. Because collagen contained little tyrosine and no tryptophan, the relation absorbance of F1 falls during ensilation whilst that of the other fractions increases.

Changes in the free amino groups, detected by the ninhydrin reaction, are shown in Fig. 4 and confirm the distribution discussed above. The relative elution volumes of the discernable peaks correspond to those of F1 and F2 and show the build-up of small peptides, or free amino acids, in F2 with time. The distribution of protein between F1 and F2 is somewhat distorted since there is a proportionately smaller response to ninhydrin by the longer chain peptides in F1 compared with free amino acids in F2

Table 4. The relative elution volumes (V_e/V_0) and molecular weights of the standards used to calibrate the chromatographic column and the fractions F1–F4

Standard or fraction	V_e/V_0	Molecular weight (daltons)
Dextran blue	1.00	1 000 000
Glucagon	1.14	3500
Bacitracin	1.32	1422
L-phenylalanine-L-tyrosine	1.75	328
L-tryptophan	2.51	204
L-histidine and glutamic acid	1.87	155*
F1	1.00	≥ 5000
F2	1.83	≈ 211
F3	2.21	≈ 141
F4	2.63	≈ 105

*Molecular weight is the average of these two amino acids.

(compare with soluble NPN/total nitrogen in Fig. 1). Johnsen & Skrede (1981) fractionated liquors from cod viscera silages on Sephadex G-15, with detection at 280 nm. Four major fractions were found: fraction 1, molecular weight greater than 1500 daltons; 2, molecular weight similar to vitamin B12 (1355); 3, molecular weight similar to phenylalanine (165) and tyrosine (181) and 4, which appeared to elute after more of the amino acids had passed down the column. The amino acid composition of the fractions showed that glycine was predominantly in fractions 1 and 2, which also contained no tyrosine, and so were similar in amino acid composition to collagen. It appears that as with silverbelly silage, collagen degradation products were of high molecular weight despite the greater degree of solubilization (> 90%) in these cod silages. Also, as with silverbelly silages, the greatest proportion of the soluble nitrogen was found in the fraction corresponding to small peptides and free amino acids (fraction 2).

Conclusions

Autolysis in silverbelly silage shows certain similarities to that in cold water fish silages and also some differences. Although the enzymes involved may be similar in both types of silage, the extent of autolysis would appear to be determined by environmentally related differences between the raw materials, particularly water temperature.

The presence of high and low molecular weight fractions indicates that both exo- and endopeptidases are present. Pepsin is the only digestive endopeptidase likely to be active in an acid silage, whilst digestive exopeptidases have alkaline pH optima and so are unlikely to be active. Other enzymes likely to be active in a silage will be the cathepsins, released in the mincing process, which are proteases active at acid pH and occurring in most organs. (Barnard, 1973). This combination of enzymes would give rise initially to soluble polypeptides which could be broken down further by exopepti-

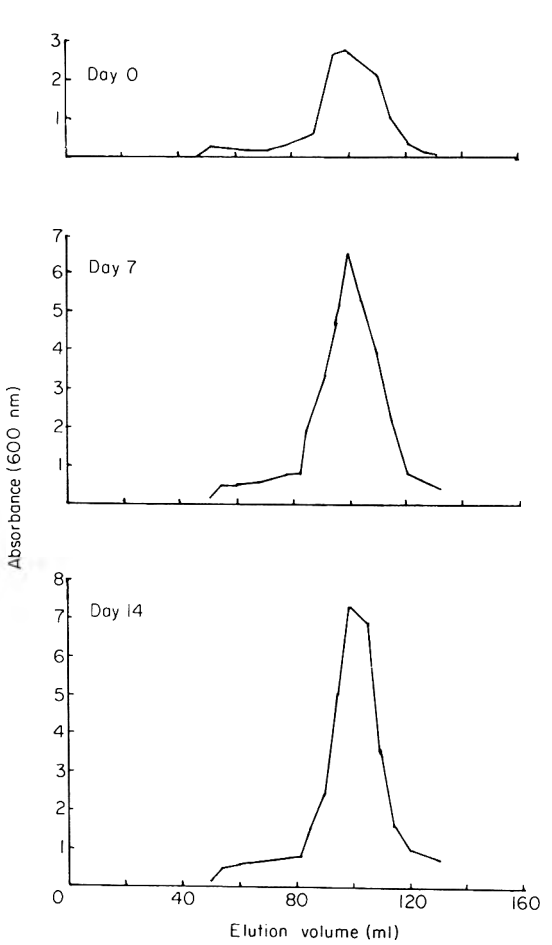

Figure 4. Relative quantity (as indicated by intensity of ninhydrin reaction) and distribution of free amino groups in 0, 7 and 14 day old silage liquors after separation of Sephadex G-25. V_0 was 60 ml.

dases. The presence of cystine in the undigested sediment of both warm water and cold water fish silages indicates common non-collagenous digestion-resistant proteins characterized by disulphide cross linking.

The presence of soluble hydroxyproline predominantly in fraction F1 of the liquor (in both cold and warm water fish silages) suggests limited enzymic degradation of collagen. A specific collagenase is presumably not involved, as this would cleave the α-chains of collagen in the helical region, giving rise to small peptides and hence a larger proportion of hydroxyproline in the F2 fraction. Non-specific proteases such as pepsin and cathepsin B1, which has a pH optimum of 3.5 for action on insoluble collagen, are known to cleave intermolecular cross links in the telopeptide region of collagen (Burleigh, Barrett and Lazarus, 1974) giving rise to soluble polypeptides, which would accumulate in the F1 fraction. Although not identified in fish, a cathepsin B1-type enzyme probably exists.

Table 5. The amino acid composition (g/16 g N) of whole fish, fish collagen and fractions F1 and F2 + F3 + F4 after 0 and 14 days ensilation at 30°C

Amino acid	Whole* fish	Fish† collagen	F1		F2 + F3 + F4	
			Day 0	Day 14	Day 0	Day 14
Lysine	7.94	3.11	7.75	9.66	2.61	8.55
Histidine	2.21	0.69	1.54	2.27	2.12	2.29
Arginine	6.57	7.79	3.24	8.41	8.71	7.16
Aspartic	8.85	5.69	13.49	8.50	3.51	7.83
Threonine	3.85	2.75	3.42	2.71	3.52	4.17
Serine	3.81	4.05	4.66	3.88	2.87	3.66
Glutamic	14.04	9.96	13.02	12.54	7.97	12.13
Proline	4.58	12.68	4.17	11.88	6.36	4.01
Glycine	6.83	20.45	5.87	13.75	11.32	6.51
Alanine	6.22	10.03	7.92	7.65	15.06	8.19
Valine	3.99	2.19	4.14	1.89	6.46	5.62
Isoleucine	3.75	1.39	3.09	0.93	4.98	3.87
Leucine	6.82	2.91	6.17	1.64	8.13	7.15
Tyrosine	3.04	0.18	1.39	0.00	0.00	1.08
Phenylalanine	3.51	2.22	6.15	0.86	2.51	3.84

*Whole minced silverbelly prior to addition of formic acid.
†Lungfish skin collagen from Eastoe & Leach (1977).

However, the pattern of collagen breakdown demonstrated here and in cod viscera silages (Johnsen & Skrede, 1981) strongly suggests that the combination of acid pH and elevated temperature is primarily responsible for the solubilization of collagen in silages. The striking difference in the extent of autolysis, in terms of total protein and collagen breakdown, between silverbelly silage and cold water fish silages could be explained by the relative resistance of collagen in these fishes to acid pH and temperature. Because the physical integrity of the issues is due to the connective tissues, limited collagen breakdown would lead to limited breakdown of other proteins.

The presence of some hydroxyproline in F2 may be due to a mechanism in which the combined effect of acid pH, incubation temperature and non-specific proteases make localized areas of the α-chains susceptible to further degradation. Cathepsin B1 has been shown to cleave both intramolecular bonds in the telopeptide region and α-chains in the helical region of soluble collagen with a pH optimum of 4.5–5.0 (Burleigh *et al.*, 1974). It was also concluded that once attacked by cathepsin B1, the α-chains were rapidly degraded to low molecular weight peptides. The results of this study emphasize the need for care in extrapolating results from studies with cold water fish to warm water fish.

Acknowledgments

We would like to acknowledge the Tropical Development and Research Institute (TDRI), London for obtaining the frozen silverbelly and for financial support for one of us (GMH).

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(Received 8 November 1984)

Journal of Food Technology (1985) **20**, 573–580

Silage from tropical fish 2. Undigested fraction

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Summary

The solubility of the undigested fraction (sediment) of a silage made from silverbelly (*Leiognathus* sp.) in sodium dodecyl sulphate, guanidine hydrochloride and 2-mercaptoethanol, alone and in combination was evaluated. The sediment was probably composed of muscle proteins cross linked by disulphide bonds and stabilized by hydrophobic associations and hydrogen bonds. Ensilation followed by *in vitro* digestion of the sediment with pepsin/pancreatin/trypsin or *in vivo* digestion of the sediment using chicks, solubilized about 81% and 90% respectively of total nitrogen in silages up to 14 days old. The most resistant fraction was rich in collagenous derived material and bones. It is suggested that controlled ensilation can produce a highly digestible protein source without the formation of large amounts of reactive and labile free amino acids.

Introduction

Centrifugation of a fish silage in which autolysis has taken place always produces an undigested fraction or sediment. The sediment usually only represents a small proportion of the proteins in silages from cold water fish but may be a significant amount in those from some warm water fish (Hall *et al.*, 1985). The reasons for the existence of this enzyme resistant fraction are not clear and several factors may be involved. The composition of the raw material is an important consideration since visceral silages liquefy more easily than whole fish silages, and the latter liquefy more easily if there is a high ratio of flesh to skeleton (Backhoff, 1976; Gildberg & Raa, 1977). This presumably reflects the concentration and nature of the proteases present since visceral silages should contain a greater concentration of digestive enzymes and cathespins than the whole fish silages.

Enzyme deactivation appears to be an unlikely cause as proteases have high stability in silages (Gildberg & Raa, 1977); however end product inhibition is possible (McBride, Idler & McLeod, 1961). There is a similarity in the composition of the sediment from cold water and warm water fish silages in that it contains a large proportion of the fish lipids and the amino acid cystine (Hall *et al.*, 1985). The presence of lipid and disulphide bonds have been proposed as possible causes of incomplete digestion in cold water fish silages (Raa & Gildberg, 1976) and may also be of importance in warm water fish silages.

The inconsistent performance of poultry fed diets containing silages made from warm water fish may be due, in part, to the poor digestibility of the silage proteins. Thus, it is important to understand why certain proteins are resistant to autolysis and to determine if they are also resistant to *in vivo* digestion. In the present study the nature of the resistant proteins was investigated by evaluating the solubility of silage sediments

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from silverbelly (*Leiognathus* sp.) in sodium dodecyl sulphate (SDS), guanidine hydrochloride (GuHCl) and 2-meraptoethanol (2ME), alone or in combination. These reagents are known to disrupt certain linkages.

Materials and methods

Materials

Details of the raw materials, method of silage production and separation into liquor and sediment by centrifugation have been given previously (Hall *et al.*, 1985). For the present work, sediments were collected from 0, 7 and 14 day old silages, freeze dried and ground to pass through a 2 mm sieve. The total nitrogen and hydroxyproline contents of the liquor and sediment were determined. Freeze dried, defatted egg yolk (EYC) was used as a control in the solubility and digestibility studies because it has a defined protein composition and is considered to be easily digested *in vivo*.

Solubility studies

The solvents used were aqueous solutions of:

(i) 1% (v/v) 2ME (0.143 M), this reduces disulphide linkages.

(ii) 1% (w/v) SDS (0.035 M), this disrupts hydrophobic associations and hydrogen bonds.

(iii) 6 M GuHCl, at pH 10.5, prepared according to Buttkus (1974); this also disrupts hydrophobic associations and hydrogen bonds.

(iv) 1% (v/v) 2ME and 1% (w/v) SDS.

(v) 6 M GuHCl and 1% (v/v) 2ME, at pH 9.0, prepared according to Buttkus (1974).

Approximately 0.5 g of sample was suspended in 50 ml of each solvent which, as the sediments contained 9–10% nitrogen, gave maximum concentration in the solvent of 1 mg nitrogen/ml. After 16 hr at ambient temperature (≃ 20°C), with occasional shaking, the sample was filtered through a Whatman No. 1 paper, washed and the nitrogen content of the filtrate determined. For solvents containing GuHCl, soluble protein was determined from the absorbance at 280 nm and expressed in arbitrary units. The ratio of the absorbance at 280–260 nm indicated negligible interference from nucleic acids. Thus, 'protein content' was expressed as $Abs_{280} \times 1.116 \times DF$, where Abs_{280} = absorbance of sample: 1.116 is the correction factor for no nucleic acid interference (Chaykin, 1966) and DF is the dilution factor. True protein contents can be determined from absorbance at 280 nm if the tyrosine/tryptophan content of the protein is known but an extinction coefficient of 10 has been found to apply to a range of proteins (Plummer, 1978). This factor was applied here to estimate soluble protein, and hence soluble nitrogen, in solvents containing GuHCl.

In vitro *digestibility*

This was determined for 1 g samples, at 37°C, by the method of Hazell, Ledward & Neale (1978) using pepsin at pH 2 for 16 hr and pancreatin and trypsin at pH 7.5 for 24 hr.

Six digests were carried out on each sample, the digestion being stopped in one pair after the pepsin stage; the other four were given the full digestion (PPT) and two used for nitrogen solubility and the other two for nitrogen and hydroxyproline determinations. EYC was given the full digestion only. The digestion mixtures were filtered, washed and the nitrogen and/or hydroxyproline content of the filtrate determined.

In vivo *digestibility*

This was determined by examining the contents of the ileum according to the method of Varnish & Carpenter (1975). At this stage in digestion, most of the sample protein should have been absorbed. Chromic oxide was used as an indigestible marker and EYC, as a well digested source of protein, for comparison.

Seven 38 day old chicks (Ross 1) weighing 900–1040 g were used, one bird for the EYC control and duplicates for the 0, 7 and 14 day old sediments. Approximately 5–6 g of sample, contributing 0.5 g of nitrogen, were mixed with 0.05–0.06 g of chromic oxide and slurried with water. The slurry was introduced directly into the crop by means of a tube and syringe, the birds were killed 4 hr *post cibum* and dissected immediately. The duodenum, jejunum and ileum were removed together by cutting below the gizzard and above the caecae. The duodenum and jejunum were separated by cutting at the end of the duodenum loop (around the pancreas) and the jejunum and ileum separated by cutting at Meckel's diverticulum. The contents of each section were squeezed by tweezers into a container, each section was cut longitudinally and the remaining contents washed into the container. The samples were freeze-dried then oven dried at 80°C under vacuum and the residue ground to pass through a 2 mm sieve. The duodenum and jejunum were free of chromic oxide so only the ileal contents were analysed for nitrogen, hydroxyproline and chromic oxide. Kjeldahl nitrogen and hydroxyproline were determined as described previously (Hall *et al.*, 1985) and chromic oxide as detailed by Varnish & Carpenter (1975).

Results and discussion

Solubility studies

The solubilities of the sediments and defatted egg yolk (EYC) in the various solvents are given in Table 1, which illustrates the effect of time of ensilation on the solubility of the sediments in each solvent. Longer exposure to autolysis produced resistant fractions which were, on a percent basis, increasingly soluble in the solvents, indicating that the bonds susceptible to the solvents may be responsible for the resistance to the autolytic

Table 1. Solubility in different solvents of the sediment from 0, 7 and 14 day old silages and egg yolk proteins (EYC)

	Solubility*			
Solvent	0	7	14	EYC
2ME†	18.1	26.3	36.2	21.6
SDS	37.3	47.2	54.3	77.3
2ME + SDS	35.5	50.7	54.4	73.9
GuHCL	117.0 (42)‡	191.9 (77)	274.6 (104)	229.9 (73)
GuHCl + 2ME	154.6 (56)	240.5 (96)	271.0 (107)	315.6 (100)

*Solubility measured as % soluble nitrogen for solvents containing 2ME and/or SDS and in arbitrary units for solvents containing GuHCL.

†For details see 'Materials and methods'.

‡Numbers in parentheses are estimated % nitrogen solubility using the factor from Plummer (1978) for converting arbitrary units to true protein (see text for details).

proteases. Overall, the evidence suggests that enzyme resistant proteins are cross linked by disulphide bonds and also stabilized by hydrophobic associations and by hydrogen bonds. Shapiro, Vinuela & Maizel (1967) suggested that 1% SDS could break easily accessible disulphide bonds which would explain the comparable effect of SDS alone and SDS with 2ME. Solubility in solvents containing GuHCl indicates a difference in the effect of GuHCL alone and in combination with 2ME. It has been shown that GuHCL disrupts hydrophobic associations and hydrogen bonds within the protein molecule, unlike SDS which acts through a surface effect, and thereby GuHCl allows access of 2ME to disulphide bonds buried within the hydrophobic centre of the molecule (Fish, Reynolds & Tanford, 1970). Thus the effect of GuHCl with 2ME is additive. Also, solvents containing GuHCl are more effective in solubilizing sediment protein than those containing SDS.

Muscle proteins represent the greatest proportion of non-connective proteins in the whole fish and aggregation of these proteins could lead to an enzyme resistant fraction in the silage. Evidence to support this hypothesis comes from studies on the denaturation of myofibrillar proteins in frozen fish. Buttkus (1970; 1971) used 6 M GuHCl with 0.5 M 2ME to resolubilize aggregated myosin in frozen fish and proposed that denaturation was due to intermolecular cross linking through the rearrangement of disulphide and sulphydryl groups during which process the hydrophobic and hydrogen bonds within the interior of the molecule become exposed and recombine, possibly intermolecularly. Matsumoto (1980) also suggested that myosin and actomyosin could be denatured by aggregation caused by a combination of both types of bonds. However, Connell (1959) argued against the involvement of disulphide bonds showing that 1% SDS could resolubilize myofibrillar proteins from cod (*Gadus morhua*) stored for 29 weeks at $-14°C$.

During silage production the mincing process may expose the hydrophobic centres of the muscle proteins to an aqueous environment, allowing disulphide bonds to break and reform intermolecularly, the proteins thus being made resistant to enzyme attack.

The solubility of the EYC proteins in the solvents suggests that they are held by the same forces as the sediment proteins and as the EYC proteins are thought to be easily digested *in vivo*, then it follows that the sediment proteins should be easily digested.

In vitro *digestibility*

The digestibilities of the sediments are given in Table 2 in terms of total nitrogen and hydroxyproline solubilization. The digestibility of EYC was 78.8% using PPT. Considering nitrogen solubilization first, it can be seen that the effect of pepsin alone (lines 3 and 5) is considerably less for a 14 day sediment than a day 0 sediment. This is not unexpected since pepsin is presumably one of the active enzymes during ensilation. Thus the sediment resistant to autolysis should also be resistant to pepsin digestion. The beneficial effect of a two pH step digestion with a mixture of enzymes is seen by comparing the effect of pepsin alone with PPT digestion (lines 4 and 6). Indeed, if the effect of ensilation is added to that of PPT (in line 8), similar amounts of nitrogen are solubilized for 0, 7 and 14 day sediments (compare with pepsin and ensilation, line 7). The digestibility of the sediments with PPT compares favourably with that of EYC and suggests that, practically, a prolonged ensilation is not necessary to ensure high digestibility. In fact it may be detrimental because of the susceptibility of free amino acids to destruction by enzymes and lipid oxidation products.

Full digestion (PPT) of the sediments always produced an insoluble residue representing about 20% of the total silage nitrogen (Table 2, line 8). This residue was a

Table 2. Digestibility *in vitro* of the sediment from 0, 7 and 14 day old silages

	Digestibility*					
	Nitrogen			Hydroxyproline		
	0	7	14	0	7	14
(1) % total N and H soluble after ensilation	9.8	38.4	45.8	1.4	34.5	49.7
(2) % total N and H undigested after ensilation (sediment)	90.2	61.6	54.2	98.6	65.5	50.3
(3) % sediment N solubilized by pepsin	42.2	33.4	30.9	n.d.	n.d.	n.d.
(4) % sediment N and H solubilized by PPT†	77.8	70.0	68.7	36.7	23.6	9.2
(5) % total N solubilized by pepsin (3×2)	38.1	20.6	16.7	n.d.	n.d.	n.d.
(6) % total N and H solubilized by PPT (4×2)	70.1	43.1	37.2	36.2	15.4	4.6
(7) % total N solubilized by ensilation + pepsin (1+5)	47.9	59.0	62.5	n.d.	n.d.	n.d.
(8) % total N and H solubilized by ensilation + PPT (1+6)	79.9	81.5	83.0	37.6	49.9	54.3

*Measured as solubilization of nitrogen (N) and hydroxyproline (H).
†PPT = Pepsin + pancreatin + trypsin.
n.d. = Not determined.

smooth greyish mass with pieces of bone clearly visible. Solubilization of hydroxyproline was determined to see if connective tissue played a part in the resistance of this residue to digestion. As with total nitrogen, percent solubilization of hydroxyproline after undergoing full PPT digestion was less for the 7 and 14 day sediments than for the day 0 sediment. However, the effect, i.e. the degree of resistance, was more pronounced when monitored by hydroxyproline solubilization than by nitrogen solubilization. It has been suggested (Hall *et al.*, 1985) that the effect of acid pH and elevated temperature on the least stable collagen is primarily responsible for hydroxyproline solubilization in silages and a similar effect may operate during initial *in vitro* digestion i.e. at pH 2 and 37°C. However, the more stable collagenous material is unaffected. Overall, connective tissue solubilization appears to be achieved best by prolonged ensilation (compare line 1, 14 day sediment with line 8, day 0 sediment), contrary to the experience for protein solubilization generally. However, it is unlikely that a prolonged ensilation would be justified given the deleterious reactions which might occur involving free amino acids.

In vivo *digestibility*

The *in vivo* digestibilities of the sediments and EYC, in terms of nitrogen absorption, are given in Table 3. The calculation of the Digestibility Coefficient (DC) of the sample is given in line 7 and indicates that the sediment proteins are as well digested as EYC proteins. The DC measured here is 'apparent' in that no correction has been made for dilution of the sample nitrogen (exogenous) by nitrogen from the gut (endogenous) in the form of intestinal cells, bacteria and enzymes. This effect is accounted for to some extent by the use of chromic oxide as an indigestible marker and EYC as a comparison source of well digested protein. By these means the DC found for each sediment has meaning in comparison with EYC because endogenous nitrogen will be secreted by chicks fed either the sediments or EYC. As with the *in vitro* digestion, the 14 day sediment appeared to be more resistant to digestion than the day 0 sediment but the difference was not as great as in the *in vitro* digestion (compare Table 3, line 7 with Table 2, line 4). Addition of the effect of ensilation (line 8) to that of digestion (line 10)

Table 3. Digestibility *in vivo* of the sediment from 0, 7 and 14 day old silages and egg yolk protein (EYC)

		Digestibility*					
		0		7		14	
	EYC	Bird A	Bird B	Bird A	Bird B	Bird A	Bird B
(1) Sample N (mg/g DWB)	97.7	98.3	99.4	87.6	87.6	92.4	94.0
(2) Sample CHR (mg/g DWB)	10.1	9.9	9.9	10.0	9.8	9.9	10.0
(3) Ratio 1/2	9.67	9.93	10.04	8.76	8.94	9.33	9.40
(4) Ileal N (mg/g DWB)	50.8	38.9	69.0	50.7	64.5	72.0	61.3
(5) Ileal CHR (mg/g DWB)	46.9	47.7	39.1	97.4	38.5	22.9	74.5
(6) Ratio 4/5	1.08	0.82	1.77	0.52	1.68	3.14	0.82
(7) % N absorbed $\frac{(3-6)}{(3)}$	88.8	91.8	82.4	94.1	81.2	66.3	91.2
(8) % total N soluble after ensilation†	—	9.8	—	38.4	—	45.8	—
(9) % total N undigested after ensilation (sample N)†	—	90.2	—	61.6	—	54.2	—
(10) % total N absorbed *in vivo* (7×9)	—	82.8	74.3	57.9	50.0	35.9	49.4
(11) % total N absorbed‡ including ensilation (8+10)	—	92.6	84.1	96.3	88.4	81.7	95.2

*Measured as absorption of nitrogen (N) with chromic oxide (CHR) as an indigestible reference material.

†From Table 2, lines 1 and 2.

‡Assuming all soluble N is absorbed.

on whole silage nitrogen indicates high digestibilty of silage proteins (line 11). This was also suggested by the *in vitro* digestion (Table 2).

Hydroxyproline digestion was determined to see if connective tissue accumulated in the ileal contents as in the residue from *in vitro* digestion (Table 4). Hydroxyproline digestion in the 14 day sediments is less than found for the 0 and 7 day sediments (line 3) but the reduction is not as marked as in the *in vitro* digestion (see Table 2, line 4). Considerably more of the connective tissue is digested *in vivo* than *in vitro* for all three sediments. Hydroxyproline was not detected in EYC or the gut contents of the bird fed EYC, indicating that no dilution of the sediment nitrogen by endogenous connective tissue occurred.

It appears that silages derived from warm water fish produce sediments that are highly digestible and the proportion resistant to digestion consists primarily of collagenous material, particularly bone. Raa & Gildberg (1976) showed that the sediment from cod viscera silages resembled the undigested contents of the alimentary tract of cod, and that in these cold water fish silages connective tissues played no part since hydroxyproline was not detected in either fraction. The collagen of cold water fish is more labile than that of warm water fish species (Hall *et al.*, 1985) and probably accounts for the differences. Raa & Gildberg found cystine to be rich in the sediment reaffirming the importance of disulphide bonds in stabilizing the residue against enzyme attack. This hypothesis is confirmed here, with the proviso that, in the case of warm

Table 4. Digestibility *in vivo* of the sediment from 0, 7 and 14 day old silages

	Digestibility*					
	0		7		14	
	Bird A	Bird B	Bird A	Bird B	Bird A	Bird B
(1) % total H soluble after ensilation†	1.4		34.5		49.7	
(2) % total H undigested after ensilation (sediment)†	98.6		65.5		50.3	
(3) % sediment H absorbed *in vivo*	78.1	73.6	79.8	76.9	65.1	62.6
(4) % total H absorbed *in vivo* (3×2)	77.0	72.6	52.3	50.4	32.7	31.5
(5) % total H absorbed‡ including ensiilation (1+4)	78.4	74.0	86.6	84.9	82.4	81.2

*Measured as absorption of hydroxyproline (H).
†From Table 2, lines 1 and 2.
‡Assuming all soluble N is absorbed.

water fish, the connective tissue proteins play an important role in resistant autolysis and *in vivo* digestion.

In vivo digestion of silverbelly silage proteins appears to be high (about 90%) which can be attributed to the particular form of the proteins present in the silage. Over 80% of the soluble nitrogen of a silverbelly silage is TCA soluble, consisting of small peptides and free amino acids, and should be directly absorbable in the gut without further digestion. A dried whole silage could therefore be looked upon as a mixture of small molecular weight water soluble proteins and a larger molecular weight water insoluble protein, the former being quickly absorbed whilst the latter are being digested. Crampton *et al.* (1971) showed that the absorption of small peptides was more rapid than the equivalent amount of free amino acids. Thus limited hydrolysis in silage protein, controlled to give small peptides rather than free amino acids, may yield highly digestible feeds but minimize any deleterious reactions that may be undergone by free amino acids.

Acknowledgments

We would like to thank the Tropical Development and Research Institute (TDRI), London for supplying the silverbelly and for financial support for G.M. Hall. Thanks are due to Dr N. Boorman for assistance with the *in vivo* digestibility studies.

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(Received 8 November 1984)

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Increase on free fatty acids during ripening of anchovies (*Engraulis anchoíta*)

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Summary

The changes of free fatty acids (FFA) during the ripening of salted anchovy was studied. In the course of the curing process, the content of free fatty acids (FFA) in the muscle increased fifteen-fold, mostly in the first 100 days of the process. Each sample was subjected to sensory evaluations. A high correlation between FFA contents and sensory scores was found. Therefore the determination of FFA is proposed as an objective index to assess the ripening of salted anchovies.

Introduction

The need of a non-sensory index to evaluate the ripening of cured fish instead of sensory assessments, such as that proposed by Thackaberry (1979), is widely accepted.

Several methods based on the chemical changes which take place during the salting and ripening of fatty fish have been reported for different species: Atlantic herring (Plorina & Leonova, 1970); Baltic herring (Podeszewski, Stodolnik & Otto, 1975); Caspian herring (Konnova, 1966); Siberian cisco fish (Sedova & Emel'yanova, 1976); and anchovies (Baldratti *et al.*, 1977; Establier & Gutiérrez, 1972; Lee & Choe, 1974).

The total ester index has been proposed by Filsinger *et al.* (1982), as a chemical index to assess the ripening of anchovies starting at about 100 days of curing. The possibility of using the determination of total volatile basic nitrogen has also been studied (Filsinger, Barassi & Lupin, 1984).

The purposes of this work were to determine the evolution of FFA during the ripening of anchovies, and to study the possibility of using the quantitative determination of FFA content to evaluate the curing of fish throughout the process.

Materials and methods

Anchovy salting, packing and ripening in brine

Fresh anchovies caught on the sea platform during spring, were salt cured during each one of 3 consecutive years. Fresh fish were put in saturated brine, and after 24 hr, fish were beheaded, gutted and washed. Washed anchovies were packed in 10 kg tins, with a total of 240 g salt scattered in the bottom and top of the fish pack. The final fish to salt ratio was approximately 30.

A maximum pressure of 80 g/cm^2 was applied to salted fish, which were stored in a basement at 18–22°C for up to 300 days. During that period an extruded brine

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containing biological material completely covered the fish. This general procedure has already been detailed (Filsinger *et al.*, 1982). At lest twenty-four batches of salted fish were prepared each year.

Sensory assessment

Fish samples were taken at different curing times. Eighteen anchovies were taken from different cans to obtain representative samples. Each anchovy was organoleptically evaluated and scored by a panel of at least three experts. The scoring method has been reported by Filsinger *et al.* (1982). The scale ran from 0 for fresh, unprocessed fish to 8 for overripe anchovies with 6 corresponding to optimally ripe anchovies. The mean panel scores were used.

Determination of free fatty acids

Uniform muscle paste was prepared with fillets taken from the same cans used for sensory assessments. Twenty g of muscle paste were homogenized with 100 ml ethanol in a Braun MRA homogenizer. Homogenates were filtered through Whatman No. 1 paper; and 0.1 aliquots of the filtrate were used to measure FFA according to the method described by Smith (1975). Calibration curves were obtained for each analysis set, by using palmitic acid (16:0) as the standard. FFA determinations were done by quadruplicates. Results were expressed as milliequivalents of palmitic acid per 100 g wet tissue. Data points in Figs 2 and 3 are mean values.

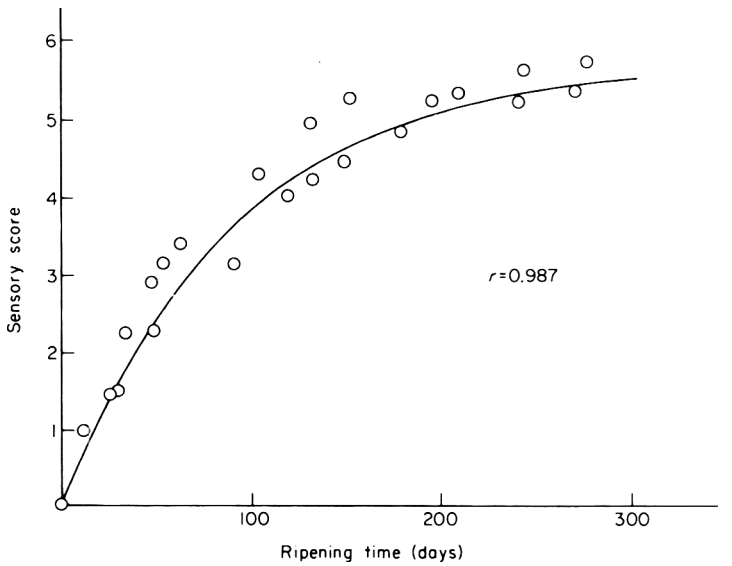

Figure 1. Sensory scores *versus* ripening time of anchovy. Each point is the panel mean score of the sensory characteristics evaluated.

Statistical analysis

Data shown in Figs 1 and 2 were fitted to pseudo first order kinetics models of the form $y = a + b\ e^{-kt}$. To fit the data, Stevens' (1951) maximum likelihood iterative method was used.

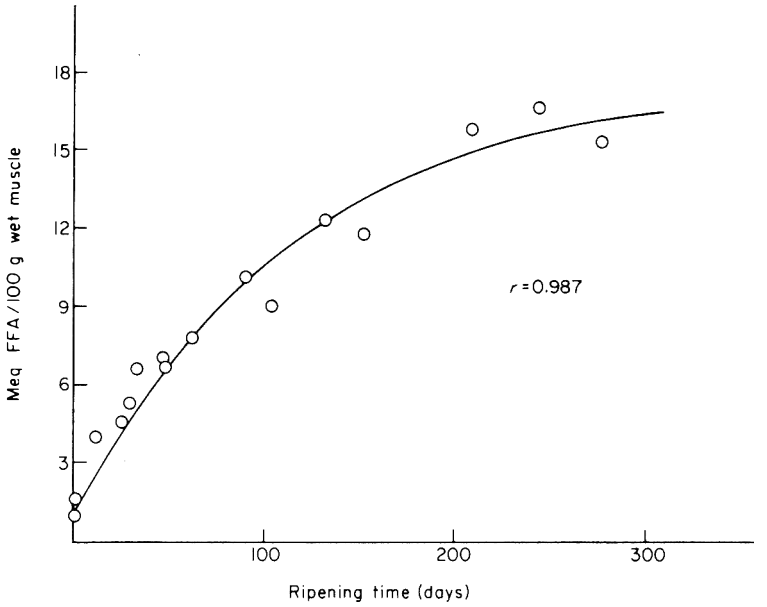

Figure 2. Changes in FFA content *versus* ripening time of the anchovies.

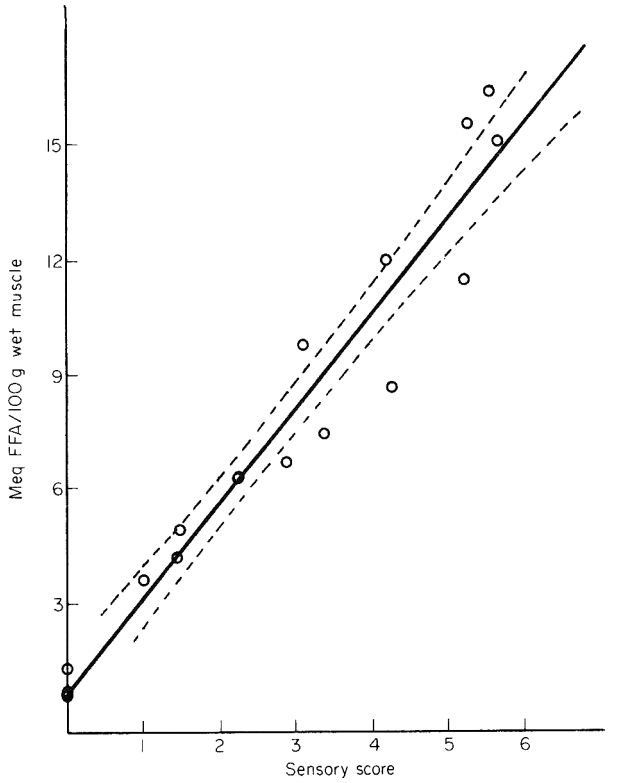

Figure 3. FFA content *versus* sensory scores for the curing of salted anchovies. The area between the dashed lines is the 95% confidence limits of the regression estimate.

Results and discussion

Figure 1 shows the increase in sensory score for three batches of anchovies caught and subsequently salted in 3 consecutive years. Over 70% of the ripening takes place in the first 100 days of the process. However, at this time, the odour and flavour of the fish are not those characteristic of the fully cured anchovy and the colour is not uniform. Actually, sensory scores reached almost constant values, indicative of complete maturity, after 200 days of curing.

Information on changes in lipids during maturation of salted fish is scarce and, in case of *Engraulis anchoíta* it is non-existent. Sedova & Emel'yanova (1976), have pointed out the importance of the characteristic aroma of cured fish. Poideszewski & Stodolnik (1976) have claimed that FFA level has a strong influence on the flavour of salted and maturated fish.

The assessment of changes in FFA could provide an objective method for measuring the maturation of salted fish. Figure 2 depicts FFA production in anchovy muscle *versus* time, for the three batches of anchovies used in sensory evaluation. A fifteen-fold increase in FFA occurred over the maturation process. Again, over 65% of this increase took place in the first 100 days period. A maximum value of about 15 mEq of FFA per 100 g wet muscle was reached after 200 days of the curing process.

Since Smith's method (1975) measures fatty acids having carbon chains over C8, the results presented here do not support a direct link between FFA production and flavour. However, a possible relationship between long chain FFA production and their subsequent degradation to shorter fatty acids cannot be totally excluded. On the other hand, the assessment of changes in FFA could provide a useful method for measuring the ripening of salted fish.

The parallel between the increase of the sensory scores and the increases in FFA, gives rise to the possibility of correlating these parameters. FFA contents are plotted against the sensory scores in Fig. 3. The high correlation coefficient obtained (0.971), would allow the estimation of curing of salted anchovies throughout the ripening process by the estimation of FFA. This would represent an advantage over the total ester index, which is not applicable for the first stages.

Acknowledgments

This work was partially supported by the Consejo Nacional de Investigaciones Científicas y Técnicas, (CONICET). H. A. Roldán is a Research Fellow of the CONICET. The authors also thank Eng. Carlos E. Del Valle for his helpful suggestions during the preparation of the manuscript.

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(Received 17 January 1985)

Journal of Food Technology (1985) **20**, 587–598

Effects of different types of carrageenans and carboxymethyl celluloses on the stability of frozen stored minced fillets of cod

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Summary

Different types of carrageenans and carboxymethyl celluloses were used as additives (5 g/kg) for minced fillets of cod. Samples of these treatments were stored at −18°C for 10 weeks and were evaluated at regular intervals for pH, water holding capacity, texture, extractable myosin and formation of dimethylamine and formaldehyde.

Except Kappa carrageenan the additions improved the water holding capacity of the raw and cooked minced fish and decreased toughening during frozen storage. The addition of Iota carrageenan resulted in samples with remarkable texture stability and so after 10 weeks of frozen storage the texture of this treatment was similar to fresh minced cod. The higher the viscosity of carboxymethyl cellulose, the more it increased the softness and water holding capacity.

Introduction

Several attempts have been made to preserve the functional properties of minced fish during frozen storage. Additions of carbohydrates, amino acids and related compounds, polyphosphates, sodium chloride and anti-oxidants have been tried (Tran & Hang-Ching, 1982; Suzuki, 1981; Codex Alimentarius Commission, 1981; Matsumoto, 1979; Ohnishi, Tsuchiya & Matsumoto, 1978). More recently, hydrocolloids such as sodium alginate, pectin, carboxymethyl cellulose, locus bean gum, guar gum, carrageenan and xanthan gum were listed as additives by the Codex Alimentarius Commission (1981, 1983).

In previous papers we described the effect of additions of different types of alginates, carrageenans, pectins, xanthan and locus bean gum on the stability of frozen stored minced fillets of whiting (da Ponte, Roozen & Pilnik, 1985a; 1985b). Remarkable changes in texture and water holding capacity were observed in the minced whiting depending on the type of hydrocolloid used. These important effects may find use in products already marketed, such as fish sticks and fish burgers and in the development of new products from minced fish. In these experiments, we pre-dissolved the hydrocolloids in water, freeze dried and ground them before adding to the minced fish. However, this is impractical because of the costs involved in the freeze drying operation. In the present study, we omitted this pre-treatment of the hydrocolloids. Three types of carrageenans were compared of which Kappa and Iota are not completely soluble and Lambda is soluble in cold water. Four types of carboxymethyl

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cellulose with similar degrees of substitution (0.80–0.95) and viscosities from 20–30 to 2000–4500 centipoises (at 1% concentration in water at 25°C) were studied.

Materials and methods

Skinned fillets of cod were bought in the local market of Wageningen on 25 January 1984. They were packed in 2 kg plastic bags, blast frozen and kept frozen at −45°C for 2 weeks. Subsequently they were thawed in cold tap water and were passed through a Kenwood mincer model A.720, using a mincing screen with a 4.4 mm mesh. The resulting minced fish was divided in portions and different hydrocolloids were spread over the surface (5 g per kg of minced fish). The hydrocolloids and the minces were gently mixed with a spoon and kept in plastic bags on melting ice for 2 hr. Hydrocolloids used were:

(i) Potassium Kappa carrageenan (HF 55758-63) and Iota carrageenan (HF 33895-96), from Copenhagen Pectin Factory Ltd (Denmark);

(ii) Lambda carrageenan, Viscarin 402, (Batch 321403), from Marine Colloids Division, FMC Corporation (Springfield, New Jersey, U.S.A.);

(iii) Carboxymethyl cellulose (CMC), Akucell AF 705, AF 1505, AF 2205 and AF 2805 from Enka bv, Industrial Colloids (Holland).

Samples of 150 g were repacked in plastic bags, blast frozen and kept at −18°C for 10 weeks. Samples of each treatment were withdrawn from the frozen storage, thawed in cold tap water and kept for 3 hr in melting ice until analyses were carried out.

Extractable myosin (EM), dimethylamine (DMA) and formaldehyde (FA) formation, texture analysis, pH measurements, water holding capacity of the raw and cooked minced fish (WHCR and WHCC), cook drip loss (CDL), protein and carbohydrate content of the supernatants in the WHCR and in the WHCC, and viscosity determinations of these supernatants were determined in the same way as described before (da Ponte *et al.*, 1985a).

Statistical analysis

Data were analysed using the statistical analysis systems computer package (Baker & Nelder, 1978). Polynomial regression lines were evaluated for each property and for each treatment. The *t*-test at the 5% level of significance was used to compare different treatments.

Results and discussion

pH measurements

In all cases (different treatments, time of storage) the pH's were between 6.5 and 6.7. The pH of the blank and the carrageenan additions showed a slight fall of 0.1 during the first 2 weeks of frozen storage and afterwards a small increase of 0.2. The pH of the CMC additions increased steadily from 6.5 to 6.7 during frozen storage.

Water holding capacity of the raw material (WHCR)

During the 10 weeks of frozen storage at −18°C, WHCR decreased in all cases, and, with the exception of the Kappa carrageenan, the WHCR of the treatments were significantly higher than the ones of the blank (Fig. 1). In the case of the CMC additions, the WHCR's correlate positively with their viscosities (Fig. 7).

The Kappa carrageenan treatment had a similar or lower WHCR than the blank.

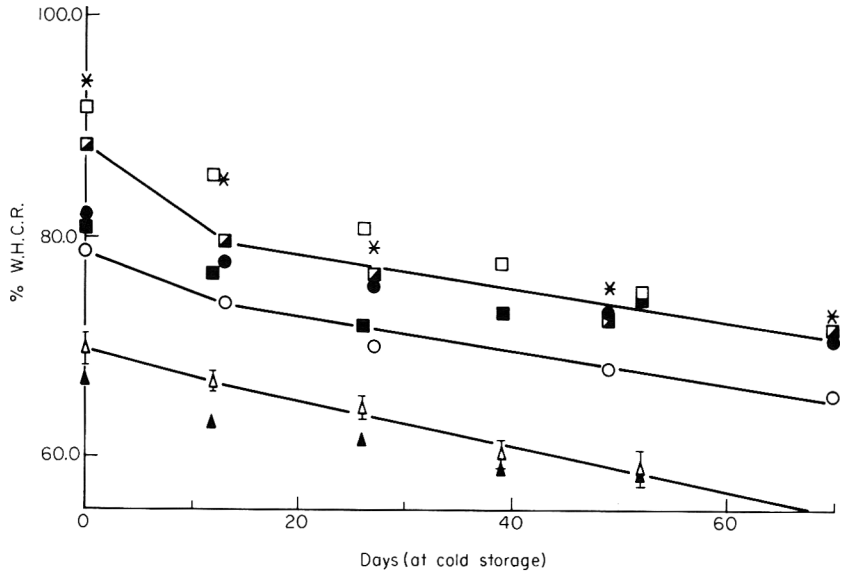

Figure 1. Effect of time and different hydrocolloids on WHCR of frozen stored minced fish muscle. △ Blank; ▲ Kappa carrageenan; □ Lambda carrageenan; ■ Iota carrageenan; O Carboxymethyl cellulose AF 705; ● Carboxymethyl cellulose AF 1505; ◪ Carboxymethyl cellulose AF 2205; and ★ Carboxymethyl cellulose AF 2805.

The mineral content of cod is about 0.320% for potassium, 0.077% for sodium, 0.016% for calcium and 0.023% for magnesium (Paul & Southgate, 1978). At this potassium content and at room temperature Kappa carrageenan is difficult to hydrate and form a solution in the raw minced fish. In a previous experiment, freeze drying helped Kappa carrageenan to hydrate and so an improvement of the WHCR of whiting was observed (da Ponte *et al.*, 1985a).

The Iota and Lambda carrageenan additions improved the WHCR. This should be ascribed to hydrated particles of Iota and Lambda carrageenan which remained in the fish residue during the WHCR determination. Similar phenomena were observed in our previous experiment (da Ponte *et al.*, 1985b) in which the use of freeze dried Iota carrageenan was shown to enhance the value of the WHCR. The supernatants obtained in that case were heterogeneous with visible lumps of hydrated particles of Iota carrageenan.

Water holding capacity of the cooked material (WHCC) and cook drip loss (CDL)

WHCC and CDL were almost constant during the ten weeks of frozen storage (Table 1a and Fig. 2). The increase of WHCC and decrease of CDL correlates with ranking of the viscosities of the CMC additions. For these hydrocolloids the differences of $(100-\overline{CDL})-\overline{WHCC}$ were similar or higher than the blank (Table 1a). The CMC additions did not seem to influence the resistance of the fish cake to the impact of the centrifugal force.

In the case of the carrageenan treatment, the difference $(100-\overline{CDL})-\overline{WHCC}$ were lower than the blank. Comparing the present results of the carrageenan additions, with previous results (Table 1b), it is clear that freeze drying improved the water holding capacity of the Kappa and Iota carrageenan significantly. Formation of separated gels of Kappa, Iota and perhaps Lambda carrageenan may explain the good resistance of the

Table 1. Comparison of the CDL and WHCC determinations (w/w %) among the different additions

(a) Actual additions to minced fillets of cod

	Blank	Kappa carrag.	Lambda carrag.	Iota carrag.	CMC 705	CMC 1505	CMC 2205	CMC 2805
$\overline{100-\text{CDL}}$*	72.6±0.8	77.1±1.9	78.0±0.9	77.6±1.9	73.4±1.6	77.1±1.8	79.6±1.4	85.7±1.5
$\overline{\text{WHCC}}$†	55.3±2.4	61.1±2.4	62.3±1.9	64.0±2.1	56.6±1.0	59.4±1.5	61.9±3.9	67.0±1.5
$(\overline{100-\text{CDL}})-\overline{\text{WHCC}}$‡	17.3	16.0	15.7	13.6	16.8	17.7	17.7	18.7

(b) Kappa and Iota carrageenan were freeze dried before adding to minced fillets of whiting (da Ponte *et al.*, 1985a; 1985b)

	Kappa carrageenan		Iota carrageenan	
	Blank	Addition	Blank	Addition
$\overline{100-\text{CDL}}$*	77.6±3.0	84.4±1.5	74.3±1.3	82.6±1.8
$\overline{\text{WHCC}}$†	59.0±3.4	69.5±1.1	57.8±0.5	74.4±1.5
$(\overline{100-\text{CDL}})-\overline{\text{WHCC}}$‡	18.6	14.9	16.5	8.2

*Average and standard deviation of different measurements of CDL and subtracted from 100% (minced tissue).

†Average and standard deviation of different measurements of WHCC.

‡Fluid expelled extra from the fish cake of the WHCC compared to the CDL.

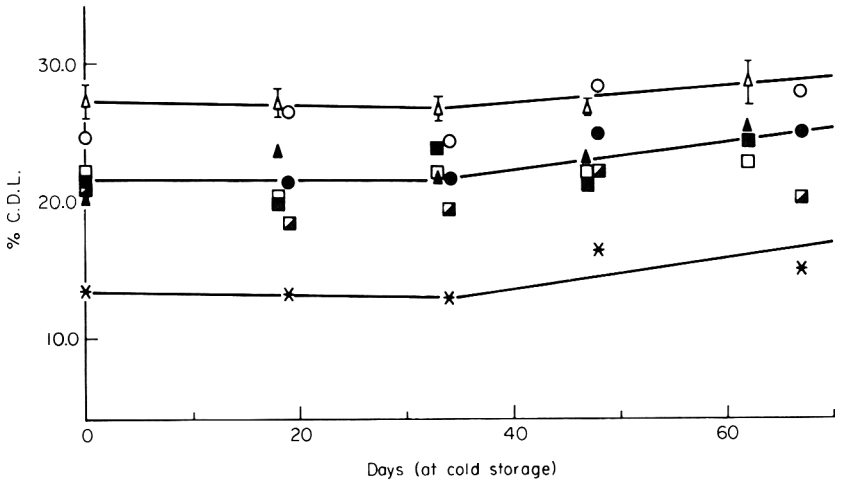

Figure 2. Effect of time and different hydrocolloids on CDL of frozen stored minced fish muscle. (See Fig. 1 for symbols.)

fish fluid against being expelled by the centrifugal force of the WHCC test. The commercial Lambda carrageenan might be a mixture of different types of carrageenan relatively rich in the Lambda type.

Texture analysis

The blank and Kappa carrageenan containing samples became much tougher (compressive strength, CS and shear stress, SS), elastic (modulus of elasticity, ME) and resilient (R) than all the other treated samples during frozen storage (Figs 3, 4, 5 and 6). In the SS determination of the raw minced fish samples, the Kappa carrageenan gave values similar or higher than the blank. It seems that the Kappa carrageenan had no effect on the texture of the raw minced fish and the initial high values of SS of this

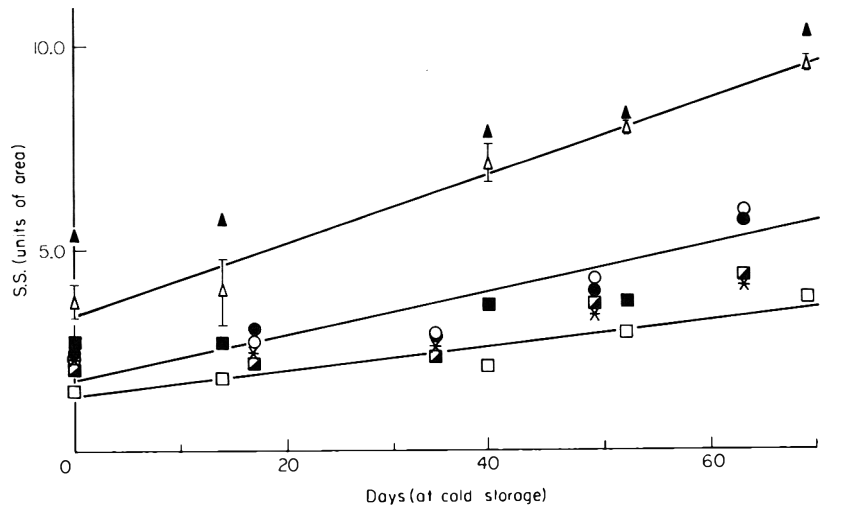

Figure 3. Effect of time and different hydrocolloids on SS of frozen stored minced fish muscle. (See Fig. 1. for symbols.)

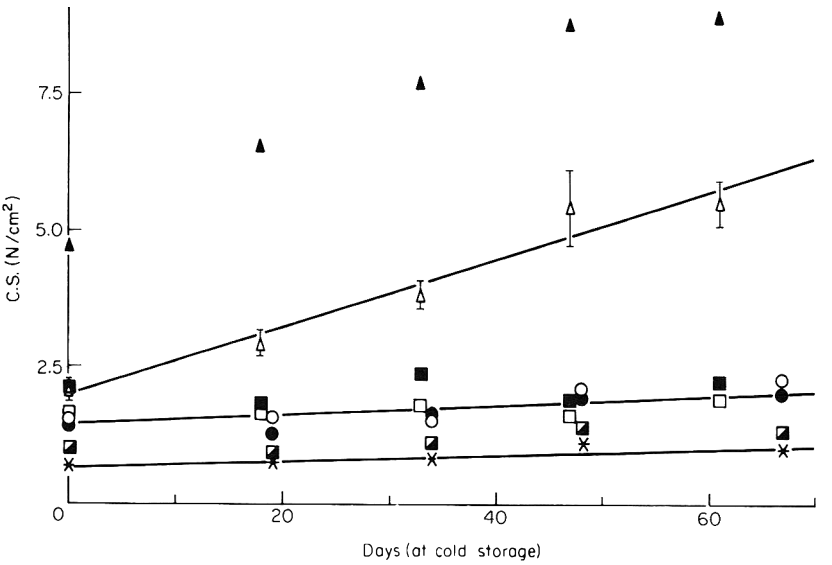

Figure 4. Effect of time and different hydrocolloids on CS of frozen stored minced fish muscle. (See Fig. 1 for symbols.)

treatment were probably due to the heterogenity of the mixture of the hydrocolloid with the minced fish. After heating, the Kappa carrageenan addition showed significantly higher values of CS, ME and R than all the other treatments. This should be attributed to the formation of a rigid gel of Kappa carrageenan separated from the fish cake and additional to the toughening of the blank.

The Iota and Lambda carrageenan additions showed remarkable stability of SS, CS, ME and R during storage. After 10 weeks the values of CS, ME and R of the Iota carrageenan treatment were (on average) similar to the initial values of the blank. The

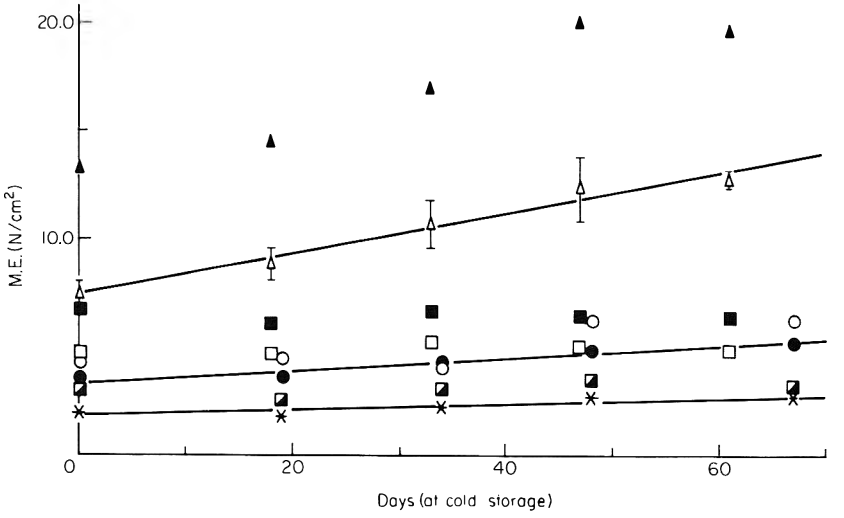

Figure 5. Effect of time and different hydrocolloids on ME of frozen stored minced fish muscle. (See Fig. 1 for symbols.)

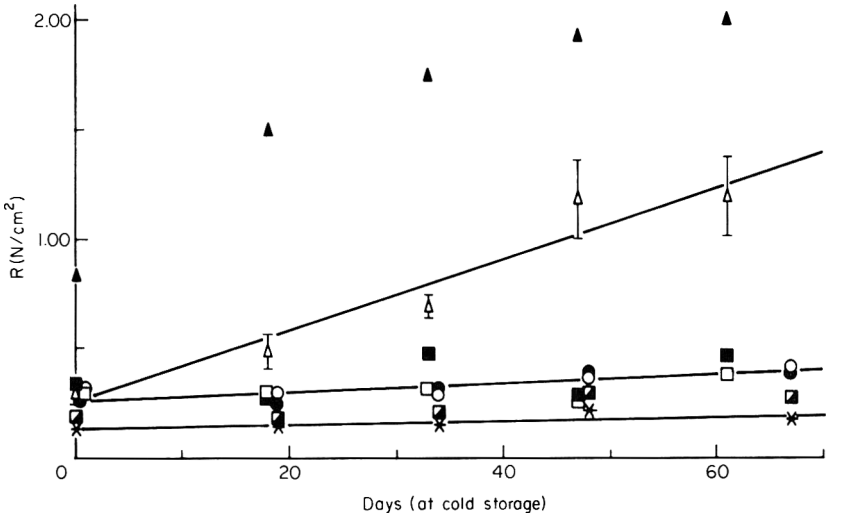

Figure 6. Effect of time and different hydrocolloids on R of frozen stored minced fish muscle. (See Fig. 1 for symbols.)

addition of Lambda carrageenan resulted in somewhat lower values than Iota carrageenan and they were in the range of the CMC treatments.

The effect of the viscosity of the hydrocolloid on the texture of the minced fish can clearly be seen in the CMC additions. With the increase of the viscosity, the minced fish became softer and less elastic. Similar results were obtained in our previous experiments with the additions of alginates and xanthan gum (da Ponte *et al.*, 1985a; 1985b).

Extractable myosin (EM)

With the exception of the Lambda carrageenan addition. all treatments exhibited similar losses of EM during the 10 weeks of frozen storage (Table 2). More experimental data are necessary to get a better understanding of the Lambda carrageenan results.

–le 2. Extractable myosin* (mg/g of fish) of frozen stored minced fish at time intervals

s at =°C	Blank	Kappa-carrag.	Lambda-carrag.	Iota-carrag.	CMC 705	CMC 1505	CMC 2205	CMC 2805
	5.0±0.3	4.3±0.2	4.2±0.2	3.4±0.3	5.4±1.4	6.4±0.1	5.2±0.8	6.9±0.4
	3.2±0.2	2.9±0.7	3.6±0.2	2.9±0.2				
					2.9±0.1	2.8±0.1	2.6±0.1	2.6±0.1
	3.2±0.2	3.5±0.2	4.1±0.1	3.3±0.2				
					3.0±0.2	3.1±0.2	2.9±0.0	2.8±0.1
	2.9±0.3	2.8±0.3	3.2±0.2	2.6±0.3				
					2.5±0.1	2.6±0.3	2.5±0.3	2.1±0.3
	2.1±0.2	1.8±0.2	2.6±0.3	1.8±0.4				
					2.0±0.4	1.9±0.4	1.7±0.1	1.7±0.2

=→ Mean of duplicate samples ± standard deviation.

Dimethylamine (DMA) and Formaldehyde (FA)

During frozen storage there was an increase of DMA and FA content in all treatments (Tables 3 and 4). Kappa carrageenan addition showed apparently the lowest increase while CMC 2805 presented the highest increase.

Table 3. Dimethylamine content* (μg DMA nitrogen/g of fish) of frozen stored minced fish at time intervals

Days at −18°C	Blank	Kappa-carrag.	Lambda-carrag.	Iota-carrag.	CMC 705	CMC 1505	CMC 2205	CMC 2805
0	12.3±0.6	8.1±0.2	10.6±1.1	8.7±0.4	10.3±0.8	9.3±0.8	10.6±0.3	10.7±0.3
20	28.9±4.0	15.8±0.5	24.2±1.3	22.6±1.7	22.0±0.9	16.5±1.1	19.8±1.3	27.6±0.9
40	25.2±1.9	15.1±1.9	28.8±2.0	26.0±2.9	24.9±1.6	18.5±1.5	23.3±6.2	26.5±3.9
53	41.7±1.1	25.3±1.4	45.6±2.0	50.2±1.0	45.3±4.8	31.3±0.3	42.7±6.1	59.6±2.0
68	41.5±1.0	30.5±1.1	64.4±0.1	50.2±2.6	53.2±2.1	36.3±2.0	42.2±3.3	67.9±2.4

*Mean of triplicate samples ± standard deviation.

Table 4. Formaldehyde content* (μg/g of fish) of frozen stored minced fish at time intervals

Days at −18°C	Blank	Kappa-carrag.	Lambda-carrag.	Iota-carrag.	CMC 705	CMC 1505	CMC 2205	CMC 2805
0	6.2±0.3	5.7±0.2	6.5±0.1	5.5±0.1	6.0±0.2	6.2±0.0	9.4±0.0	8.8±0.2
21	13.8±0.6	7.9±0.1	11.1±0.3	12.0±0.3	12.5±0.0	13.9±0.1	21.1±0.4	11.9±0.1
31	19.7±0.8	11.7±0.1	19.3±0.3	18.6±0.3	19.7±0.1	17.0±0.2	24.6±0.4	18.2±0.5
41	22.6±0.8	10.4±0.2	24.4±0.4	20.6±0.8	21.9±1.5	14.5±0.8	20.8±0.9	36.0±1.7
67	32.1±0.4	16.7±2.0	39.3±0.9	24.3±2.0	39.0±0.6	34.6±2.2	52.0±1.1	68.8±0.7

*Mean of duplicate samples ± standard deviation.

Protein, carbohydrate content and viscosity of the supernatants released in WHC determination

The supernatants of all treatments of the WHCR presented a slight decrease of protein nitrogen content in the course of the frozen storage while the protein nitrogen content of the supernatants of the WHCC were almost constant during the same time (Tables 5 and 6).

Table 5. Protein nitrogen content* (mg/ml) of the supernatants obtained from the WHCR at time intervals

Days at −18°C	Blank	Kappa-carrag.	Lambda-carrag.	Iota-carrag.	CMC 705	CMC 1505	CMC 2205
0	10.9±0.5	11.0±0.0	10.6±0.1	10.8±0.2	10.8±0.2	11.5±0.2	n.d.
12	10.6±0.1	10.2±0.3	10.4±0.2	9.8±0.4			
13					10.4±0.5	11.1±0.1	10.9±0.1
26	10.3±0.0	10.5±0.6	9.8±0.2	9.8±0.6			
27					10.5±0.4	11.2±0.5	10.5±0.0
39	9.3±0.1	9.5±0.0	9.7±0.1	9.6±0.5			
49					10.7±0.2	10.6±0.1	10.9±0.3
52	9.3±0.1	9.2±0.0	8.8±0.1	9.2±0.0			
70					10.3±0.1	10.4±0.2	10.7±0.2

*Mean of duplicate samples ± standard deviation.
n.d. = Not determined.

ble 6. Protein nitrogen content* (mg/ml) of the supernatants obtained from the WHCC at time intervals

ys at 8°C	Blank	Kappa-carrag.	Lambda-carrag.	Iota-carrag.	CMC 705	CMC 1505	CMC 2205	CMC 2805
	6.4±0.1	6.1±0.1	6.5±0.1	6.4±0.1	6.7±0.2	6.9±0.2	7.0±0.3	7.2±0.1
	6.5±0.1	6.2±0.3	5.7±0.3	6.3±0.1				
					7.0±0.1	6.8±0.2	6.9±0.0	7.0±0.3
	6.0±0.3	6.1±0.0	6.5±0.3	6.1±0.0				
					6.7±0.1	7.0±0.3	7.1±0.5	7.3±0.5
	6.8±0.2	6.4±0.1	7.0±0.1	6.7±0.1				
					6.9±0.2	6.9±0.2	7.2±0.4	7.5±0.4
	6.6±0.4	6.0±0.2	6.6±0.2	6.3±0.2				
					7.0±0.2	7.2±0.4	7.3±0.3	7.4±0.2

*Mean of duplicate samples ± standard deviation.

The carbohydrate content of the supernatants of the WHCR and WHCC are reported in Tables 7 and 8 respectively. The supernatants of the CMC additions of the WHCR showed a decrease of carbohydrate content with the increase of fluid released in the WHCR (Table 7). The heat treatment of WHCC determination caused a further increase in fluid released which was accompanied by a decrease in the carbohydrate content of the supernatants of the CMC treatments (Table 8). It seemed that competition existed for water between the fish tissue and the hydrocolloid. Whenever the capacity of the fish tissue to hold water decreased, more water became available for the CMC additions.

Table 7. Carbohydrate content (mg/ml) of the supernatants obtained from the WHCR at time intervals

Days at −18°C	Blank*	Kappa-carrag.†	Lambda-carrag.†	Iota-carrag.†	CMC 705	CMC 1505†	CMC 2205†
0	1.4	0.5	4.4	0.3	10.2	10.3	n.d.
12	1.5	0.6	4.0	0.2			
13					9.4	10.1	9.5
26	1.3	0.2	3.1	0.5			
27					8.3	9.7	9.3
39	1.5	0.8	2.8	0.1			
49					7.6	9.2	8.2
52	1.4	0.3	2.6	0.1			
70					7.8	8.6	7.9

*Equivalents of glucose obtained from the colorimetric method of Dubois *et al.* (1956).

†Calculated by subtracting the glucose content of the blank and multiplying with factors which were determined for each carbohydrate (Kappa-carrageenan = 0.67, Lambda-carrageenan = 0.59, Iota carrageenan = 0.58, CMC 705 = 0.58, CMC 1505 = 0.58, CMC 2205 = 0.62).

n.d. = Not determined.

The rheological flow curves of the supernatants of the WHCR and WHCC determinations at zero time are presented in the Figs 7 and 8. The Iota and Kappa carrageenan treatment exhibited supernatants with rheological flow curves similar to the blank and with very low carbohydrate content. The supernatants of the Lambda carrageenan used, Viscarin 402, had intermediate viscosities and carbohydrate

Table 8. Carbohydrate content (mg/ml) of the supernatants obtained from the WHCC at time intervals.

Days at −18°C	Blank*	Kappa-carrag.†	Lambda-carrag.†	Iota carrag.†	CMC 705†	CMC1505†	CMC 2205†	CMC 280
0	1.7	0.3	2.7	0.5	6.8	7.4	7.5	6.8
12	1.8	0.3	2.4	0.2				
13					7.0	7.5	6.3	6.2
26	1.9	0.1	2.8	0.2				
27					6.7	5.8	5.9	6.0
39	2.1	0.6	3.3	0.3				
49					6.7	6.3	6.2	6.9
52	2.0	0.6	2.9	0.1				
70					6.4	6.9	6.7	6.3

*Equivalents of glucose obtained from the colorimetric method of Dubois *et al.* (1956).

†Calculated by subtracting the glucose content of the blank and multiplying with factors which were determined for ea carbohydrate (Kappa-carrageenan = 0.67, Lambda-carrageenan = 0.59, Iota-carrageenan = 0.58, CMC 705 = 0.58, CM 1505 = 0.58, CMC 2205 = 0.62 and CMC 2805 = 0.56).

contents. Probably only the Lambda carrageenan fraction was in solution and the other types of carrageenans present in Viscarin 402 remained in the fish cake as hydrated particles for the raw sample and as separated gel for the cooked sample.

The volumes of supernatants of the WHCR of the CMC 2805 addition are too small

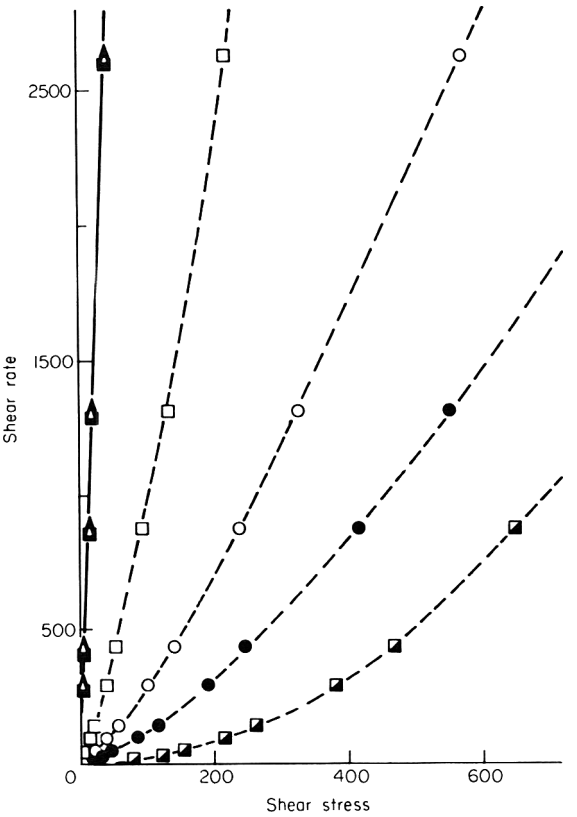

Figure 7. Rheological flow curves of the different supernatants obtained from the WHCR at zero time. (See Fig. 1 for symbols.)

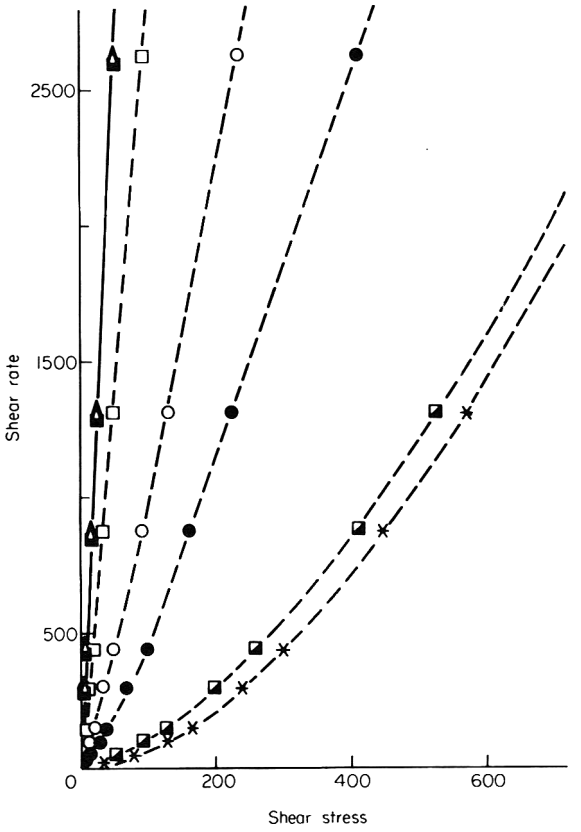

Figure 8. Rheological flow curves of the different supernatants obtained from the WHCC at zero time. (See Fig. 1 for symbols.)

to determine the protein nitrogen and carbohydrate content and the rheological flow curves. The same is true for the supernatant of the WHCR at zero time of CMC 2205 addition, which was only enough for the determination of the rheological flow curve.

Conclusions

Viscosity of the fish fluid influenced the texture and water holding capacity of the minced fillets of cod. Carboxymethyl celluloses with high viscosity made samples softer in texture and higher in water holding capacity.

Iota carrageenan caused remarkable texture stability during frozen storage. Kappa, Iota and Lambda carrageenan significantly improved the WHCC due to the formation of a separated gel, while only Iota and Lambda carrageenan significantly improved the WHCR.

The carboxymethyl cellulose can be blended directly with the minced fish while freeze drying increased the effects of Kappa and Iota carrageenan.

Acknowledgments

We are indebted to the Ministry of Foreign Affairs of The Netherlands for financial

support and to the suppliers of the hydrocolloids. D.J.B. da Ponte is very grateful to the University of Azores for the permission and partial support of his stay at the Department of Food Science of the Agricultural University of Wageningen.

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(Received 18 January 1985)

Journal of Food Technology (1985) 20, 599–613

Compositional differences of black, green and white pepper (*Piper nigrum* L.) oil from three cultivars

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Summary

The composition of volatile oil from black, green and white pepper products of a local Sri Lankan cultivar has been compared with that for the Panniyur and Kuching cultivars introduced from India and Sarawak respectively. The Local Sri Lankan cultivar is rich in β-caryophyllene, a major proportion in sesquiterpene hydrocarbons, and is more suitable for the perfumery industry than are the other two cultivars. The high proportion of oxygenated compounds in the Panniyur cultivar makes an important contribution to the overall odour quality of its pepper oil while the Kuching cultivar, containing a higher monoterpene concentration with a significant content of pinenes, is rich in the top peppery notes. Knowledge of the variation in the composition of pepper oils from different cultivars is important when pepper is used as a component of food flavourings.

Introduction

Pepper (*Piper nigrum* L.) is one of the world's most important spices, used for both its aroma and pungency. The constituents of pepper responsible for its value as a food additive are the steam volatile oils for aroma and non-steam volatile alkaloids for the characteristic pungency (Govindarajan, 1977; Purseglove *et al.*, 1981). Black, green and white pepper are the three different forms of pepper products available in the market, although most of the pepper oil in commerce is distilled from black pepper. Oil production from white pepper is not common, due to the higher cost of the raw material and the lower oil yield. Little information is available for green pepper oil.

Pepper oil is comprised mainly of monoterpene hydrocarbons (70–80%) with smaller amounts of sesquiterpene hydrocarbons (20–30%) which appear to possess the main desirable attributes of pepper flavour. Although the oxygenated terpenes are relatively minor constituents, comprising less than 4%, they contribute to the characteristic odour of pepper oil (Lewis, Nambudiri & Krishnamurthy, 1969a). A study of the aromatic substances present in pepper oil was begun during the last century, but until the advent of gas chromatography and other modern analytical techniques, work on the composition of essential oils of pepper using chemical isolation and derivatization techniques could detect only a few of the major components.

The presence of terpenoids in black pepper oil including terpine hydrate, α-phellandrene and β-caryophyllene were reported over 80 years ago. Later, a vast number of additional substances were identified including those shown in Table 1. Debrauwere &

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Table 1. Constituents identified in black pepper oil

Constituents	Reference
Monoterpene hydrocarbons	
Camphene*	2, 3, 8, 11, 12, 14
Δ^3-Carene	2, 4, 10–12, 14, 15
p-Cymene*	2–4, 8, 10–15
Limonene*	1–4, 10–12, 14
Myrcene*	2–4, 8, 10–12, 14, 15
cis-Ocimene	2, 12
α-Phellandrene*	1–4, 10–12, 14, 15
β-Phellandrene	2–4, 10–12
α-Pinene*	1–4, 8, 10–12, 14
β-Pinene*	1–4, 8, 10–12, 14, 15
Sabinene*	2–4, 8, 10–12
α-Terpinene*	2, 4, 10–12
γ-Terpinene*	2–4, 10–12, 14
Terpinolene*	2, 4, 10–12, 14, 15
α-Thujene	2, 4, 10–12
Sesquiterpene hydrocarbons	
α-cis-Bergamotene	5, 6, 8, 12, 15
α-trans-Bergamotene	5, 6, 8, 12, 15
β-Bisabolene	5, 11, 12, 14, 15
δ-Cadinene	5, 14, 15
γ-Cadinene	14
Calamenene*	5, 14
β-Caryophyllene*	1, 3, 5, 8, 11, 12, 14, 15
α-Copaene*	5, 11, 12, 14, 15
α-Cubebene*	5, 11, 12, 14, 15
β-Cubebene	14, 15
ar-Curcumene	7
δ-Elemene*	5, 14, 15
β-Elemene	5, 11, 12, 14, 15
β-Farnesene*	5, 11, 12, 14
α-Guaiene	14
α-Humulene*	3, 5, 8, 11, 12, 14, 15
Isocaryophyllene	5
γ-Muurolene	5, 12, 14
α-Santalene	5, 12, 15
α-Selinene	5, 8, 11, 14
β-Selinene	5, 8, 11, 12, 14, 15
α-Patchoulene isomer	15
Oxygenated monoterpenes	
Borneol	13
Camphor	13, 15
Cavacrol	13
cis-Carveol	7, 10
trans-Carveol	7, 10
Carvone	7, 10, 13, 15
Carvetonacetone	13
1, 8-Cineole	13
Cryptone	1, 7
p-Cymene-8-ol	7, 10, 13, 15
p-Cymene-8-methyl ether	13
Dihydrocarveol	1

Dihydrocarvone	13
Linalool	7, 10–13, 15
Myrtenal	13
Myrtenol*	13
cis-Sabinene hydrate	9, 10, 15
trans-Sabinene hydrate	9, 10, 15
β-Pinone	13
1-Terpinen-4-ol*	7, 10–13, 15
1-Terpinen-5-ol	13
α-Terpineol	7, 10, 13, 15
Phenyl ethers	
Eugenol	10, 15
Methyl eugenol	7, 10, 15
Myristicin	7, 10
Safrole	7, 10
Anethole	15
Oxygenated sesquiterpenes	
β-Caryophyllene alcohol	13
Caryophyllene ketone	11
Caryophyllene oxide*	13
Epoxy-dihydrocaryophyllene	1
Nerolidol	7, 10
Eudesmol	15
Miscellaneous	
Butyric acid	13
Hexanoic acid	13
Benzoic acid	13
Cinnamic acid	13
Piperonic acid	13
Piperonal	1, 13
Piperidine	1

*Identified in black, white and green pepper oil in the present study.

1, Hasselstrom *et al.* (1957); 2, Ikeda *et al.* (1962); 3, Nigam & Handa (1964); 4, Wrolstad & Jennings (1965); 5, Muller, Creveling & Jennings (1968); 6, Russel *et al.* (1968); 7, Russell & Jennings (1969); 8, Lewis *et al.* (1969a); 9, Russell & Jennings (1970); 10, Richard & Jennings (1971); 11, Richard *et al.* (1971); 12, Russell & Else (1973); 13, Debrauwere & Verzele (1975b); 14, Debrauwere & Verzele (1976); 15, Artem'ev & Mistryukov (1979).

Verzele (1975a) found that black pepper oil is a complex mixture of hydrocarbons, acids, esters, carbonyl compounds, alcohols and oxides. The compositional variability of the volatile oil of black pepper from different cultivars has been examined by several authors (Lewis *et al.*, 1969a, 1969b; Datta *et al.*, 1971; Richard, Russell & Jennings, 1971; Russell & Else, 1973).

In this study we have analysed the composition of black, green and white pepper oils from a local Sri Lankan pepper cultivar and from two cultivars introduced to Sri Lanka, with particular emphasis on the major hydrocarbons.

Materials and methods

Black, green and white pepper samples from a local Sri Lankan cultivar and two cultivars introduced to Sri Lanka (Panniyur and Kuching from India and Sarawak, respectively) and harvested at different maturities (Table 2), were obtained from the Minor Export Crops Research Station, Matale, Sri Lanka. Details of the selection of different berry maturities and the preparation of black, green and white pepper products have been outlined by Rathnawathie & Buckle (1984). Authentic samples of α- and β-pinene, α-phellandrene, ρ-cymene, α- and γ-terpinene, terpinolene, limonene, β-caryophyllene, humulene and β-farnesene were obtained from the Tropical Development and Research Institute, London, U.K. or from Keith Harris & Co. Ltd, Sydney, Australia.

Table 2. Volatile oil content of black, green and white pepper products

Age of berries (months)	Volatile oil content (% v/w dry wt basis)		
	Panniyur	Kuching	Local
Black pepper			
2	2.64	2.34	1.98
3	3.00	2.78	2.64
4	3.57	3.01	2.78
5	3.05	2.91	2.64
6	2.80	2.71	2.44
Green pepper			
4	3.21	3.16	3.08
4.5	3.76	3.53	3.35
5	3.39	2.98	2.89
5.5	2.99	2.81	2.76
White pepper			
5	2.92	2.84	2.53
5.5	2.83	2.73	2.45
6	2.62	2.58	2.25

Analyses

Moisture was determined by Method 30.005 of the Association of Official Analytical Chemists (AOAC, 1980). Volatile oil was determined by the American Spice Trade Association (ASTA) analytical method 5.0 (Anon., 1968). Steam distilled oil samples were stored at 5°C for subsequent gas-liquid chromatographic analysis. All chemicals and solvents were of analytical reagent grade.

The chemical constituents of volatile oils were examined by a Varian Aerograph Model 2100 gas-liquid chromatograph, containing a 4 × 3 mm (i.d.) glass column packed with 10% Carbowax 20 M on Chromosorb WAW 80/100 mesh. The operating parameters were as follows: nitrogen carrier gas flow rate, 50 ml/min; injector temperature, 200°C; flame ionization detector: hydrogen flow rate 30 ml/min, air flow rate 300 ml/min, temperature 250°C; initial column temperature, 60°C, final temperature, 200°C, rate 4°C/min; chart speed, 1 cm/min; sample size, 0.5 μl.

Authentic samples were separately injected together with tridecane (0.5 μl) as internal standard. Calibration graphs were plotted for peak area ratio (peak area of authentic sample/peak area of internal standard) and the volume of authentic sample. All pepper oil samples were injected together with the internal standard. The proportion of major hydrocarbons in the volatile oil was calculated by reference to calibration curves. Where authentic samples were not available, calibration curves for authentic compounds chemically related and/or eluting close to the unknown compound were used.

For confirmation of particular peaks, gas chromatography was conducted with a capillary column on a Schimadzen GC 6-AMP instrument fitted with flame ionization detector, helium carrier gas at 2 ml/min and SCOT-FFAP (70×0.5 mm i.d.) column programmed from 80°C at 2°C/min. For combined gas chromatography–mass spectrometry the gas chromatograph was connected to an AEI-MS12 mass spectrometer through an all-glass straight split. Mass spectra were recorded at 70 ev ionizing voltage with an ion source temperature of 150°C. The spectra were recorded on a VG Digispec Display data system and compared to those in Standard data bases (Heller & Milne, 1978/1980; Stenhagen, Abrahamsson & McLafferty, 1974).

Table 3. Reproducibility of the glc procedure for analysis of constituents in pepper volatile oil*

Peak no.	Mean peak area ratio†	Error‡ (%)
2	0.065	5
3	0.006	16
4	0.107	5
6	0.108	5
7	0.054	7
8	0.032	9
9	0.195	5
10	0.017	12
11	0.022	14
12	0.023	13
17	0.024	13
19	0.025	12
24	0.145	4
26	0.013	15
27	0.013	15
29	0.015	13
30	0.006	16
31	0.006	16
36	0.053	8
40	0.014	14

*Black pepper oil (six months) of the Kuching cultivar.

†Five replicate samples were analysed.

‡Error (%) = $\frac{\text{Maximum deviation of peak area ratio}}{\text{Mean value of peak area ratio}} \times 100$.

Reproducibility

Five replicate samples of black pepper oil (0.5 μl) from berries of the Kuching cultivar at 6 months maturity were injected together with the internal standard (0.5 μl) and analysed using the above mentioned conditions. The error was calculated and the results shown in Table 3.

Results and discussion

Volatile oil content

Results for volatile oil content (Table 2) showed no common trends for the three pepper products analysed. Black pepper of the three cultivars showed a rise in volatile oil content for berries harvested up to 4 months maturation and thereafter the level gradually decreased. Mathai (1981) also observed the accumulation of volatile oil in black pepper of the Panniyur cultivar. He found a steep rise in volatile oil content in berries up to 4.5 months of age and then a gradual increase up to 7.5 months; thereafter the oil content decreased with increased maturity. Though Mathai (1981) examined berries up to 8.5 months of age, under Sri Lankan conditions fully mature black pepper berries are normally obtained 6 months after fruit setting, but the trend of accumulation of volatile oil in black pepper during development showed similar responses in both studies. Mathai (1981) and Rathnawathie & Buckle (1984) found that the sudden rise of starch content in black pepper berries toward the end of maturation was related to the proportionate fall in oleoresin and non-volatile ether extract contents during the same stage of development. Similar observations were noted for the accumulation of volatile oil in black pepper. For green pepper, the volatile oil content reached a maximum level in berries 4.5 months old and decreased with further maturation for all cultivars. Irrespective of the cultivar, green pepper contained the highest level of volatile oil which is proportionately related to the lower level of starch present in green pepper than in the other products (Rathnawathie & Buckle, 1984). The volatile oil content of white pepper decreased with further maturation of all cultivars from 5 to 6 months of age. The outer skin forms about 25% of the dry berry weight of pepper and consists mainly of fibre and some of the essential oil cells (Purseglove *et al.*, 1981). Since the pericarp is removed during the preparation of white pepper, these samples contained lower levels of volatile oil than did black and green pepper products, irrespective of the cultivar.

Of the three cultivars examined, Panniyur showed the highest level of volatile oil in black, green and white pepper products of comparable maturity, while the local Sri Lankan cultivar contained the lowest. The general pattern of accumulation of volatile oil during the maturation of pepper berries of the three cultivars analysed shows a similar response to that of the content of non-volatile ether extract (Rathnawathie & Buckle, 1984).

Constituents in volatile oils

Reproducibility of peak area ratio for the glc analysis of the constituents present in pepper volatile oil varied from 84 to 96% depending on the size of the peak (Table 3). Smaller peaks (with average peak area ratio < 0.025) showed a lower reproducibility (error 12–16%) than did the larger peaks. The error varied for major peaks (Peak area ratio > 0.05) from 4 to 8%.

Typical chromatograms of black pepper oil at 6 months maturity from Panniyur, Kuching and Local cultivars are shown in Fig. 1. The major monoterpenes present in

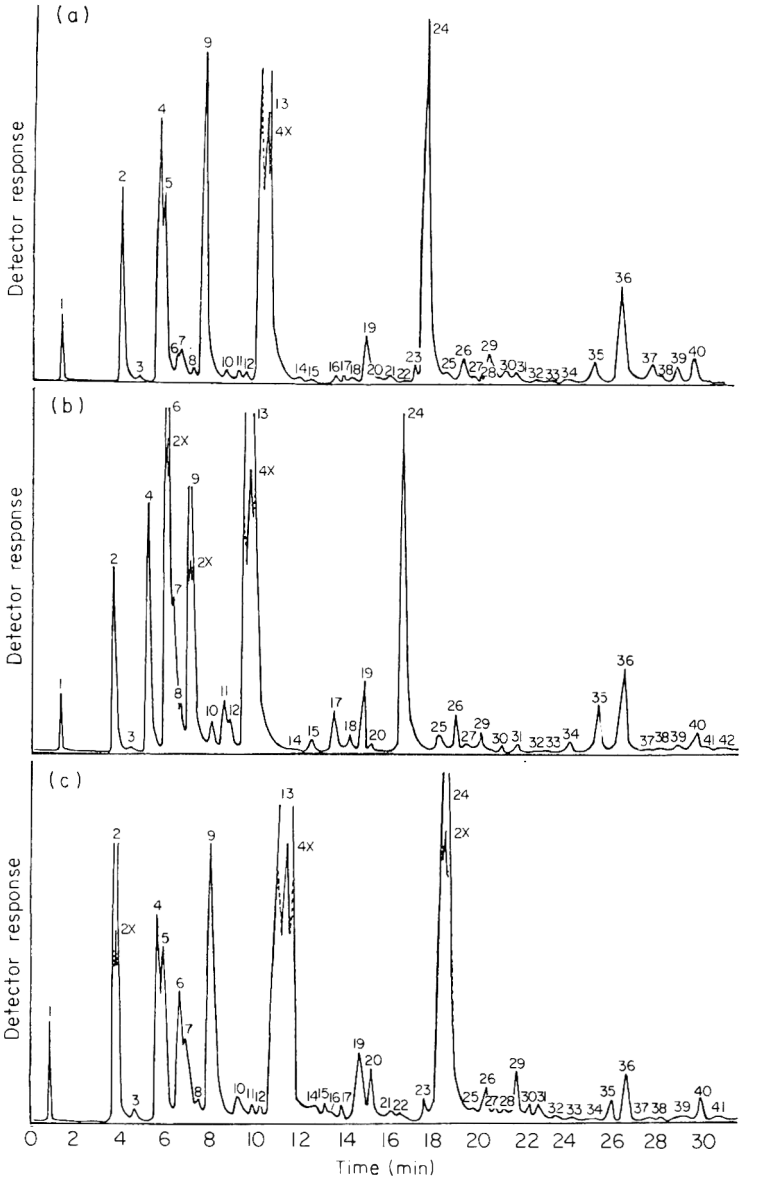

Figure 1. Gas-liquid chromatogram of volatile oil from (a) Panniyur, (b) Kuching and (c) Local cultivars; run on 10% carbowax 20M, programmed from 60° to 200°C at 4°C/min; internal standard: tridecane; peak numbers are described in Table 4.

pepper oil are α- and β-pinene, sabinene, myrcene, α-phellandrene and limonene; the sesquiterpenes are β-caryophyllene, β-farnesene and humulene. A large number of other mono- and sesquiterpenes and oxygenated compounds are also present in small amounts. The wide variation in the level of individual mono- and sesquiterpenes in pepper oil reported by various authors (Table 1) is evidently due to the geographical and varietal variation in the raw material (black pepper) used for distillation. Our study also shows the effect of variety on the composition of pepper oil. In the present study we

have identified eleven monoterpene hydrocarbons, seven sesquiterpene hydrocarbons and five oxygenated compounds in black, green and white pepper oils from Kuching, Panniyur and Local cultivars (Table 4) as detailed in Tables 5, 6 and 7 respectively. The different totals of identified compounds in different samples of each cultivar are derived from the varying levels of unknown fractions, contained in many small peaks which were not identified. The unknown fraction varied from 1.3 to 3.0%, 10.1 to 12.6% and 10.8 to 14.1% for Kuching, Panniyur and Local cultivars, respectively. Among the cultivars analysed, Kuching showed the highest monoterpene hydrocarbon concentration (55.1–67.9%), was devoid of sabinene but contained the highest level of α-phellandrene irrespective of the type of oil.

Table 4. Constituents identified in volatile oils from the Panniyur, Kuching and Local cultivars

Peak no.*	Constituent
2	α-Pinene
3	Camphene
4	β-Pinene
5	Sabinene
6	α-Phellandrene
7	Myrcene
8	α-Terpinene
9	Limonene
10	γ-Terpinene
11	ρ-Cymene
12	Terpinolene
13	Tridecane†
16	α-Cubebene
17	δ-Elemene
19	α-Copaene
23	1-Terpinen-4-ol
24	β-Caryophyllene
26	Pinocarveol
27	Humulene
29	Farnesene
30	Myrtenol
31	Calamenene
36	Caryophyllene oxide
40	Humulene oxide

*See Fig. 1.
†Internal standard.

Of the compounds identified in pepper oils and listed in Table 4, pinocarveol and humulene oxide have not been reported previously in similar products. Both compounds were present only in low concentrations and may have been formed during berry drying and processing, oil extraction or storage.

Kuching cultivar

Of the three cultivars examined, Kuching contained the lowest levels of sesquiterpenes (25.9–32.1%) and oxygenated compounds (4.2–11.6%) and was devoid of α-cubebene and 1-terpinen-4-ol, irrespective of the type of oil. The levels of α- and

β-pinene, limonene and β-caryophyllene in black pepper oil from the Kuching cultivar fluctuated during berry development and showed the lowest and highest levels at 4 and 6 months of age, respectively, although the highest and lowest levels of caryophyllene oxide were found at 4 and 2 months respectively.

The proportion of all components in green pepper oil from the Kuching cultivar fluctuated except for limonene and β-caryophyllene which increased while caryophyllene oxide decreased with increasing berry maturity. In white pepper oil from the Kuching cultivar, the levels of α- and β-pinene, limonene and β-caryophyllene increased, while the levels of myrcene and caryophyllene oxide decreased with increasing maturity. The α-phellandrene content of the Kuching cultivar decreased with increased maturity, irrespective of the type of oil.

Panniyur cultivar

The proportions of α- and β-pinene and β-caryophyllene in black pepper oil from the Panniyur cultivar were similar to the levels of those components in the Kuching cultivar; the highest and lowest levels of caryophyllene oxide were found in berries at 4 and 6 months maturity, respectively, and the highest levels of sabinene and limonene were found at 5 and 6 months maturity, respectively. The proportion of the components present in green pepper oil from the Panniyur cultivar was similar to that found for the Kuching cultivar, except for an increase with increasing maturity in the level of α-pinene and a decrease in the levels of α-copaene and β-farnesene.

The levels of α- and β-pinene, limonene, β-caryophyllene and caryophyllene oxide in the white pepper oil from the Panniyur cultivar were similar to the levels in the Kuching cultivar; the levels of most of the other components decreased with further berry maturity.

Local cultivar

Unlike the Panniyur and Kuching cultivars, the proportions of α-pinene and limonene in black pepper oil from the Local cultivar showed a gradual increase with maturation; but the levels of α-copaene and α-phellandrene were similar to those found in the Panniyur and Kuching cultivars, respectively. The highest and lowest levels of β-caryophyllene were found in the Local pepper berries at 6 and 4 months respectively, as for the other two cultivars. The proportions of most of the other components fluctuated with further berry maturity.

Jansz *et al.* (1984) quantitatively analysed eight components including bisabolene (which was not detected in this study) in black pepper oil of a Sri Lankan cultivar during berry development. They also found similar fluctuations in the levels of β-pinene, sabinene and β-caryophyllene in agreement with the present study.

The levels of most of the components present in green pepper oil from the Local cultivar fluctuated with increased berry maturation; but the proportions of limonene and β-caryophyllene were similar to those of the other two cultivars. In white pepper oil from the Local cultivar, the levels of α-pinene, limonene, α-copaene and β-caryophyllene were similar to the levels in the Kuching and Panniyur cultivars while the β-pinene level in the Local cultivar decreased with increasing berry maturity.

Biochemical transformation of volatile oil constituents

The biosynthesis of terpenes in higher plants is complicated by the morphological and physiological variations in plants as caused by environmental and other factors.

Since the concentration of monoterpenes in all plants increases from the young plant stage through to the flowering period, both in leaves and in flowers, the composition of the terpene mixtures produced also changes (Loomis, 1967; Lawrence, 1973). According to the biogenetic isoprene rule of Ruzicka, Eschenmoser & Heusser (1953) almost all of the monoterpenes are derived from geraniol or its geometric isomer nerol. Geraniol may cyclize via intermediates to α-pinene, β-pinene and limonene. Due to ring opening mechanisms, α-pinene leads to limonene and ocimene, while β-pinene leads to limonene and myrcene (Davies, Giovanelli & Rees, 1964). Limonene which has the typical monocyclic monoterpene structure, can be isomerized to yield α-phellandrene and α-terpinene. As the result of oxidation or reduction reactions the monocyclic terpene limonene is modified to yield ρ-cymene (Bonner, 1950). According to Moore, Columbic & Fisher (1956), autoxidation of α-pinene can give a variety of compounds including trans-carveol; it is possible that pinocarveol found in black pepper oil in this and previous studies may be an artifact, produced during the preparation of the oil.

Table 5. Composition of Kuching cultivar pepper oil (% v/v)

	Age of berries (months)											
	2	3	4	5	6	4	4.5	5	5.5	5	5.5	6
Constituent	Black pepper					Green pepper				White pepper		
Monoterpene hydrocarbons												
α-Pinene	5.7	6.0	5.5	6.1	6.6	6.1	7.3	5.6	6.1	5.6	6.4	7.
Camphene	0.6	0.6	0.6	0.9	0.6	0.6	0.6	0.9	0.6	0.9	0.6	0.
β-Pinene	5.8	6.6	5.4	6.3	7.2	6.2	7.0	7.4	7.1	6.4	6.6	7.
α-Phellandrene	18.2	17.4	16.7	11.9	11.4	14.7	14.2	12.5	11.8	11.5	10.8	10.
Myrcene	6.5	6.5	5.9	6.1	4.8	5.9	6.6	5.9	5.3	6.5	5.3	4.
α-Terpinene	2.8	2.5	1.9	2.4	3.0	2.4	1.4	1.4	2.9	1.7	1.7	2.
Limonene	19.4	17.4	15.9	16.8	20.0	16.4	17.1	18.9	19.1	17.0	17.2	18.
γ-Terpinene	1.9	1.6	1.4	1.7	1.2	1.7	1.6	1.6	1.9	1.7	1.8	1.
ρ-Cymene	2.8	1.6	2.5	3.1	2.7	2.8	2.1	3.1	2.1	1.7	2.0	1.
Terpinolene	4.2	4.1	3.4	2.6	3.4	3.5	3.4	3.8	4.1	2.6	2.8	2.
Total monoterpenes	67.9	64.3	59.2	57.9	61.0	60.3	61.3	61.1	60.0	55.1	55.2	57.
Sesquiterpene hydrocarbons												
δ-Elemene	4.9	5.1	5.6	5.8	4.8	5.7	4.9	4.0	4.4	6.0	5.6	5.
α-Copaene	6.1	7.2	6.5	5.7	4.5	6.7	5.2	4.0	4.4	5.2	5.1	4.
β-Caryophyllene	9.9	10.3	9.5	15.4	15.9	10.0	11.8	16.2	16.3	16.2	17.1	17.
Humulene	1.1	1.1	2.0	1.2	0.8	0.4	0.7	0.6	0.4	2.3	1.2	0.
β-Farnesene	3.9	4.5	2.4	2.4	2.0	3.0	3.0	2.7	2.4	1.8	2.4	2.
Calamenene	tr	tr	tr	0.6	0.6	0.9	0.9	0.9	0.7	0.6	0.7	0.
Total sesquiterpenes	25.9	28.2	26.0	31.1	28.6	26.7	26.5	28.4	28.5	31.8	32.1	31.
Oxygenated compounds												
Pinocarveol	1.3	1.5	2.3	1.1	1.5	2.3	1.9	1.7	1.5	2.3	2.5	1.
Myrtanol	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
Caryophyllene oxide	2.2	3.0	8.4	7.0	6.0	7.9	7.3	6.6	5.9	7.0	6.6	5.
Humulene oxide	0.7	0.9	0.9	1.2	1.2	0.7	0.9	0.9	1.4	1.2	0.9	1.
Total oxygenated compounds	4.2	5.4	11.6	9.3	8.7	10.9	10.1	9.2	8.8	10.5	10.0	7.
Unknown fractions	2.1	2.1	3.1	1.7	1.7	2.1	2.1	1.3	1.7	2.6	2.7	3.
Total of all constituents	97.8	97.9	96.8	98.3	98.3	97.9	97.9	98.7	98.3	97.4	97.3	97.

*tr = Trace.

According to Ruzicka *et al.* (1953) the formation of most sesquiterpenes follow the farnesol rule. Beta-caryophyllene, the major sesquiterpene in pepper oil, is derived from farnesol which also yields humulene. Caryophyllene oxide, a major oxygenated compound, could not be detected in some preparatively isolated pepper oil fractions; it can be rearranged to a bicyclic aldehyde found in previous studies (Govindarajan, 1977).

Effect of processing methods on volatile oil constituents

The level of individual hydrocarbons varied with the type of product, but no common trend was apparent for individual components within and between cultivars. Only berries at 5 months maturity were used for the preparation of each of the three pepper types.

ıble 6. Composition of Panniyur cultivar pepper oil (% v/v)

	Age of berries (months)											
	2	3	4	5	6	4	4.5	5	5.5	5	5.5	6
ɔnstituent	Black pepper					Green pepper				White pepper		
onoterpene hydrocarbons												
Pinene	3.6	4.5	2.7	5.5	8.4	4.1	4.5	6.3	6.4	6.8	7.3	8.4
amphene	tr*	0.3	0.3	tr	0.3	0.3	tr	tr	tr	0.3	0.3	tr
Pinene	8.8	8.7	6.9	7.8	10.1	9.2	9.1	10.6	9.4	9.0	9.2	10.1
ıbinene	5.8	5.5	6.2	7.4	6.2	6.9	7.1	8.5	6.8	10.7	9.3	7.6
Phellandrene	0.9	0.9	0.6	tr	tr	0.8	0.9	0.7	0.7	tr	tr	tr
yrcene	1.2	1.2	1.4	1.2	1.2	1.2	1.2	2.2	2.2	3.6	3.6	2.2
Terpinene	1.4	1.2	0.9	0.9	1.2	1.2	1.2	1.2	1.8	1.1	1.2	1.2
ımonene	12.1	11.9	13.0	18.9	20.6	14.5	17.3	17.6	20.8	19.7	20.4	20.8
Terpinene	0.6	0.5	0.5	0.5	0.6	0.5	0.5	0.6	tr	0.3	tr	tr
-Cymene	tr	tr	tr	tr	tr	tr	0.5	0.5	0.5	0.6	0.5	0.5
erpinolene	0.5	0.3	0.3	0.3	0.5	0.3	0.3	0.5	0.4	0.3	0.4	0.3
otal monoterpenes	34.9	35.0	42.3	42.3	49.1	39.0	42.6	48.6	49.0	52.4	52.2	50.9
esquiterpene hydrocarbons												
-Cubebene	0.3	0.3	0.3	tr	tr	tr	tr	tr	tr	0.3	tr	tr
Elemene	0.6	0.6	0.6	0.3	0.3	0.6	0.5	0.3	0.3	0.3	0.4	0.6
-Copaene	9.1	8.1	8.2	6.7	4.2	8.9	7.7	6.0	4.8	5.0	3.6	4.1
-Caryophyllene	16.4	18.8	10.0	15.9	20.3	10.3	14.3	14.8	20.0	12.4	14.1	19.6
umulene	4.6	2.5	5.0	1.8	1.8	1.8	2.5	1.8	2.5	4.6	3.9	1.8
-Farnesene	7.1	8.0	7.7	4.2	3.0	7.8	5.5	3.4	3.3	4.0	4.3	2.7
alamenene	0.6	tr	1.0	0.7	0.6	1.0	0.6	0.7	tr	tr	tr	tr
otal sesquiterpenes	38.6	38.3	33.2	29.6	30.2	30.4	31.1	27.0	30.9	26.6	27.3	28.8
)xygenated compounds												
-Terpenen-4-ol	1.5	1.5	1.7	2.0	0.9	0.8	1.3	0.6	0.6	0.6	0.6	0.5
inocarveol	1.6	1.4	1.3	1.4	1.3	0.8	1.5	1.5	1.6	1.0	1.0	1.0
lyrtanol	3.0	3.0	2.0	1.0	1.2	2.0	1.0	1.3	1.2	1.2	1.2	1.0
aryophyllene oxide	7.0	6.4	15.2	10.0	5.4	14.2	10.1	7.8	5.8	6.5	6.1	5.4
lumulene oxide	0.9	1.8	1.2	1.5	1.8	0.9	1.1	1.1	0.9	0.9	1.1	1.2
otal oxygenated compounds	14.0	14.1	21.4	15.9	10.6	18.7	15.0	12.3	9.8	10.2	10.3	9.1
Jnknown fraction	12.4	12.6	12.6	12.1	10.1	11.9	11.3	12.1	10.3	10.8	10.3	12.1
otal of all constituents	87.6	87.4	87.4	87.8	89.9	88.1	88.9	87.9	89.7	89.2	89.8	88.8

tr = Trace.

For the Kuching cultivar, green pepper oil contained higher levels of β-pinene, α-phellandrene and limonene than the other two products at 5 months maturity. The levels of α-phellandrene and limonene in black pepper oil were higher than those in white pepper oil at 6 months maturity (Table 5). Green pepper oil at 5.5 months maturity contained a higher proportion of α-phellandrene than did white pepper oil at 6 months maturity.

For the Panniyur cultivar, the highest level of β-caryophyllene was contained in black pepper oil, in which the proportions of α- and β-pinene and sabinene were lower than in the other two products at 5 months maturity. The proportions of α- and β-pinene were similar for black and white pepper products at 6 months maturity (Table 6). The level of β-caryophyllene in green pepper oil at 5.5 months maturity was similar to the level in white pepper oil at 6 months maturity.

Table 7. Composition of Local cultivar pepper oil (% v/v)

	Age of berries (months)											
	2	3	4	5	6	4	4.5	5	5.5	5	5.5	6
Constituent	Black pepper					Green pepper				White pepper		
Monoterpene hydrocarbons												
α-Pinene	9.2	9.3	9.3	10.3	10.8	9.5	9.5	9.4	10.3	9.5	10.1	10.5
Camphene	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.5	0.5	0.6	0.6	0.5
β-Pinene	9.2	7.1	7.1	7.3	7.2	9.1	9.2	9.4	7.3	8.4	8.2	7.3
Sabinene	6.8	6.5	6.5	7.0	5.4	5.9	6.8	6.5	6.3	5.8	5.4	5.3
α-Phellandrene	5.0	4.8	4.8	4.0	3.6	4.8	3.7	4.0	3.5	4.1	3.8	3.4
Myrcene	3.0	3.0	3.0	3.6	2.4	3.0	2.4	2.4	3.0	3.6	2.8	2.4
α-Terpinene	1.4	0.9	0.9	1.1	1.2	1.0	1.1	1.2	1.5	0.8	1.1	1.3
Limonene	8.7	9.9	9.9	12.2	12.6	9.5	9.7	9.9	10.9	10.2	10.9	12.3
γ-Terpinene	1.2	1.0	1.0	1.0	1.2	1.2	1.4	0.6	0.6	1.0	1.1	1.3
ρ-Cymene	tr*	0.3	0.3	tr	tr	0.3	tr	tr	tr	0.3	tr	tr
Terpinolene	0.8	0.6	0.6	0.4	0.5	0.6	0.6	0.6	0.8	0.5	0.6	0.7
Total monoterpenes	44.9	43.9	44.0	47.6	45.5	45.5	45.0	44.5	44.7	44.8	44.6	44.8
Sesquiterpene hydrocarbons												
α-Cubebene	tr	tr	tr	tr	tr	0.3	tr	0.3	0.3	0.6	0.6	0.6
δ-Elemene	2.3	1.8	2.2	1.9	1.8	2.3	2.0	1.9	1.8	1.9	1.9	1.8
α-Copaene	8.9	8.5	7.3	6.1	6.0	7.2	7.3	5.8	5.6	7.2	6.3	5.6
β-Caryophyllene	17.7	19.7	17.6	23.6	26.0	17.6	18.9	22.2	23.2	21.2	22.2	23.
Humulene	0.5	0.5	0.4	0.4	0.5	0.6	0.6	0.7	0.7	0.6	0.7	0.
β-Farnesene	5.4	6.0	5.4	2.4	2.4	6.0	5.4	4.5	2.4	3.9	3.9	2.
Calamenene	0.6	0.6	0.6	0.6	0.6	0.9	1.2	0.7	0.7	0.6	0.7	0.
Total sesquiterpenes	35.4	37.1	33.5	35.0	37.3	34.9	35.4	36.1	34.7	36.0	36.3	34.
Oxygenated compounds												
1-Terpinen-4-ol	1.2	1.3	1.3	0.8	0.8	1.2	1.6	1.0	1.3	1.8	1.8	1.
Pinocarveol	1.2	1.8	2.3	0.9	0.9	1.8	1.1	2.1	1.1	1.0	0.9	1.
Myrtanol	tr	tr	tr	tr	tr	0.6	2.1	0.8	0.9	tr	0.6	0.
Caryophyllene oxide	2.6	3.8	4.8	3.2	3.2	3.0	3.2	2.9	3.0	3.8	2.9	3.
Humulene oxide	0.6	tr	tr	tr	0.6	0.6	0.8	0.8	0.7	0.6	0.7	0.
Total oxygenated compounds	5.6	6.9	8.4	4.9	5.5	7.7	8.8	7.6	7.0	7.2	6.9	7.
Unknown fraction	14.1	12.1	14.1	12.5	11.7	11.9	10.8	11.8	13.6	12.0	12.2	12.
Total of all constituents	85.9	87.9	85.9	87.5	88.3	88.1	89.2	88.2	86.4	88.0	87.8	87.

*tr = Trace.

For the Local cultivar, black pepper oil contained higher levels of α-pinene, limonene and β-caryophyllene than did the other two products at 5 months maturity, and the proportion of β-caryophyllene in black pepper oil was higher than in white pepper oil at 6 months maturity (Table 7). Green pepper oil at 5.5 months contained a higher level of sabinene than did the other two products from berries at 6 months maturity.

The variations in the composition of green pepper oil may be due to the cooking of the berries and/or oven drying at 55°C during preparation. The processing procedures (cooking of berries and removing the pericarp) are also possible sources of the changes in the composition of white pepper oil. Further studies are needed to determine whether the longer ripening period together with the processing procedures result in a marked difference in white pepper oil composition compared with that of the unripe berries harvested for the preparation of black and green pepper oils.

For the three cultivars analysed, information is available only for black pepper oil from matured berries of the Panniyur cultivar, except for the study of Jansz *et al.* (1984). The present observations on black pepper oil from this cultivar are in agreement with the results of Lewis *et al.* (1969a, 1969b), except for higher proportions of sabinene + myrcene and the absence of camphene and humulene in their study. Richard *et al.* (1971) also showed that black pepper oil from the Panniyur cultivar contained low levels of monoterpene hydrocarbons, were almost devoid of phellandrene and α- and γ-terpinene, but contained large amounts of β-pinene and limonene as observed in this study. However, the sesquiterpenes selinene and bisabolene were not detected in the present study. Jansz *et al.* (1984), who also analysed black pepper oils of Sri Lankan cultivars, observed similar levels of particular components as found in the current study, except for Δ^3-carene and β-bisabolene which were not detected in the present study.

The presence in the volatile oil of a high proportion of monoterpenes is important to obtain strong top peppery notes. The proportion of pinenes is related to either of two top notes which are described as refreshing/piney (desirable) and terpentine/eucalyptus (undesirable). The high content of pinenes is related to the 'turpentine-like' off-odour of pepper oils (Lewis *et al.*, 1969b; Govindarajan, Dhanaraj & Narasimhan, 1973). Each product of the Kuching cultivar is rich in the top peppery notes since it has the higher monoterpene concentration with a consequential lower content of pinenes.

The high boiling fraction containing β-caryophyllene and other sesquiterpenes and related polar compounds (Pangborn, Jennings & Noelting, 1970) and the low boiling monoterpene alcohols (Russell *et al.*, 1968) contributes largely to the odour of pepper. The Panniyur and Local cultivars, containing a higher proportion of sesquiterpene hydrocarbons, have strong pepper odours. Caryophyllene is one of the major sesquiterpenes which smells sweet and flowery and is desirable in products for perfumery applications. The Local Sri Lankan cultivar, rich in β-caryophyllene, appears to be more suitable for the perfumery industry than the other two cultivars. The oxygenated compounds appear to be very important in contributing 'body' to pepper odour and are mostly responsible for the characteristic spicy notes (Lewis *et al.*, 1969a, 1969b; Richard *et al.*, 1971). The high proportion of oxygenated compounds in the Panniyur cultivar makes an important contribution to the overall odour quality of pepper oil from this cultivar.

Acknowledgments

The authors wish to thank Dr V.J. Jacob, Senior Advisor UNDP/FAO Project and Dr W.S. Alles, Project Director, Research Station, Department of Minor Export Crops, Matale, Sri Lanka, for the supply of pepper samples. Malinie Rathnawathie thanks FAO, Rome for a postgraduate fellowship.

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(Received 12 June 1984)

Journal of Food Technology (1985) **20**, 615–622

Effects of chilling hot boned meat with solid carbon dioxide

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Summary

The effect of carbon dioxide chilling on drip, appearance and sarcomere length was investigated by cooling hot beef *semitendinosus* muscles from one end with solid carbon dioxide, thus obtaining a range of cooling rates covering carbon dioxide and conventional chilling within single muscles.

Drip ranged from 3 to 12% and correlated with the chilling rate except where freezing and subsequent thawing occurred. Statistically significant differences in appearance were found but are commercially unimportant. Sarcomere length and cooling rate were found to be unrelated but this may be due to limitations in the apparatus used.

Introduction

Using solid carbon dioxide (CO_2) to chill hot boned meat in mobile abbatoirs has been shown to be feasible, economically advantageous and produces meat of good bacteriological quality (Gigiel, 1985). However during the process the meat was subjected to very rapid rates of cooling, which produced steep temperature gradients and partial freezing followed by thawing. These could affect its acceptability, due to increased drip production and changes in colour and texture.

Exudate (drip) is a major cause of weight loss during cutting and storage, and detracts from the appearance of fresh meat displayed for sale (Malton & James, 1983). Taylor & Dant (1971) have shown that rapid chilling produces a two- to three-fold reduction in drip when compared to slow chilling. Since CO_2 cooling is faster than air blast cooling it should reduce the drip potential of meat, but the partial freezing and thawing that occur at the surface during CO_2 chilling will have the opposite effect. Partial freezing of pork during ultra rapid chilling produced a four-fold increase in drip (James, Gigiel & Hudson, 1983).

The development of the bright red colour which gives fresh meat its desired appearance is due to the oxidation of myoglobin to oxymyoglobin (MacDougall, 1972) the rate being affected by the concentration of oxygen and the temperature. If the concentration of oxygen is reduced by the sublimation of CO_2 the rate of oxidation will be reduced. Brooks (1933) and Haines (1933) showed that high levels of CO_2 delayed colour development but the normal process was resumed once concentrations fell below 20%. Our earlier work on CO_2 chilling (Gigiel, 1985) showed that the concentration of CO_2 in the boxes of meat decayed to 60% during the first 2 hr after packing and, assuming an exponential rate of decay, would be below 20% after 8 hr. Furthermore, as the direct effect of CO_2 is limited to the surface layers of the meat, it is unlikely that there will be any effect on the subsequent colour development of the inner tissues.

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On the other hand, large variations in cooling rate during rigor, as found in CO_2 chilling, could have an effect on the stability and uniformity of the appearance, and particularly the reflectance, of the subsequently cut meat during retail display. MacDougall (1982) reported very large changes in lightness in beef chilled by traditional methods, attributed to changes in protein structure brought about by differences in cooling rate.

Marsh & Leet (1966) showed that increased toughness in cooked meat could result from cold shortening, induced by rapid pre-rigor cooling, and that the effect was localized if large temperature gradients occurred in the meat. It was therefore likely that some regions of hot boned CO_2 chilled meat would show considerable shortening, which should be indicated by substantial variation in sarcomere length.

The large scale method used in the feasibility trials for chilling 25 kg boxes of meat was not suitable for experimentally quantifying the effect of CO_2 chilling on drip potential, appearance and sarcomere length, because of the large quantity of meat that would be needed to take into account variation between animals and muscles. This paper describes a method which overcame this problem by producing a complete range of chilling rates in individual excised semitendinosus muscles, cooled from one end using pellets of solid CO_2.

Materials and methods

Four semitendinosus muscles were removed hot at 30 min post-mortem from two Friesian × Hereford heifers, approximately 18 months old, slaughtered conventionally. Each muscle was trimmed of excess fat tissue and inserted into an 80 mm diameter acrylic tube, 234 mm long, and the ends cut off square. Samples were removed from the trimmed ends of the muscle at 1 hr post-mortem and the pH measured using the method described by Gigiel (1985). The cylinder was insulated along its length and at one end with 13 mm of flexible, closed cell insulation (Armaflex) and 150 mm of expanded polyurethene foam and placed vertically in a room at 0°C. Ten copper constantan thermocouples were then inserted into each muscle. Each thermocouple was fixed to a separate nylon cord, coated with epoxy resin and positioned by threading the cord through a large needle and drawing it through pairs of holes in the tube, perpendicular to the cylinder axis, until the thermocouple lay on that axis. A section through the apparatus is shown in Fig. 1. Solid CO_2 pellets equivalent to 25% of the muscle weight were placed on the open end of the cylinder under an insulated cover and the cylinder left for 48 hr for the muscle to cool and equalize in temperature. Temperatures were recorded to ± 0.5°C at 0.5 hr intervals using a Solartron data logger.

After equalization the meat was removed from the apparatus in a room at 0°C and cut into fifteen slices (approximately 15 mm thick). Each slice was closely covered with an oxygen permeable polyethylene film and exposed to air for 1 hr at 0°C. The colour was then measured at three positions on each face (except the face on the first slice which had been in contact with the CO_2 using a Hunterlab colour difference meter (Taylor & MacDougall, 1972) and the reflectance measured at 3 positions within each slice using the Meat Research Instiute Fibre Optic Probe (FOP) (MacDougall & Jones, 1975).

A 10 mm diameter sample was then removed from the centre of each slice with a cork borer. Fibres were teased from the sample and the sarcomere length measured using the laser diffraction technique described by Voyle (1971). The pH of the remainder of the sample was measured using the method described by Gigiel (1985).

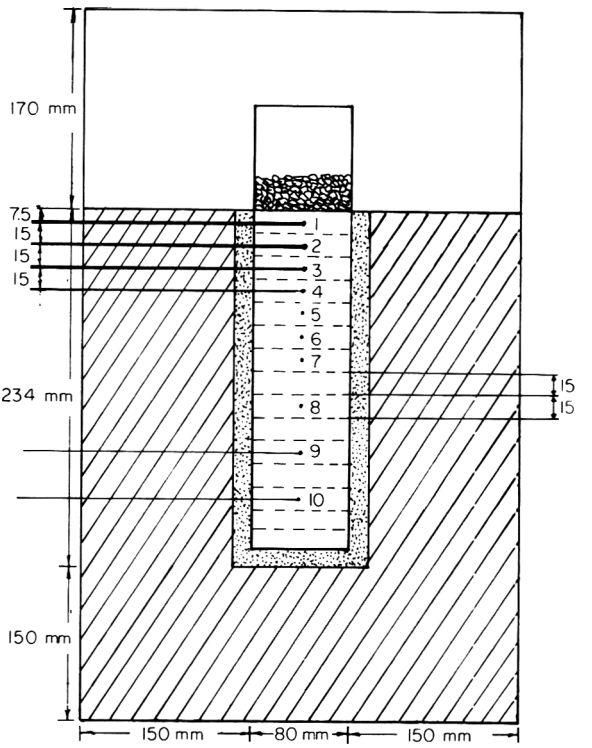

Figure 1. Section through the apparatus used to chill cylinders of meat from one end with solid CO_2.

Drip measurements were then carried out on the remaining annulus of muscle, using the method described by Taylor (1982), over 5 days storage at 0°C, weighing being carried out to an accuracy of ±0.01 g. The colour and reflectance of each slice were then measured again as described above.

Results

A graph of the temperature at different positions along the length of a typical meat cylinder during chilling and temperature equalization is shown in Fig. 2. The temperature 7.5 mm from the CO_2/meat interface fell very rapidly to below 0°C, reaching a minimum of −15°C 4 hr after the start of chilling. It then rose as heat continued to flow from the warmer, more distant parts of the cylinder, until the temperatures equalized at 0°C. Further from the CO_2/meat interface the temperature fell more gradually and to higher minimum, meat at distances greater than 52.5 mm remaining unfrozen. The average time for the temperature at each position to fall to 7°C is shown in Fig. 3, temperatures for positions which did not coincide with a thermocouple being estimated by interpolation.

The drip loss from each slice over 5 days was expressed as a percentage of the original slice weight and the average values were plotted against position in the cylinder in Fig. 4. An analysis of variance showed that position along the cylinder was highly significant ($P < 0.001$). Drip was a minimum of 3% in the slices 82.5 mm from the CO_2/meat interface and increased towards the CO_2 with a maximum of 12% at

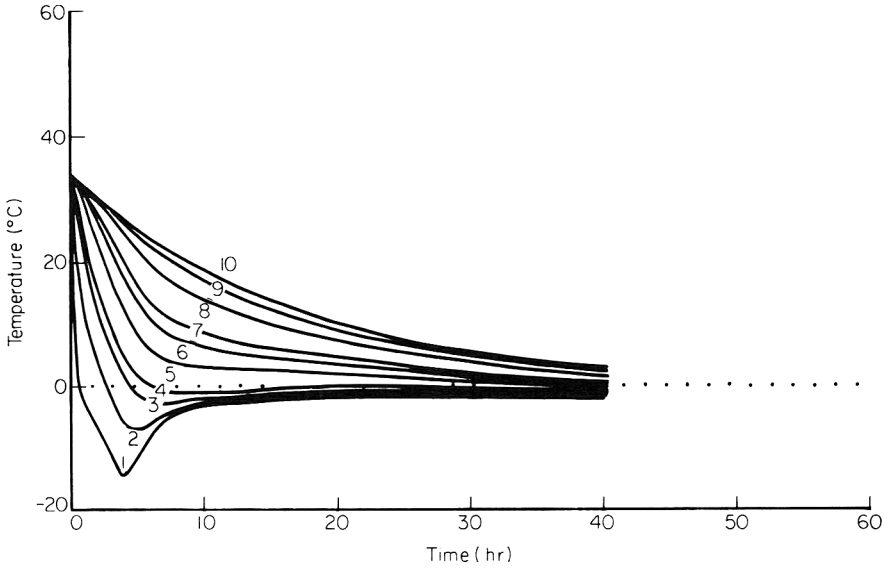

Figure 2. Temperatures at different positions along the length of a typical meat cylinder during chilling and temperature equalization. Numbers refer to thermocouple positions in Fig. 1.

22.5 mm. Beyond 82.5 mm drip increased linearly with position to a maximum of 6%, 217.5 mm from the interface with a high linear correlation ($r = 0.92$) between drip loss and chilling time to 7°C.

The results for the colour measurements were expressed in units of lightness, hue and saturation and for the reflectance in FOP units (Table 1). Four separate analyses of

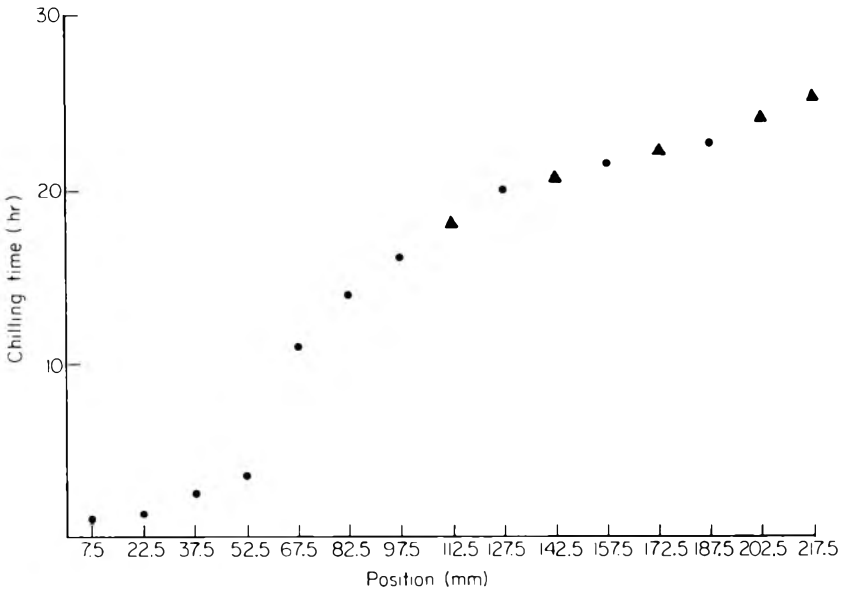

Figure 3. The average time for the temperature to fall to 7°C at each position in the meat cylinder when cooled from one end with solid CO_2. ● Actual temperature readings. ▲ interpolated values.

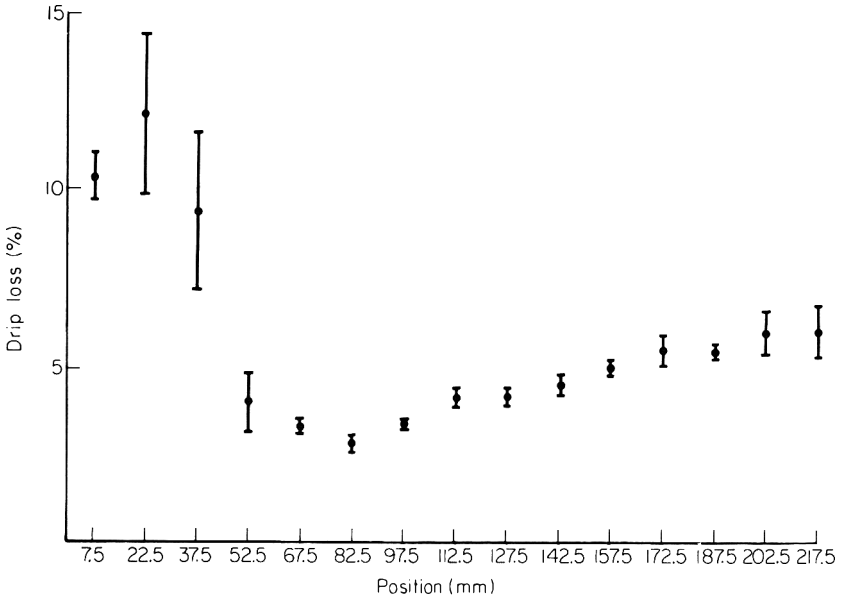

Figure 4. The average drip loss after 5 days at each position in the meat cylinder. Error bars represent ± 1 s.e.m.

variance showed that there were significant differences ($P < 0.05$) between animals which, when removed, left small significant differences ($P < 0.05$) due to position along the meat cylinder. The overall range of the values was small and subjectively would only be noticed in a careful side by side comparison between the two extremes of the range.

Table 1. The mean lightness, hue, saturation and fibre optic probe value at 2 and 7 days post-mortem and sarcomere length and pH at 2 days post-mortem at different positions in a meat cylinder, chilled from one end with solid CO_2

Position (mm from interface)	Lightness		Hue		Saturation		FOP		Sarcomere length	pH
	2	7	2	7	2	7	2	7	2	2
7.5	29.03	31.66	29.37	32.80	18.57	18.24	27.3	29.1	2.23	5.60
22.5	29.52	32.64	30.23	32.81	18.11	19.68	26.0	31.1	2.59	5.52
37.5	29.72	32.70	31.23	32.37	17.56	19.88	26.5	32.8	2.50	5.53
52.5	29.81	33.15	29.22	32.81	17.11	20.66	28.6	33.6	2.33	5.53
67.5	31.01	32.82	30.37	33.34	17.37	21.21	29.7	34.1	2.31	5.55
82.5	31.63	33.29	31.09	33.34	18.63	21.99	29.9	33.4	2.34	5.52
97.5	31.92	33.85	32.66	31.09	17.55	22.56	29.9	34.6	2.43	5.52
112.5	32.22	31.72	30.95	33.57	19.26	23.05	30.9	33.7	2.41	5.50
127.5	31.54	33.63	30.66	35.06	19.52	22.68	30.3	34.0	2.54	5.49
142.5	31.54	32.83	30.08	30.08	19.17	23.72	30.0	32.7	2.62	5.50
157.5	32.05	33.35	30.94	32.05	18.78	23.50	29.6	32.7	2.63	5.49
172.5	31.38	33.68	29.94	22.76	18.79	23.81	29.6	31.9	2.66	5.52
187.5	31.03	33.80	30.37	32.19	19.10	22.99	29.2	32.4	2.54	5.50
202.5	31.91	34.03	30.37	30.18	20.34	24.36	28.7	33.2	2.36	5.47
217.5	31.94	34.55	30.37	30.18	19.30	22.15	30.0	33.5	2.18	5.48

The faces of the first slices which had been in contact with the CO_2 were very dark and dry and were not included in the above analysis.

The average sarcomere lengths are shown in Table 1 and plotted against position in Fig. 5. An analysis of variance showed that differences between muscles was significant and much larger than the significant differences due to position. The range in sarcomere length was small, from 2.18 to 2.66 μm, the shortest being from the ends of the cylinder with a secondary minimum occurring at 97.5 mm from the CO_2/meat interface.

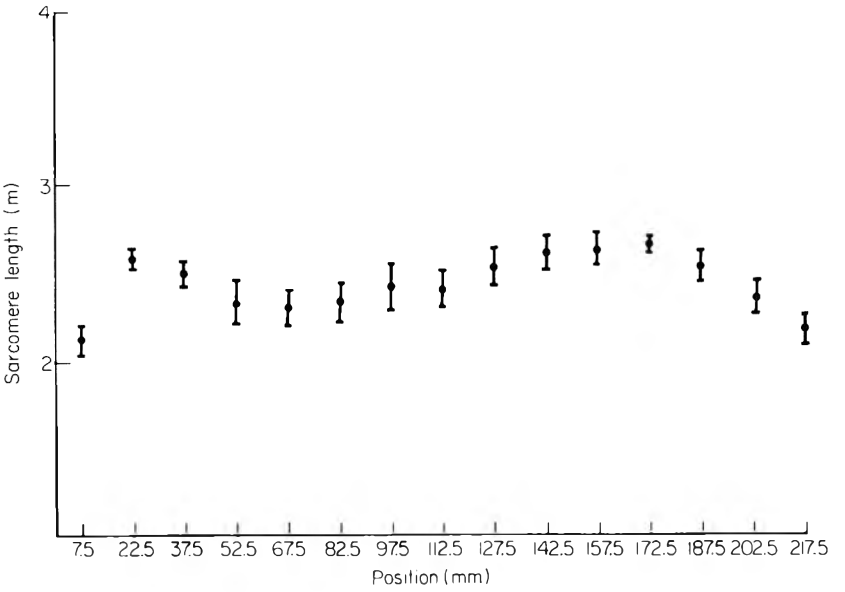

Figure 5. The average sarcomere length of slices at different positions along the meat cylinder. Error bars represent ± 1 s.e.m.

pH values for each muscle at 1 hr post-mortem ranged from 6.7 to 6.9 and values for each slice at 2 days post-mortem are shown in Table 1. An analysis of variance showed no significant difference ($p < 0.05$) between muscles or slices.

Discussion

In this investigation the range of cooling rates which occur during CO_2 chilling of hot boned meat and in conventional systems was achieved within single muscles so that the effect on drip, appearance and sarcomere length could be evaluated without large interactions due to muscle and animal differences. In earlier commercial trials approximately 12% of the CO_2 chilled boxed meat froze and thawed during the process and this is represented in the present work by the first 60 mm of the muscle cylinder. The remainder of the boxed meat reached 7°C within 15 hr, without freezing, corresponding to the layer from 60 to 90 mm in the cylinder. Meat in the conventionally chilled control carcasses did not freeze and took between 5 and 22 hr to fall to 7°C; this is represented in the present work by slices between 60 and 210 mm from the CO_2/meat interface.

The drip measurements in this experiment can be used to explain why there was no significant difference in drip loss between the treatments during the commercial CO_2

chilling trials (Gigiel, 1985). The small proportion of CO_2 chilled meat which froze during the trials would have lost approximately 10% drip, if it had been sliced, whilst the bulk of the meat which did not freeze, would have lost only 3%, giving an overall average of 3.7%. The conventionally chilled meat would have lost from 3 to 5%, if it had been sliced, depending upon its position in the carcass. Therefore the overall average drip loss in each process would have been similar.

The actual drip loss in the commercial trials was less than these predicted values, because the ratio of cut surface area to volume was very large in the slices used in the experimental determinations, but small in the large pieces of meat packed in the commercial boxes.

Although no overall difference in drip was found between CO_2 and conventionally chilled meat, selective cuts from meat close to the CO_2 during chilling will produce excessive drip compared to the remainder. Since uniformity is important in quality judgments, such sampling may result in whole batches being downgraded.

The statistically significant differences in appearance which were found along the length of the cylinder were small and less than differences between animals. There is therefore no important difference in appearance between meat produced using the CO_2 process and conventionally chilled meat, except for the drying which occurred at the CO_2/meat interface. This was due to the surface remaining uncovered for 2 days during temperature equalization in the experimental situation and was not noticed in the earlier work in wrapped and boxed meat (Gigiel, 1985).

The lack of relationship between sarcomere length and chilling rate was unexpected. It indicates no abnormal contraction and infers that meat texture was not adversely affected by the rapid chilling rate. One possible explanation is that the radical constraint exerted on the muscle by the acrylic cylinder prevented the increase in diameter which must occur if shortening takes place. Thus, although the method used in this experiment was successful in limiting muscle and animal variation when quantifying drip potential and appearance, it would appear to be unsuitable for investigating the possible occurrence of cold shortening and thaw-rigor. Assessment of these requires an alternative method which does not impose any radical constraint upon the muscle.

The results of this experiment are not only applicable to the CO_2 chilling process. They can be used to assess quality differences in beef subjected to different time-temperature histories during chilling. In particular the results showing a linear relationship between drip and chilling time to 7°C can be used to quantify differences in drip potential between slow and fast chilling using conventional refrigeration.

Using CO_2 to chill hot boned muscle has considerable potential in the recovery of meat from animals in remote areas, and a commercial project for such applications is currently being evaluated. Of the quality attributes measured in this paper, drip is considered to be the most important since it has a direct bearing on the economics of the process, while colour and texture are considered unimportant for the particular market for which the meat is intended. This paper has shown that, although the amount of drip from CO_2 chilled meat from different parts of one box may differ considerably, there is no overall difference in drip between CO_2 and conventionally chilled meat.

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(Received 17 November 1984)

Journal of Food Technology (1985) **20**, 623–629

Utilization of Fick's second law for the evaluation of diffusion coefficients in food processes controlled by internal diffusion

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Summary

Several processes in the food industry, such as leaching and drying are usually controlled by internal diffusion. In many cases these processes have been interpreted on the basis of constant diffusion coefficients calculated through a series solution of Fick's second law which converge rapidly for large values of time. This work presents a numerical solution of Fick's second law for various types of concentration dependence of the diffusion coefficient. The results indicate that the above mentioned conventional treatment of experimental data, may not be sufficient to establish whether or not the process is characterized by a constant or variable diffusion coefficient.

Introduction

Several processes in the food industry depend upon internal diffusion; among them drying and leaching. Drying of foods is usually controlled by internal diffusion of water (Van Arsdel & Copley, 1963; King, 1968). Leaching, which is the transfer of solutes from a solid to an adjacent fluid, may be also controlled by internal diffusion of solutes (Schwartzberg, 1975; Schwartzberg & Chao, 1982). Extraction of sugar, vegetable oils and coffee, and desalting of pickles, are examples of the application of leaching in the food area.

Several works have been reported in the literature on the determination of solute(s) or water diffusivities during the above processes, and solutions to Fick's second law have been frequently used to determine such diffusivities. Generally, the diffusion coefficients have been reported based on data interpreted by solutions to the diffusion equation assuming a constant diffusion coefficient. Useful solutions of Fick's second law for this situation include series solutions which converge rapidly for large values of time. This is best illustrated by considering diffusion out of spheres or an infinite slab. Solution of Fick's second law for constant diffusivity and constant boundary conditions is for the first case (spheres):

$$c^{\#} = \sum_{n=1}^{\infty} \frac{6}{(n\pi)^2} \exp\left(-(n\pi)^2 \frac{D\theta}{R^2}\right) \tag{1}$$

where the dimensionless concentration, $c^{\#} = (\bar{c} - c_\infty) \div (c_0 - c_\infty)$, and $\bar{c}$ is the average concentration of the substance (i.e. water or solute) diffusing within the food. For values of $D\theta/R^2$ ($=F_0$) larger than 0.1, the first term of the series solution can usually be

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used with little error (Schwartzberg, 1975). Consequently, when the logarithm of c^* is plotted against time, a straight line should be obtained and the diffusivity (solute or water) can be obtained from the slope of that line. For an infinite slab solution of Fick's second law becomes:

$$c^* = \sum_{n=1}^{\infty} \frac{8}{\pi^2(2n+1)^2} \exp\left[-(2n+1)^2 \frac{\pi^2 D\theta}{4R^2}\right] \tag{2}$$

For values of $D\theta/R^2 > 0.1$ equation (2) reduces to the first term of the series solution. Consequently, and as mentioned for spheres, when the logarithm of c^* is plotted against time, a straight line should be also obtained and the diffusivity can be obtained from the slope of the line.

This approach, either for infinite slab or spheres, has been utilized by a large number of workers to calculate diffusivities from experimental drying data (Saravacos & Charm, 1962; Jason, 1965; Del Valle & Nickerson, 1968; Holdsworth, 1971; Pitkin & Carstensen, 1973; Vaccarezza, Lombardi & Chirife, 1974; Palumbo *et al.*, 1977; Mazza & Le Maguer, 1980; as well as many others); or from leaching experiments (Beverloo, Leniger & Weldring, 1962; Pflug, Fellers & Gurevitz, 1967; Schwartzberg, 1975; Schwartzberg & Chao, 1982). In many cases experimental data adhered fairly well to the linearity predicted by the analytical solution of Fick's second law for large values of time. Consequently, this was interpreted as constancy of the diffusion coefficient.

Solute or water diffusion in solid foods during leaching or drying are recognized as complex phenomena. They are influenced by structural considerations, shrinkage and interactions which may be expected to deviate the diffusion behaviour from that predicted by formal solutions of the differential equations for diffusion in solids. For instance, constancy of diffusivities should be rare and some sort of 'concentration dependent' diffusivities should arise in most practical situations. However, and as mentioned before, this was not always observed; on the contrary, a large number of drying and leaching data exist that suggest a constant diffusion coefficient when analysed via the asymptotic solutions of Fick's second law.

The objective of the present work is to analyse the solution of Fick's second law regarding its application for evaluating diffusivities in internal diffusion controlled processes.

Results and discussion

Nomenclature

c = concentration;
$\bar{c}$ = average concentration;
c_∞ = equilibrium concentration;
c_0 = initial concentration;
c^* = $(c-c_\infty)/(c_0-c_\infty)$; dimensionless;
$c^{\#}$ = $(\bar{c}-c_\infty)/(c_0-c_\infty)$; dimensionless;
D = diffusion coefficient;
D_0 = initial diffusion coefficient (at $\theta = 0$);
D^* = $D(c^*)/D_0$; dimensionless;
$D_s^{\#*}$ = diffusion coefficient evaluated from the slope of logarithm $c^{\#}$ *versus* F_0+
F_0 = Fourier number, = $D\theta/R^2$;

$k_1; k_2$ = constants;
R = sphere radius or slab half thickness;
r = space coordinate; dimensionless;
D^{**} = diffusion coefficient; calculated through equation (7) or equation (10)
ρ = space coordinate;
θ = time.

Mathematical solution of Fick's second law with diffusion coefficient varying with concentration

Fick's second law which can be used to analyse unsteady state diffusion in solids is:

$$\frac{\partial c}{\partial \theta} = -\nabla[D(c)\nabla c] \tag{3}$$

In spherical coordinates equation (3) becomes (in non-dimensional form)

$$\frac{\partial c^*}{\partial F_0} = \frac{1}{r^2}\frac{\partial}{\partial r}\left[D^* r^2 \frac{\partial c^*}{\partial r}\right] \tag{4}$$

and for infinite slab

$$\frac{\partial c^*}{\partial F_0} = \frac{\partial}{\partial r}\left[D^* \frac{\partial c^*}{\partial r}\right] \tag{5}$$

where the following dimensionless variables were introduced,

$$F_0 = \frac{D_0\theta}{R^2}$$

$$r = \frac{\rho}{R}$$

$$c^* = \frac{c - c_x}{c_0 - c_x}$$

$$D^* = \frac{D(c^*)}{D_0}.$$

The boundary conditions are as follows:

(i) at the centre: $F_0 > 0$, $r = 0$, $\frac{\partial c^*}{\partial r} = 0$;

(ii) at the surface; $F_0 > 0, r = 1; c^* = 0$;

(iii) at the start; $F_0 = 0, 0 \leq r \leq 1; c^* = 1$.

In order to solve equations (4) and (5) three types of concentration dependence of the diffusion coefficient were arbitrarily selected:

exponential; $D^* = \exp[k_1(c^{*k_2} - 1)]$ (6),

potential; $D^* = (1 - k_1)c^{*k_2} = k_1$ (7),

hyperbolic; $D^* = (1 - k_1)/(1 - k_1 c^{*k_2})$ (8).

Values of k_1 were selected to obey the following conditions,

when $c^* = 1$; $D^* = 1$;

when $c^* \to 0$; $D^* = 10^{-3}$.

For the case of diffusion in the sphere values of k_2 were taken as 0.1, 0.5 and 0.001 for the exponential, potential and hyperbolic law, respectively; for the infinite slab values taken were 0.5 and 0.1 (exponential); 1 and 0.5 (potential) and 0.01 and 0.001 (hyperbolic). Figure 1 illustrates the type and magnitude of the concentration dependence of the diffusion coefficient, as described by equations (6)–(8) with numerical values of k_2 used for the spherical case.

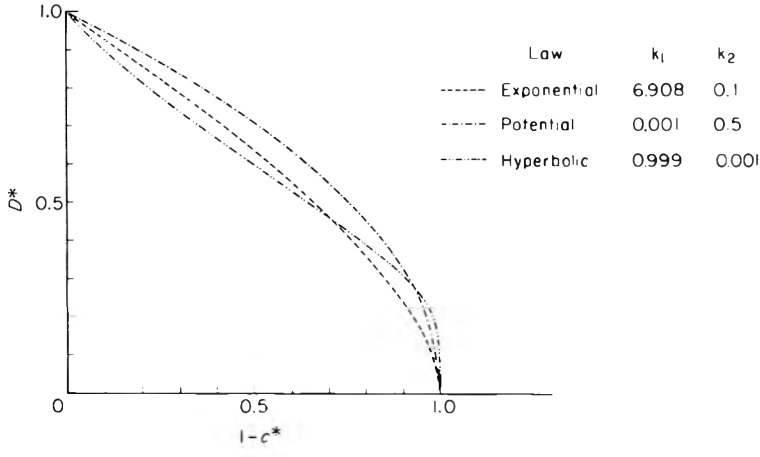

Figure 1. The various forms of concentration dependence of diffusion coefficient used in the numerical solution of Fick's second law.

Equations (4) and (5) were numerically solved using a Crank–Nicolson implicit method (Crank & Nicolson, 1947). The results were plotted in terms of dimensionless average concentration (c^*) *versus* the F_0 number for the various concentration dependences of the diffusion coefficient; this is shown in Figs 2, 3 and 4. For the purposes of comparison the analytical solution of equations (4) and (5) with constant diffusivity (equations (1) and (2)) are also plotted in each figure. It can be observed that the plot of logarithm c^* *versus* F_0 for the numerical solutions of equations (4) and (5) follows a 'straight' line behaviour with little error, i.e. as if the data followed the analytical solutions of Fick's law for constant diffusivity. Table 1 shows the correlation coefficients of the 'apparent' straight lines, the corresponding intercepts and the values of a hypothetical 'constant' diffusion coeffiient computed from the slopes of the correlated straight lines. For the purposes of comparison the same table shows values of the diffusion coefficients corresponding to the various concentration dependencies considered, calculated as follows:

$$D^{+*} = \frac{\int_0^1 D^* c^* r^2 \, dr}{\int_0^1 c^* r^2 \, dr} \quad \text{for the sphere} \tag{9},$$

$$D^{+*} = \frac{\int_0^1 D^* c^* \, dr}{\int_0^1 c^* \, dr} \quad \text{for the slab} \tag{10}.$$

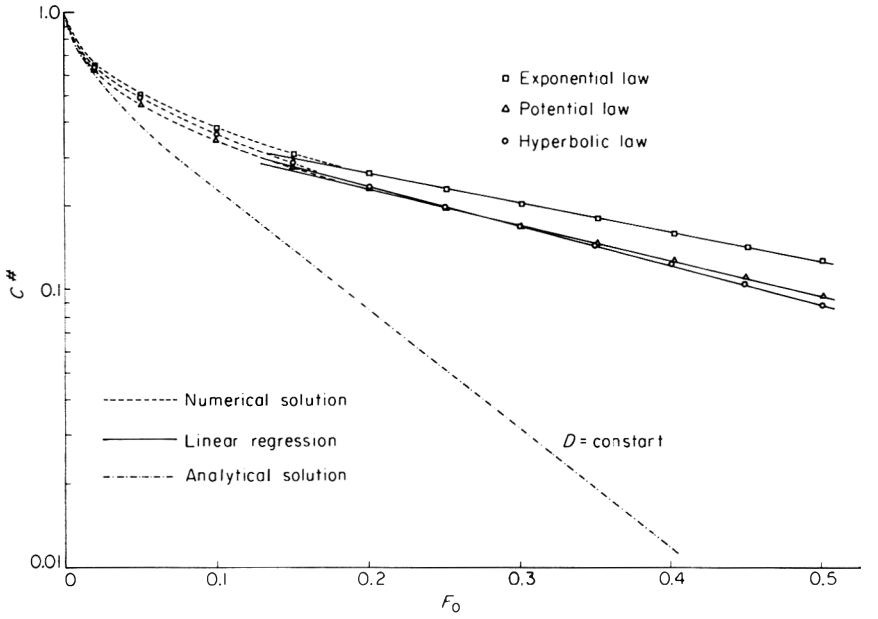

Figure 2. Comparison of the analytical solution of equation (1) with numerical solutions of equation (4) allowing for the variation of the diffusion coefficient with concentration.

These coefficients were evaluated at $F_0 = 0.15$ and $F_0 = 0.50$ for the sphere, and at $F_0 = 0.2$ and $F_0 = 0.9$ for the slab (arbitrary end points of the straight lines selected for this study). There are various comments which can be made about the results shown in Table 1; as follows. Correlation coefficients for the 'straight' line periods are high (above 0.99) despite the fact that the diffusion coefficient was allowed to decrease by a

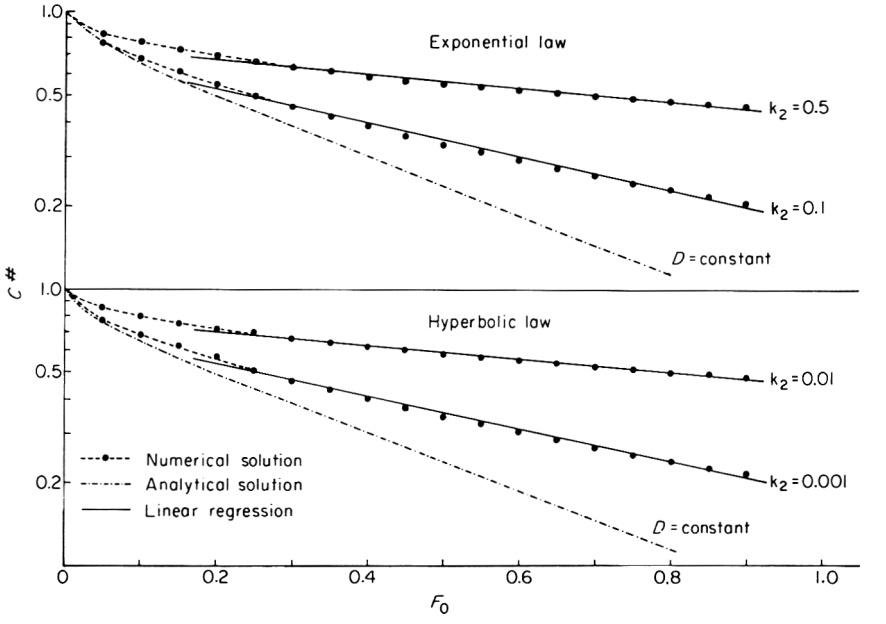

Figure 3. Comparison of the analytical solution of equation (2) with numerical solutions of equation (5) allowing for the variation of the diffusion coefficient with concentration.

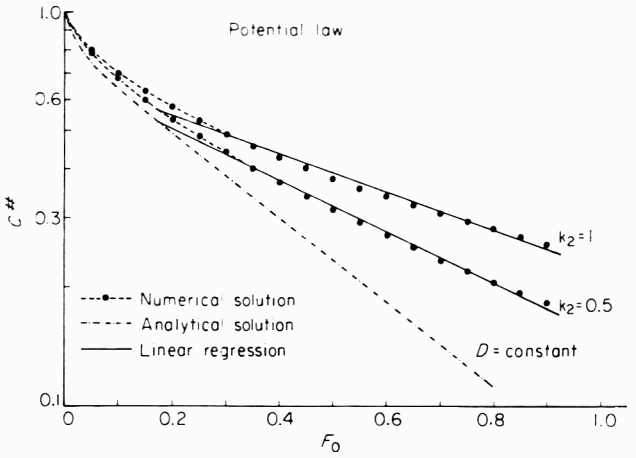

Figure 4. Comparison of the analytical solution of equation (2) with numerical solutions of equation (5) allowing for the variation of the diffusion coefficient with concentration.

factor of 3 to 6 from the beginning of each run. Diffusion coefficients calculated from the slope of these apparent straight lines ($D_s^{\#*}$) do not resemble the correct values, calculated from equations (9) or (10), neither at the beginning or the end of the periods considered. These results suggest that experimental drying or leaching data may apparently adhere fairly well to the linearity, suggesting that the process is governed by a constant diffusion coefficient, while the reverse may be true.

Thus, the following conclusion may be readily obtained regarding the kinetic analysis of experimental drying or leaching processes controlled by internal diffusion. The existence of a straight line relationship between logarithm $c^{\#}$ and time, either for slab or sphere geometries, does not necessarily mean that the process is governed by a

Table 1. Comparison of diffusion coefficients values calculated from the plots shown in Figs 2, 3–4

Functionality for D(c)	k_2	Intercept	$D_s^{\#*}$	$c \times c^{\dagger}$	$D^{**}(0.2)^{\ddagger}$	$D^{**}(0.9)^{\ddagger}$	$D^{**}(0)/D^{**}(0.9$
Infinite slab							
Exponential	0.5	0.7495	0.2309	0.989	0.4939	0.1694	5.90
	0.1	0.6898	0.5568	0.996	0.7495	0.4086	2.45
Potential	1	0.6824	0.4525	0.993	0.6814	0.3127	3.20
	0.5	0.6952	0.6177	0.997	0.7956	0.4670	2.14
Hyperbolic	0.01	0.7847	0.2279	0.994	0.4082	0.1635	6.12
	0.001	0.7009	0.5485	0.997	0.7198	0.4257	2.35
Sphere							
					$D^{**}(0.15)^{\ddagger}$	$D^{**}(0.5)^{\ddagger}$	$D^{**}(0)/D^{**}(0.5$
Exponential	0.1	0.4259	0.2494	0.998	0.5590	0.3657	2.7
Potential	0.5	0.4241	0.3070	0.9993	0.6149	0.3956	2.53
Hyperbolic	0.001	0.4632	0.3411	0.9996	0.5329	0.3396	2.94

†Correlation coefficient.

‡Brackets denotes the value of Fourier number at which the diffusion coefficient was evaluated.

constant diffusivity and that its value may be obtained from the slope of the line. Variations of the diffusion coefficient by a factor of 3 to 6 may not be revealed by this analysis and the slight departure of the data from the linearity, may be attributed to experimental error. Of course this analysis is true in the range of c^* studied in this work; if the drying or leaching experiments are performed down to low values of c^*, a curvature of data will become apparent indicating that the diffusion coefficient is not constant. Vaccarezza & Chirife (1975) advanced this explanation but they did not support it with numerical calculations.

Acknowledgments

The authors acknowledge the financial support from Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina.

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(Received 19 November 1984)

Journal of Food Technology (1985) **20**, 631–636

Simplified method for the prediction of water activity in binary aqueous solutions

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Summary

This work describes the application of a simplified method to predict the water activity (a_w) of binary aqueous solutions in a range of concentrations relevant to intermediate moisture foods. The water activity as a function of molality for several solutes (electrolytes and non-electrolytes) is described by the equation, $a_w = 1 - K\,m$, where K is a constant and m is the molality. Values of K are reported for fifteen non-electrolytes and eight electrolytes. The accuracy of water activity prediction by this method satisfies the actual needs in food research.

Introduction

In recent years, the need for water activity (a_w) control in intermediate moisture foods (IMF) has stimulated research into the prediction of the a_w of aqueous solutions of present or potential interest in this area (Ross, 1975; Sloan & Labuza, 1976; Benmergui, Ferro Fontán & Chirife, 1979; Chirife, Ferro Fontán & Benmergui, 1980; van der Berg & Bruin, 1981). A number of approaches have been taken in order to correlate a_w data in single electrolyte and non-electrolyte solutions, and they have been reviewed by Chirife & Ferro Fontán (1980) in connection with intermediate moisture foods.

It is the purpose of the present work to use a simplified method to predict the a_w of binary aqueous solutions in a range of most interest to intermediate moisture food technology.

Results and discussion

The thermodynamic properties of binary electrolyte and non-electrolyte solutions have been studied both experimentally and theoretically by many workers over the past 50 years (Scatchard, Hamer & Wood, 1938; Ellerton & Dunlop, 1966; Robinson & Stokes, 1961; Ellerton *et al.*, 1964; Stokes and Robinson, 1966; Robinson & Stokes, 1965; Pitzer, 1973; Bromley, 1973; Platford, 1979). It has been customary to report the results in terms of the osmotic coefficient, φ, and most workers usually also provided theoretical or empirical equations to predict the effect of solute concentration on the osmotic coefficient. The osmotic coefficient is related to water activity through the relationship,

$$a_w = \frac{p}{p_o} = \exp\left(-\varphi 0.018\, m\, v\right) \qquad (1)$$

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where: φ, osmotic coefficient; m, molality; and υ, number of moles of kinetics units ($\upsilon = 1$ for non-electrolytes).

Thus, if the relationship between φ and molality is known, one can readily calculate a_w at any desired solute concentration (molality). Data on such relationships for certain substances are already available in the literature (i.e. Pitzer, 1973; Bromley, 1973; Ellerton *et al.*, 1964); however, calculation is usually tedious since a great deal of mathematical manipulation is involved. A highly simplified method is shown here.

Lupin, Boeri & Moschiar (1981) showed that equation (1) may be expanded as the series:

$$a_w = 1-(\varphi 0.018\,\upsilon)m+\frac{(\varphi 0.018\,\upsilon)^2 m^2}{2!}-\frac{(\varphi 0.018\,\upsilon)^3 m^3}{3!}+\ldots\ldots$$

and thus if,

$$(\varphi 0.018\,\upsilon) \ll 1, \tag{2}$$

the following relationship holds true:

$$a_w \cong 1-(\varphi 0.018\,\upsilon)m. \tag{3}$$

In order to satisfy condition (2), φ and/or υ must be small numbers. For non-electrolytes $\upsilon = 1$, while for 1–1 and 2–1 electrolytes (which are those of most interest regarding a_w control in foods) it becomes 2 or 3 respectively.

An examination of the values of osmotic coefficients for solutes of interest to the food area, either electrolytes or non-electrolytes in the a_w range of present concern (Robinson & Stokes, 1965; Stokes & Robinson, 1966), suggests that condition (2) may apply. For example, for NaCl, KCl, sucrose and glucose solutions above $a_w \cong 0.85$ at 25°C, the maximum value of ($\varphi 0.018\,\upsilon$) approaches 0.040, 0.0354, 0.027, and 0.021 respectively. The osmotic coefficient varies with molality; however, if the changes are relatively small in a given interval of molality, equation (3) may be written:

$$a_w \cong 1-\mathrm{k}M \tag{4}$$

where K = constant = $\bar{\varphi} 0.018\,\upsilon$, where $\bar{\varphi}$ is some sort of average osmotic coefficient. Consequently, experimental a_w data for diversity of solutes (electrolytes and non-electrolytes) were fitted by equation (4); the maximum molality to which the data were fitted corresponded either to $a_w \cong 0.85$, or to saturation in the case of compounds with limited solubilities. In some cases the maximum molality was given by the availability of experimental data. Following Benmergui *et al.* (1979) and Chirife *et al.* (1980), experimental data were taken from the compilations of Teng & Lenzi (1974), Robinson & Stokes (1965) and Pitzer & Mayorga (1973). No attempt was made to correlate data for $a_w < 0.85$ since it was well established that at these a_w values the aqueous solute concentration was already too high to be compatible with acceptable taste and other desired physical properties of the IMF (Flink, 1978; Benmergui *et al.*, 1979). Present efforts for development of IM foods for human consumption are directed to microbial control at higher a_w; i.e. around 0.90 (Fox & Loncin, 1982; Leistner, Rödel & Krispien, 1981). For this reason a_w predictions in this work were restricted to IMF range.

A linear regression analysis was used to calculate the slope (K) and intercept of experimental data corresponding to fifteen non-electrolytes and eight electrolytes, fitted by equation (4). The results are shown in Table 1, which also shows the correlation coefficient, the maximum molality to which the data were fitted; the

Table 1. Application of equation (4) to experimental water activity data

Product	K (mol $^{-1}$)	Intercept	r^2	m_{max}*	$(a_w)_{min}$†	$\|\epsilon\|_{max}$†	(% RMS)§
Non-electrolytes							
α-Alanine	0.01811	1.0001	0.9999	1.829	0.967	0.0001	0.43
β-Alanine	0.02048	1.0041	0.9975	6.887	0.859	0.004	4.26
α-Aminobutiric acid	0.01908	1.0005	0.9997	2.131	0.959	0.0005	0.73
Erythrytol	0.01824	1.0003	1.0000	5.459	0.900	0.0002	0.25
Glucose	0.01959	1.0013	0.9998	7.665	0.850	0.001	1.28
Glycerol	0.01723	0.99785	0.9994	8.532	0.849	0.002	3.10
Glycine	0.01523	0.99889	0.9998	3.330	0.948	0.001	1.09
Malic acid	0.01888	1.0008	0.9996	5.860	0.889	0.001	1.36
Maltose	0.02070	1.0012	0.9980	3.007	0.937	0.002	2.89
Mannitol	0.01806	1.0000	1.0000	1.288	0.977	0.0001	0.16
L-Proline	0.02200	1.0034	0.9986	6.892	0.848	0.003	2.83
Sorbitol	0.01859	1.0005	0.9998	3.815	0.929	0.001	0.76
Sucrose	0.02476	1.0045	0.9958	5.931	0.851	0.006	5.09
D-Tartaric acid	0.02279	1.0036	0.9977	5.908	0.865	0.004	4.17
Xylose	0.01840	1.0002	1.0000	3.431	0.937	0.0002	0.32
Electrolytes							
Sodium H adipate	0.03226	0.9999	1.0000	0.736	0.975	0.0001	0.14
Sodium butyrate	0.04403	1.0046	0.9991	3.535	0.848	0.004	2.69
Sodium chloride	0.03710	1.0037	0.9982	4.013	0.851	0.004	3.04
Sodium formate	0.03465	1.0012	0.9998	3.676	0.873	0.001	1.06
Sodium fumarate	0.05133	1.0036	0.9971	2.083	0.894	0.003	4.17
Sodium sulfate	0.03476	1.0011	0.9944	3.960	0.854	0.009	5.88
Potassium acetate	0.04283	1.0055	0.9973	3.580	0.848	0.005	4.16
Potassium chloride	0.03248	1.0007	0.9998	4.471	0.854	0.001	1.37

* Maximum molality to which the data were fitted;
† Minimum a_w to which the data were fitted;
† Maximum absolute difference between predicted and observed a_w;
§ Relative root mean square in percent (defined in text).

maximum absolute difference between predicted and observed a_w and the value of the relative root mean square in percent (% RMS). The value of the (%RMS) indicates the quality of the fit and is defined as follows:

$$\% \text{ RMS} = \sqrt{\frac{\sum_{1}^{N} \left[\frac{(a_w)_e - (a_w)_p}{1-(a_w)_e} \right]^2}{n}} 100$$

where n, number of experimental points; $(a_w)_e$, experimental water activity; and $(a_w)_p$, predicted water activity.

It is noteworthy that the deviation between observed and calculated a_w values is expressed on a $(1-a_w)$ basis rather than in terms of a_w itself. The error in the quantity $1-a_w$ is much more significant than the error in a_w, since it gives directly the error in a_w lowering (Ferro Fontán *et al.*, 1980). For this reason it is advisable not to use equation (4) for a_w prediction at very high values, i.e. above $a_w = 0.99$ because the relative error on a $(1-a_w)$ basis becomes important. Nevertheless, this is irrelevant since at these very

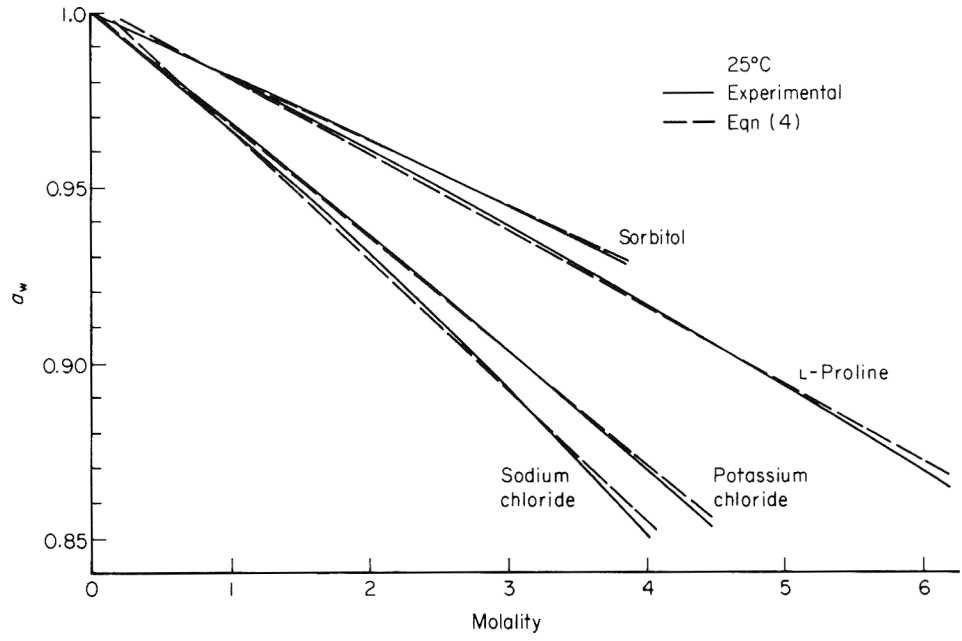

Figure 1. Comparison of observed and predicted (equation (4)) water activity as a function of solute molality for various single component aqueous solutions at 25°C.

high water activities Raoult's law applies quite well. The relatively low values of the (% RMS) suggest the goodness of fit for all cases examined. It can also be seen in Table 1, that the intercepts of the correlated straight lines are very close to the value of 1, as predicted by equation (4).

Figures 1, 2–3 compare predicted (equation (4)) and observed water activity as a

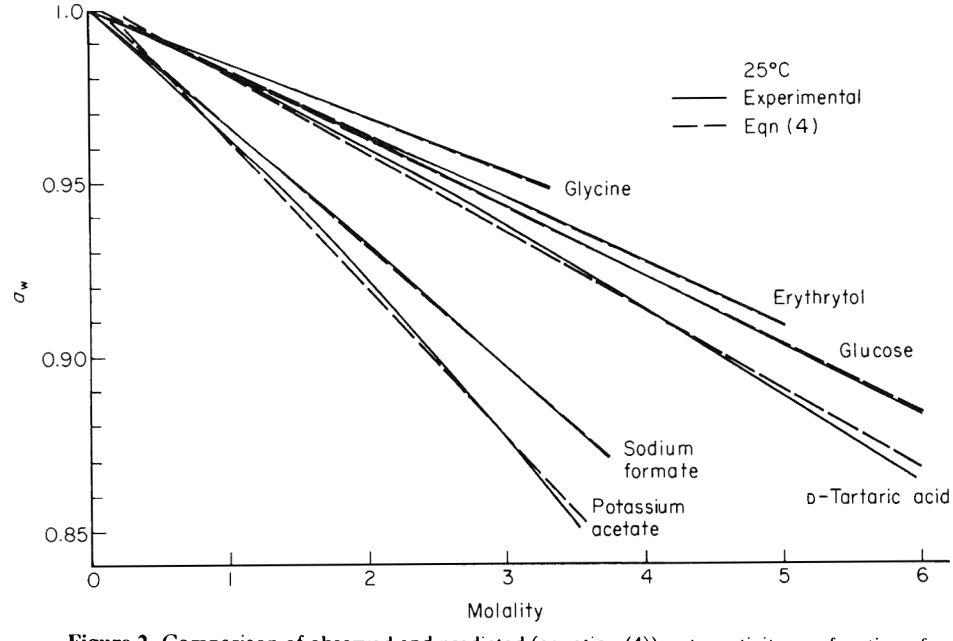

Figure 2. Comparison of observed and predicted (equation (4)) water activity as a function of solute molality for various single component aqueous solutions at 25°C.

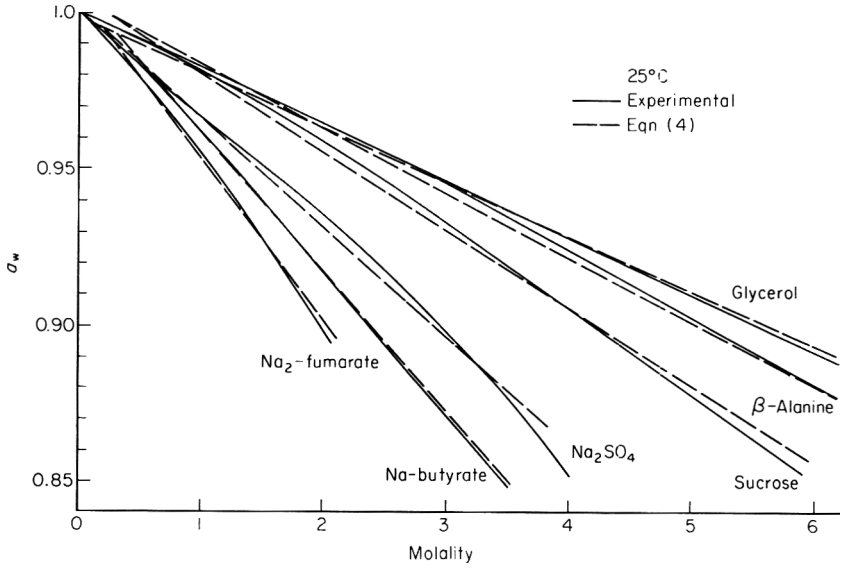

Figure 3. Comparison of observed and predicted (equation (4)) water activity as a function of solute molality for various single component aqueous solutions at 25°C.

function of molality for various solutes of interest. It can be seen that there is a good agreement between the predicted straight line behaviour and reality.

It may be concluded that equation (4) constitutes an extremely simple, but still accurate mathematical method, to predict the a_w of binary solutions in a range relevant to IM foods. As was shown in Table 1, maximum absolute differences between predicted and observed a_w values are usually within 0.005 a_w. This accuracy is adequate for food research, where a_w values are usually measured to ± 0.005 a_w, or at best to ± 0.002 a_w (Troller & Christian, 1978; Favetto *et al.*, 1983).

Acknowledgments

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

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(Received 28 February 1984)

Journal of Food Technology (1985) **20**, 637–645

Fire and explosion hazards in the spray drying of milk

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Summary

Recent reports in the literature indicate that incidents involving fires and explosions in the spray drying of milk products are increasing in number. Whilst milk powder is not highly explosible, the conditions for a dust explosion, apart from the ignition source are almost always present in certain areas of a spray dryer during its normal operation. Self-ignition of deposits is known to be possible at the high temperatures in a spray dryer. At best, this can cause product degradation and discolouration whilst at worst it can provide the source of ignition for an explosion. This paper is an attempt to draw together recent work on fire and explosion prevention and protection for milk powders.

Introduction

The observation has been made more than once, that the number of fires and explosions in operations involving the spray drying of milk products has been on the increase in recent years (Duane & Synnott, 1981; Pisecky, 1900; Pineau, 1984). The losses to the industry are thought to be very large. Pineau (1984) reports that of thirty-five major accidents occurring between 1967 and 1982 in the French dairy industry, fourteen involved fire and explosion. Whilst spray drying of milk is known not to be a particularly hazardous process, product degradation due to charring and minor fires occurs persistently; occasionally the result is the more dramatic event of a major dust explosion with all the associated consequences. This paper is an attempt to draw together recent work on fire and explosion prevention and protection and to suggest means by which these risks and losses may be reduced.

Causes of dust explosions

A dust explosion occurs when a finely divided combustible solid is dispersed in air and subjected to an ignition source. In practice several requirements have to be met before an explosion occurs: the dust must be airborne in sufficient concentration to fall within the explosible range; the ignition source must be strong enough to initiate combustion; and there must be sufficient oxygen in the atmosphere to support combustion. In addition, finer particles and higher ambient temperatures are more likely to lead to explosions. Once ignition has occurred, the propagation of the flame front through the dust suspension causes the very rapid temperature and pressure increases which give rise to explosive effects.

In a milk spray dryer, in certain regions at least, all the conditions for an explosion apart from the existence of an ignition source are always present during normal operation. Milk powder is not a highly explosible dust, nor is it readily ignited.

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This paper was first presented to a seminar on Developments in Milk Powder Technology, Portlaoise, Ireland on 6 December 1984.

Nevertheless it is essential to consider how potential sources of ignition might arise and how these circumstances can be avoided.

Self-ignition in milk powders

Self-ignition (or spontaneous combustion) is believed to be far and away the most important cause of fires and explosions in milk powders (Pisecky; Pineau, 1984). It arises as a result of the oxidation of powder deposits exposed to elevated temperatures. Milk powder contains a number of oxidizable constituents such as fats, proteins and lactose. In addition filled powders have other, possibly more reactive, fats added to them. It is well known in other contexts that these constituents are readily oxidised; and that milk powders deteriorate unless great care is taken in processing and storage to ensure that, for example, flavour and reconstitution properties are not lost.

All these oxidation reactions are exothermic (heat generating) and in common with most chemical reactions the rate of heat generation increases very rapidly with ambient temperature. Though these reactions are known to occur in milk powder stored at room temperature, the rate of heat generation is imperceptibly small. However, at the much higher temperatures of a spray dryer the rate of heat generation will be about 1000 times faster at 100°C than at 20°C, and at 200°C more than 100000 times faster. Given the right conditions deposits of milk powder can self-heat to temperatures in excess of 700°C presenting a serious ignition source for the dust cloud. It is therefore important to have an understanding of these conditions.

Any accumulation of material which is generating heat must lose that heat to the surroundings. If the deposit of powder is sufficiently small, all the heat generated can be lost and no hazard will arise, though discolouration may occur. However, if the deposit is thicker, the heat generation increases without a significant increase in the heat loss and eventually a critical thickness may be reached where the heat generated cannot escape to the surroundings. This results in a rapid rise in temperature culminating in ignition. Because the rate of heat generation is so strongly dependent on temperature the critical thickness for self-ignition of a deposit is also dependent on temperature.

In a spray dryer there are several regions where powder can accumulate. Bulk powder may accumulate in the base; layers may be deposited on the walls; piles may build up in the corners where the dryer cone slopes and on the exhaust and fines return pipes. In principle the downward flow of the air should prevent powder settling in the hot regions near the top of the dryer and under the roof. However the turbulence introduced by a rotary atomizer tends to give rise to eddies which can throw the powder upwards so layers, albeit thinner layers, can even accumulate there. Duane & Synnott (1981) have prepared a diagram from measurements made in a spray dryer which shows the temperature distribution on the various surfaces where powder may accumulate. This is given in Fig. 1.

Various workers (Pisecky; Saprygin & Kiselev, 1968; Beever, 1984) have studied the variation in critical size with ambient temperature for self-ignition in milk and milk based powders. A summary drawn from the results is given in Table 1. The results are presented in terms of critical thickness of layer for two ambient temperatures: 200°C, the hottest likely to be met in the spray dryer and 100°C, a surface temperature found lower in the dryer. The results, where they are not given in the references are calculated from the results quoted using the theory of thermal ignition (Bowes, 1984). This theory allows extrapolation to be made from small scale tests carried out in ovens in the laboratory to the rather different conditions encountered in

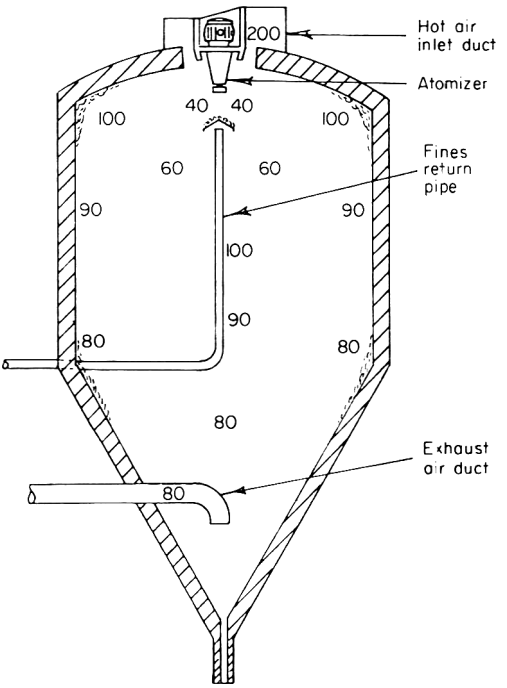

Figure 1. Section through spray dryer for milk products. Typical temperature distribution (°C) and areas of powder deposition are indicated.

the industrial situation. It is the basis for the routine tests carried out at the Fire Research Station to assess the self-heating hazards of industrial powders (Beever, 1982).

It will be noticed first of all from Table 1 that over all the powders tested there is approximately a factor of 10 difference between the critical thicknesses at 100°C and

Table 1. Critical thicknesses for self-ignition of various powders

Products	Minimum thickness for self-ignition at 200°C (cm)	Minimum thickness for self-ignition of layer at 100°C (cm)	Reference
Skimmed milk	1.7	17	1
	1.1	15	2
	0.9	12	3
Coconut-oil filled milk, 26%	1.3	13	4
22%	1.4	14	1
Tallow filled milk, 30%	1.1	20	4
	1.5	15	1
Whole milk	1.0	17	2
	0.7	10	3
Buttermilk (a)	0.9	13	4
(b)	0.8	10	4
Filled milk (formulation unknown)	1.4	14	2
Whey	1.3	32	2

1. Beever (1984); 2. Fire Research Station (unpubl.); 3. Saprygin & Kiselev (1968); 4. Duane & Synnott (1981).

200°C. This arises as the result of the very much faster rate of heat generation at the higher temperature. As can be seen, the results in Table 1 cover a wide range of milk products; those which have lower critical thicknesses are more hazardous than those with higher critical sizes. No clear trend emerges. It would be expected that skim milk with its lower fat content would be less hazardous than filled milks and that the greater the fat content, the greater the hazard. However, this does not appear to be the case. Apart from being able to say that whole milk and buttermilk probably present more of a problem than other products, a ranking of the rest cannot be made.

However, it is apparent that the skim milk tested by Saprygin & Kiselev (1968) is more hazardous than the other two quoted and also the whole milk tested by the Fire Research Station (Beever, 1984). In addition the three powders tested by Beever (1984) from one creamery appear less hazardous than comparable powders tested by other authors. The test method used by all the workers was very similar so these differences cannot be attributed to that cause. It therefore appears that the actual source of the milk for a product may well be of more importance in determining the hazard than the details of the composition. On the other hand it is known that fat-filled powders are more sticky and so critical thicknesses of deposit are more likely to build up. This may justify the assertion made by Pisecky that fat filled powders create more problems

It will be noticed that over the whole range of powders tested, there is a difference by a factor of about 2, at both temperatures, in the critical thicknesses of the most hazardous and least hazardous powders. This factor is sufficiently small for precautionary measures to be adopted which need not necessarily be altered as the product is changed.

If, in the spray drying of milk products, it is found that the deposits of the thicknesses indicated can build up in the parts of the dryer which are at those temperatures, then in principle there is a risk of fires due to self-ignition. The times to ignition are likely to be of the order of an hour or less at 200°C and a few hours at 100°C. In practice, however, deposits are not formed instantaneously and a gradually accumulating layer will tend to oxidize as it is formed and present less of a hazard. The figures given in Table 1 therefore are probably reasonable for rapidly formed layers but will be underestimates for layers which build up gradually. However, the use of figures such as these allows for a safety margin. It has been suggested (Pisecky) that the greatest risk of self-ignition is in accumulations near the outlet which would be at a temperature of about 80°C. Critical thicknesses there would be 20–40 cm and the time to ignition many hours. It does not seem likely that such deposits could build up and remain in place for such a time unnoticed and a watchful eye should be kept on them. It is worth noting that low temperature self-ignition of larger quantities of milk products is relevant to the processing and storage of the powders after leaving the dryer. If stored or packed warm from one drying process, a large pile may, instead of cooling to room temperature, self-heat to ignition.

Other sources of ignition

Apart from self-ignition there are several other possible sources of ignition which in the case of milk powders are probably of considerably less importance. It is possible that incandescant particles may enter the dryer in the air supply. This is probably only likely in a direct fired dryer. Gibson & Schofield (1977) have shown that such particles would have to be 3–5 mm in diameter at temperatures of about 600°C to present a problem.

Such particles can readily be removed by filters and such filters probably already exist to remove foreign matter from the air supply in order to maintain product quality.

Friction between moving parts is a possible source of sufficient heat to initiate combustion. There are few places where this can occur, namely around the atomizer and possibly in rotary valves. Incorrect assembly or cleaning could give rise to this problem. Alternatively it is possible that the atomizer could work loose from its mounting and fly off, hitting the dryer wall. A mechanical spark so generated might be sufficient to ignite the dust cloud. This hazard can be reduced by installing vibration monitors on the shaft of the atomizer, monitoring the temperature of the bearings and/or the energy consumption of the motor.

Finally, it is possible, in principle, that the dust cloud could be ignited by an electrical spark. Leaving aside the problem of electrical equipment failure which seems unlikely, the problem of static remains. Spray drying can generate static as can the subsequent movement of the powder. Milk powders are not very susceptible to static build-up and are not readily ignited by an electrical spark. Nevertheless, as a matter of good practice the whole of a spray dryer and its associated equipment is normally earthed.

Avoidance of ignition

All of the last mentioned ignition sources can be eliminated without practical difficulty; the installation and maintenance of filters should remove incandescant particles; correct monitoring maintenance and cleaning procedures should reduce the risk of mechanical failure resulting in heating or sparks; bonding should remove the small risk of ignition by static. The risk of fire and explosion arising from self-ignition is not so readily removed.

In some spray dryers it appears not to be possible to operate without the build-up of powder deposits. The situation tends to be worse with fat filled powders. The installation of knocking hammers, vibrators, scrapers or air brooms may solve the problem though of course any malfunction of these would have to be watched for. If these are found to be impracticable or not to be effective then the operating intervals will have to be adjusted such that the dryer is cleaned before deposits of critical thickness have built up on the walls. Cleaning must be carried out much more regularly with sticky powders. Until it can be established why nominally similar milk powders give quite different results in similar tests, it is probably best to use the worst case figures in Table 1 as guidelines for safe operation.

Even in dryers which normally run clean it is possible for powder deposits to occur. In particular, the procedure adopted for starting up drying operations seems to be most important. If the feed is switched to product before the dryer is fully heated up, damp patches on the walls will encourage deposition. Once a layer has started to build up it provides a good basis for further deposits. For the same reason, cleaning must be thorough and should be checked visually, to avoid leaving damp patches of product on the walls, to which powder may adhere.

During the course of self-heating charred lumps may fall from the dryer walls into the product. The appearance of such charred particles at the outlet should be watched for and always investigated, particularly if they occur fairly soon after starting up when they would be least expected.

With spray dryers which discharge into a system of fluid bed instantizers, it has been observed that a large number of oversize spheres appearing on the sieve is often

associated with fire or overheating problems within the system. Sometimes these spheres are black or discoloured clearly indicating problems but sometimes they are superficially white but are charred inside. It is not clear what causes these spheres to arise but possibly hot material falling from the drier wall into the outlet may preferentially attract a coating of powder in the instantizer, growing to larger than normal size. Whatever the cause it is worth breaking open such spheres to see if they are charred inside.

Temperature sensors should be installed at least at the inlet and the exhaust of the dryer. These should be sufficiently sensitive to respond to temperature changes and give warning of sudden change rapidly. Devices with response times of several minutes are in use and these are not sensitive enough to give early warning of fire. Fires spread very rapidly through fluid bed instantizers and detection devices should be installed.

Fire protection

Once a fire has been detected by smell, by the appearance of charred material at the outlet or by operation of some form of detector, action must be taken at once to avoid the risk of explosion. A fire within the dryer itself need not be particularly serious as the space is confined and the amount of material limited. Fire fighting however may be extremely difficult unless some extinguishing system has been incorporated into the dryer chamber. Fire can spread very rapidly through the instantizers and these should also be fitted with an extinguishing system. Once a fire has been detected the air flow should be switched off at once, the product feed switched off or switches to water and the extinguishing system activated. The same applies to ancillary equipment into which burning material may have passed.

Water is the commonest extinguishing agent though carbon dioxide is sometimes used. For dryers greater than 8.4 m in diameter Pineau (1984) suggests a water flow rate of 10 l/sec. Even if this does not extinguish the fire, this rate of flow over the dryer walls will minimize heat damage to the structure. The extinguishing nozzles should be designed and maintained so that they do not become blocked with powder. Fire fighters opening the dryer for access with a hose should be prepared for falling burning debris and should be aware of the danger of dust explosion. Smouldering fires are very difficult to extinguish and it may take many hours before the fire is entirely out.

Explosion protection

Since it is not possible to remove entirely the risk of self-ignition in a milk spray dryer then it must be assumed that there is a risk of explosion. Once an explosion has been initiated, to minimize damage to plant and risk to personnel, it must be contained, suppressed or vented.

Containment, i.e. making the dryer and ancillary equipment so strong that an explosion can be sustained without damage, is not suitable for spray dryers larger than the laboratory scale because of the strength required.

Explosion suppression involves the early detection of an explosion and the injection of inert or inhibiting material into the chamber to quench flame spread and consequent pressure rise. A large number of injection points may be needed to protect the whole volume of a spray dryer and in some instances only the cone is protected, since this is the region where the powder concentration is likely to give an explosible mixture. At the

present time volumes up to 150 m^3 can be protected by suppression methods, or larger if the cone only is suppressed. However the installation of such a system is expensive.

Explosion venting is the method most commonly used for dealing wth dust explosions. Although vents are in widespread use the technology is not fully developed and there are different methods for calculating the area of a vent for a given dust in a known volume. A detailed account of explosion venting has recently been given by Schofield (1984).

The system which has been used in the U.K. and U.S.A. for many years is the vent ratio method. The vent ratio is defined as:

$$\text{vent ratio} = \frac{\text{Area of vent}}{\text{Volume of vessel}}.$$

The appropriate vent ratios, based on the explosibility of milk powders are given in Table 2. It is assumed in Table 2 that the plant can withstand an overpressure of 0.14 bar. The disadvantage of this method is that it demands very large vent areas in the larger dryers which may be difficult and costly to accommodate.

Table 2. Vent ratio as a function of volume of equipment for milk powder

Volume range (m^3)	Vent ratio (m^{-1})
< 30	1/6.1
30–300	Linearly reduced from 1/6 to 1/25
300–600	Half area of top
> 700	Full area of top

For vessels which are stronger and can withstand a minimum of 0.2 bar the cubic law method of vent sizing developed by Bartknecht (1981) and summarized by Schofield (1984) can be used. This method is now widely used in Europe. This method yields smaller vent areas and is suitable for large dryers providing the design strength is high enough.

Pineau (1984) has recently carried out large scale tests on whole milk powder using a 100 m^3 chamber. Taking his measured values of pressure rise as a function of vent area, smaller vents would appear to be appropriate than would be calculated by either of the above methods. This was found to be particularly true for explosions which only involved a fraction of the chamber volume. In a spray dryer it is unlikely that the whole of the volume will contain an explosible concentration of dust and this therefore reflects the real situation. On the basis of this work it would seem that there is some justification in strong plant for choosing slightly smaller vents than the cubic law method suggests.

Vents on spray dryers are usually installed in the roof but can take the form of hinged doors on the walls. If these are used they need to fit flush with the wall such that dust cannot accumulate on ledges. Because they need to be dust tight, yet open readily, considerable care is needed in the design of door catches.

If the vent is operated by a dust explosion, burning dust will be ejected vigorously by the explosion. Personnel and equipment in front of the vent will be at risk from flame and pressure effects. Vents to the outside of a building will be safe, providing that access

to the area into which the vent operates is restricted when the plant is in operation. Ducts leading from vents to the outside of a building are not recommended for spray dryers because such ducts increase the severity of the explosion. Stronger plant or larger vents are required if ducts are to be used and a full list of design considerations for ducts is given by Schofield (1984).

Case histories

(i) A company approached the Fire Research Station for assistance following a series of fires in an installation spray drying milk products. An examination of the records showed that over the 18 months prior to the investigation eight incidents had occurred and one of these had been a fairly serious explosion. In the case of the explosion the cause was assumed to be the atomizer flying off and hitting the dryer wall. The fires however, were of unknown cause. All the company products had been involved in the incidents; no particular one standing out as hazardous.

At the Fire Research Station routine self-heating tests were carried out on the materials and predictions of situations likely to give rise to fires were made. Following the investigation recommendations were made with respect to starting up procedures and cleaning intervals. No further fires have occurred. This investigation is reported in detail by Beever (1984).

(ii) Reay (1979) quotes a case where the atomizer wheel in a spray dryer handling dairy products was given too much lubricating oil. The oil soaked into powder deposited on the underside of the roof of the dryer and during subsequent operation the oil-soaked powder spontaneously ignited. The burning material fell to the bottom of the dryer but fortunately no explosion occurred. However one entire batch of the product had to be discarded.

(iii) The Fire Research Station was approached by a company which had suffered an explosion in a spray dryer operating with skimmed milk. Subsequent investigation by the company led to the conclusion that a deposit around the fines return pipe had ignited and had been the source of the explosion. The pipe itself was at about 40°C and the air in the vicinity was assumed to be at 200°C based on results published in the literature on self-ignition of milk. Calculations were carried out to predict the critical thickness of deposit under these circumstances. It was found to be 2.6 cm. This was felt to be within the bounds of possibility. If the air in the vicinity of the pipe was as low as 125°C, the critical thickness was calculated to be 11 cm which was too large to be likely. The company took these figures and went back to investigate further.

(iv) Reay (1979) quotes a case of a fire due to self-ignition in a series of interconnected fluid bed dryers in a milk powder plant. The fires were rapidly controlled by the internal water spray systems. However further smouldering became evident in almost horizontal sections of the exhaust trunking to the cyclone and it was some hours before this was extinguished. Considerable deposits of powder had accumulated in this trunking. This incident shows the need to reduce the length of horizontal trunking on dryer exhausts to a minimum. If this is not possible, access doors should be provided for regular cleaning and the air velocity should be kept as high as possible.

(v) A company involved in the spray drying of milk products approached the Fire Research Station to have their products tested for explosibility. It was pointed out to them that self-ignition in deposits in the dryer could provide the necessary source of ignition for an explosion to occur. Tests were therefore carried out on the powders,

prediction of critical thickness were made and preventive measures suggested. The company was also interested in storing milk powders in bulk in silos, which would be filled with freshly dried powder at 40–50°C. Using theory developed at the Fire Research Station it is now possible, based on the results of fire tests already carried out to predict the critical size of silo, above which the material will self-heat to ignition and below which it will cool to ambient. The critical diameter can be calculated to be 5.3 m for whole milk powder at 50°C for an ambient temperature of 20°C.

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(Received 21 December 1984)

Journal of Food Technology (1985) **20**, 647–649

Aflatoxins in imported edible nuts: some data 1982–84

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Summary

Results of 188 analyses for aflatoxin in edible nuts imported into the U.K. during 1982–84 are presented. Most samples (140/188, 74%) had aflatoxin B_1 content ≤ 5 μg/kg. Brazilian peanuts may be identified as consistently unreliable as regards aflatoxin contamination.

Introduction

Awareness of the existence of mycotoxins has led to ever-increasing concern over their presence in food materials of all types. In edible nuts, contamination by the aflatoxins, mycotoxins produced by toxigenic strains of *Aspergillus flavus* Link ex Fries and *A. parasiticus* Speare, is a potential problem (MAFF, 1980).

Much attention has been paid in the U.K. to controlling the level of aflatoxin contamination in edible nuts reaching retail sale, by governmental testing on import and at retail outlets. However, much of the monitoring is carried out voluntarily by edible nut traders and processors themselves with a self imposed maximum limit of 5 μg/kg aflatoxin B_1 (lower in some cases) being tolerated rather than the 30 μg/kg limit dictated by the code of practice agreed between the MAFF and the edible nuts trade.

This laboratory tests both raw imported kernels and processed nuts for its clients, and the data presented here represents a summary for those kernels tested at import where the country of origin has been defined. This information may provide at least a partial update on the current situation. It is important that importers should be made aware of potentially unreliable sources of kernels in order to curb potential losses.

Materials and methods

Samples of edible nuts provided were usually 1–2 kg in weight. It is assumed that some acceptable sampling scheme had been adhered to when taking the sample. Kernels were decorticated if necessary and milled in a mechanical mill which had previously been cleaned with chloroform and allowed to dry.

Quantitative analysis for aflatoxins was done on oilseed meals which had been defatted by two 2 hr periods of continuous extraction with redistilled light petroleum (boiling point 40–60°C) with an intermediate micropulverization stage. The defatted powders were wetted with distilled water and aflatoxins were extracted by shaking vigorously with chloroform (2.5 ml/g of original meal) for 30 min (Jones, 1972). Thin layer chromatography was done on Whatman LK6D precoated plates using chloroform-acetone (9:1, vol/vol) as the developing solvent. Identification and quantification of aflatoxins was based on a standard method (Anon., 1976).

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Results and discussion

Sampling and analysis procedures for aflatoxins have been extensively investigated and reliable protocols designed based on statistical evaluation (Coker, unpubl. and 1984). While these procedures recommended by the Tropical Development and Research Institute are statistically acceptable, from the small to medium sized importer's point of view, the cost required to generate large weight samples from each parcel and have these analysed is prohibitive. Compromise is inevitable and as an independent commercial analyst, this laboratory accepts samples of typically 1–2 kg edible nuts and analyses these on the assumption that they are representative of the parcels sampled.

Tables 1 and 2 summarize the data for samples tested during the last 3 years. The results for peanuts are limited to those samples tested where a country of origin has been specified. It must also be considered that this laboratory analyses only a fraction of the parcels of edible nuts being imported into the U.K. since many processors do their analyses in house and other independent analysts also carry out this work. The relatively small sample size used is a further possible limitation on accuracy.

Table 1. Aflatoxin B_1 content of 171 parcels of edible peanuts imported into the U.K. during calendar years 1982–84*

Country of origin	No. of samples analysed	Number of samples containing levels of aflatoxin B_1† in the stated range (μg/kg)								
		≤ 5	6–11	11–25	26–50	51–100	101–200	201–300	301–400	> 400
Australia	1	1	0	0	0	0	0	0	0	0
Brazil	64	28	1	6	5	9	11	1	3	0
China	40	37	1	1	0	0	0	1	0	0
Egypt	1	1	0	0	0	0	0	0	0	0
Gambia‡	1	0	0	0	1	0	0	0	0	0
India	9	6	0	1	1	1	0	0	0	0
Malawi	14	14	0	0	0	0	0	0	0	0
Senegal	9	6	0	1	0	1	0	0	0	1
S. Africa	3	3	0	0	0	0	0	0	0	0
Sudan	4	2	0	0	0	0	0	1	1	0
U.S.A.	23	23	0	0	0	0	0	0	0	0
Vietnam	1	1	0	0	0	0	0	0	0	0
Zimbabwe	1	1	0	0	0	0	0	0	0	0
Totals	171	123	2	9	7	11	11	3	4	1

*Number of samples analysed in each year: 1982, 35; 1983, 43; 1984, 93.

†Aflatoxin B_1 was the major contaminant in all cases where present.

‡Parcel, although of HPS peanut type, imported for use as crushing stock.

Table 1 indicates that most parcels of peanuts meet the statutory limit of 50 μg/kg (82% all sources, 94% excluding Brazil) and even the voluntary limit of 5 μg/kg (72% all sources, 89% excluding Brazil). There has been a clear increase in concern with the aflatoxin problem as shown by the number of samples analysed in each year (footnote to Table 1). Of those sources with a sufficiently large number of samples tested to be statistically viable, only Brazilian peanuts show consistent unreliability for aflatoxin content, with no evidence of improvement (Brazilian samples with ≤ 5 μg/kg aflatoxin B_1: 1982, 45%; 1983, 37%; 1984, 48%).

In conclusion, with noted exceptions, it appears from the limited data presented that most imported edible nuts are within the required quality standards as regards aflatoxin

Table 2. Aflatoxin B_1 content of seventeen parcels of edible nuts other than peanuts imported into the U.K. during calendar years 1982–84

Variety	No. of samples analysed	Number of samples containing levels of aflatoxin B_1 in the stated range (μg/kg)		
		< 5	5	> 5
Almonds (Californian)	4	4	0	0
Brazils (in shell)	1	1	0	0
Walnuts (Indian halves)	12	10	2	0

contamination, but that odd parcels may be badly or very badly contaminated. The situation therefore requires continued monitoring and perhaps action to stimulate concern at problem sources.

Acknowledgment

I thank Frank Hackett for technical assistance.

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(Received 17 January 1985)

Journal of Food Technology (1985) **20**, 651–657

Equation for fitting desorption isotherms of durum wheat pasta

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Summary

This work was undertaken to obtain water vapour desorption data for durum wheat pasta (spaghetti) between 40° and 90°C by an equilibrium method using sulphuric acid solutions. Sorption isotherms were also measured for semolina of the same composition. From these data the net heat of desorption, calculated by the Clausius-Clapeyron relationship, and BET monolayer values X_m were determined. In the range of water activity (a_w) investigated, i.e. $0.10 < a_w < 0.90$, the results were correlated to an Oswin type empirical relation $X = f(a_w, T)$ with a mean precision of 7%. This equation should be of use in modelling pasta drying.

Introduction

Equations for fitting water sorption isotherms are very useful for the analysis of dehydration and subsequent conservation of foodstuffs. For example the prediction and modelling of drying times or the determination of the shelf life of the dried product after packaging can be derived.

Furthermore, sorption data obtained at different temperatures allow for the calculation of thermodynamic functions of sorbed water such as sorption heats; such data are of paramount importance in studying the energetic aspects of drying.

It is not surprising therefore that numerous models and equations, both theoretical and empirical, have been published in the attempt to interpret corresponding sorption data. All such equations have been reviewed by Chirife & Iglesias (1978) and Chirife (1983).

Due to the development of high temperature drying processes in the pasta industry there is interest in studying and modelling the drying of these foodstuffs. Experiments have shown that the drying rate is governed by the resistance to water transport within the solid, and that the drying rate depends on the equilibrium humidity at the external surface (Andrieu & Stamatopoulos, 1984).

On the other hand, the mechanical properties of dry pasta are very dependent on the air humidity during drying, i.e. on the humidity at the external surface (Görling, 1960).

To our knowledge, there are no data concerned with the water vapour desorption of extruded pasta; published data concern only wheat or durum wheat semolina (Boquet, Chirife & Iglesias, 1978; Bushuk & Winkler, 1957; Iglesias & Chirife, 1982). Thus, desorption isotherms were measured at six temperatures, in the range 40–90°C, for an extruded pasta product.

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After the comparative study of Boquet *et al.* (1978) for starchy products, the following equations were selected to fit our experimental results:

Bradley relation:

$$\ln a_w = K_1 \cdot (K_2)^X, \tag{1}$$

where K_1 and K_2 are constants;

Henderson relation:

$$1 - a_w = \exp -(K' \cdot X^{n'}); \tag{2}$$

Oswin relation:

$$X = K \cdot \left\{ \frac{a_w}{1 - a_w} \right\}^n, \tag{3}$$

where X is the solid humidity (dry basis)

In these two last empirical equations, the constants K′ and n′ or K and n, characteristic of each product, are temperature dependent.

In order to obtain thermodynamic sorption parameters i.e. the monolayer value X_m and the net desorption heat ΔH_{des}, we also fitted our results in the activity range $0.10 < a_e < 0.50$, to the well known theoretical BET equation:

$$\frac{a_w}{(1 - a_w)\,X} = \frac{1}{X_m \cdot C} + \frac{(C-1)}{X_m \cdot C} \cdot a_w \tag{4}$$

Due to the good fit observed for many foodstuffs with the GAB equation (Bizot, 1983) we also correlated our results with this three parameter relation derived from the BET model:

$$\frac{X}{X_m} = \frac{K \cdot C \cdot a_w}{(1 - K \cdot a_w)(1 - K \cdot a_w + K \cdot C \cdot a_w)} \tag{5}$$

where: X_m represents the monolayer sorbed quantity; C represents the Guggenheim constant, a function of the heat of adsorption; and K represents the correction factor.

Materials and methods

Desorption runs were made with spaghetti (diameter = 1.5 mm; extrusion pressure = 80×10^5 Pa) and with durum wheat semolina of the same composition. The sample weight was about 5 g. The durum wheat semolina was a standard industrial semolina with a protein content of 14.5% (w/w).

Equilibrium sorbed water was determined by sample weighing using a Sartorius balance with a precision of 0.1 mg, after equilibration over sulphuric acid solutions in sealed glass vessels; these vessels were maintained at a constant temperature, ±0.5°C, in a ventilated oven for 1 week.

Dry matter was calculated by weighing after dehydration at 130°C (cf. standard NF V 03 707). The experimental a_w range was obtained by varying the acid concentration (Multon, Bizot & Martin, 1984; Rüegg, 1980). This method was preferred as the activities of such solutions are well known over a large temperature range with a good precision and the activity range could be varied continuously by varying the sulphuric acid concentration. Uncertainty of the solid humidity estimate was better than 1% and

repeatability of the equilibrium sorbed quantity, X, was about 2% in all cases.

Results and discussion

Desorption runs for extruded pasta were done at 40, 50, 60, 70, 80 and 90°C and at each temperature, ten a_w values, in the range of $0.10 < a_w < 0.90$, were studied. Corresponding curves for X as a function of a_w, where X is in kg water/kg dry matter, are shown in Fig. 1. On the BET classification, these curves are of type II as it can be seen that the influence of temperature on the sorbed quantity decreased as the activity tends to 1.

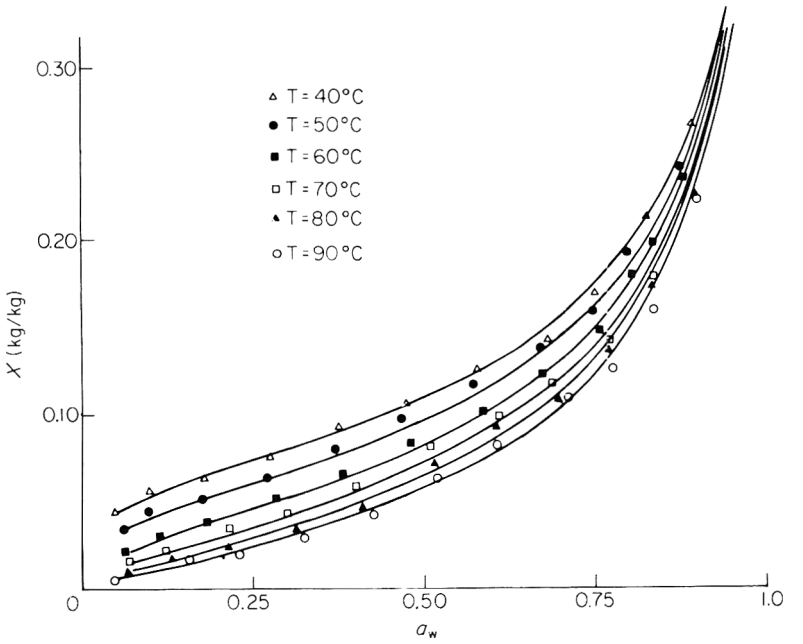

Figure 1. Equilibrium desorption humidity X (kg water/kg dry matter) for pasta as a function of water activity, a_w.

In Fig. 2 it can be seen that the amount of water sorbed by durum wheat semolina is higher than the amount sorbed by pasta under the same conditions. This difference may be explained by the influence of extrusion pressure and by a modification of the particle surface state due to hydration during pasta manufacture.

Complementary experiments showed that the particle size distribution of semolina had no significant influence on the isotherm.

As found by Bushuk & Winkler (1957) for wheat flour and its components, we found that the semolina composition (e.g. potein content) had a significant influence on the amount of water sorbed.

Data correlation

In order to derive a simple relation between X, a_w and temperature, which is easily used in a computer program, we fitted these data to some of the many equations published in the literature (Chirife & Iglesias, 1978).

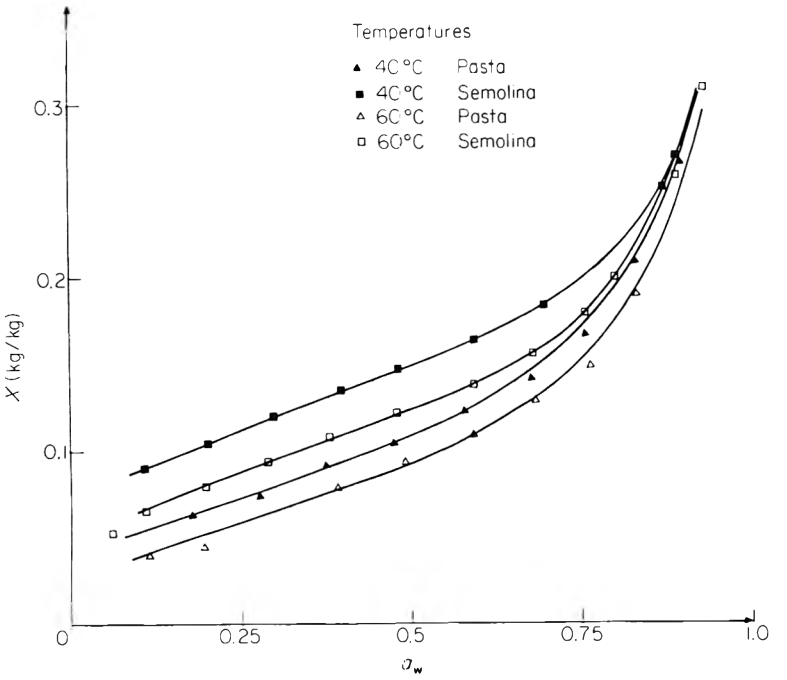

Figure 2. Desorption data of extruded pasta (spaghetti) and semolina of the same composition.

Among the two parameter equations we selected those of Bradley, Henderson & Oswin over the whole range of activities investigated, and the BET equation for activities lower than 0.5.

Optimal parameters were obtained by the least square method. Fitting precision was estimated by calculating mean relative error (MRE) defined by:

$$\text{MRE} = \frac{X_{\text{mes}} - X_{\text{cal}}}{X_{\text{mes}}} 100$$

where mes = measured value and cal = calculated value. The GAB relation, which is a three parameter relation well suited to food products, was also evaluated (Bizot, 1983).

Mean values of MRE for the different correlations are given in Table 1. These results show that among the two parameter relationships an Oswin type equation gives the best fit over the whole range investigated. Fitting precision is about the same as for the GAB relation which (as indicated before) is a three parameter relation. Furthermore using an Oswin type relation, the constants K and n can be easily and accurately found as a function of temperature:

$$n = 0.078 + 0.00732 \times T$$

and:

$$K = 0.154 - (1.22 \times 10^{-3}) \times T$$

So, we can propose in the range $0.10 < a_w < 0.90$ the final equation:

$$X = (0.154 - 1.22 \times 10^{-3} \times T) \left\{ \frac{a_w}{1 - a_w} \right\}^{0.078 + 7.32 \times 10^{-4} \times T} \quad (6)$$

where T is expressed in °C.

Table 1. Fitting precision of the different equations

Equation	Bradley	Henderson	Oswin	BET for $a_w < 0.5$	GAB (three parameters)
MRE %	17.7	7.8	6.3	5.5	6

The mean absolute relative error of this latter relation is about 7% for the data concerning durum wheat pasta (spaghetti). The GAB equation, with empirical correlation of the constants X_m, C and K as a function of temperature, does not give a better precision. Thus, the Oswin type equation given by (6) was preferred.

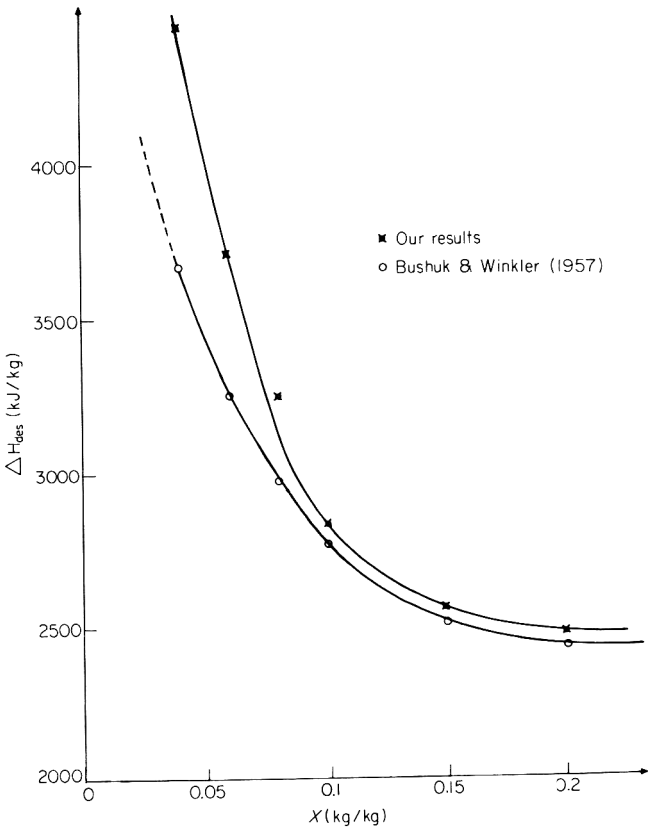

Figure 3. Extruded pasta total desorption enthalpy ΔH_{des} as a function of the water content of the solid (X).

Net desorption enthalpy was calculated from the slopes of isosteric straight lines (X = constant) and the Clausius-Clapeyron relation:

$$-\ln a_w = \frac{\Delta H_{des}}{RT}. \quad (7)$$

As shown in Fig. 3, this net sorption enthalpy decreases asymptotically towards zero as the amount of sorbed water increases; furthermore, these values are very close, but slightly higher, than the values obtained by Bushuk & Winkler (1957) for water vapour adsorption on wheat flour. For $0.04 < X < 0.18$ the plot of $\ln \Delta H_{des}$ against ($\ln X$) is a straight line; consequently the desorption enthalpy can be found from the following equation:

$$\Delta H_{des} = 7145 \times X^{-0.39} \quad (8)$$

where ΔH_{des} is expressed in kJ/kg sorbed water.

Thus, we can deduce that in the study of industrial pasta drying, in the less favourable case when $X = 13.6\%$, the net desorption enthalpy is about 300 kJ/kg, i.e. 12% of the heat of vapourization of pure water. Consequently this sorption enthalpy can be neglected in any energy balance of the drying process.

BET monolayer values obtained by the least square method, in the range $a_w < 0.50$, are shown in Table 2.

Table 2. BET equation parameters

Temperature (°C)	40	50	60	70	80	90
BET monolayer X_m	5.83	5.44	5.40	4.95	3.78	3.51
Xm (Bushuk & Winkler, 1957)	5.6	5.2				

As expected (Iglesias & Chirife, 1976; Iglesias, Chirife & Lombardi, 1975) these values decrease slowly with temperature. They are also very close to published literature values by Bushuk and Winkler (1957).

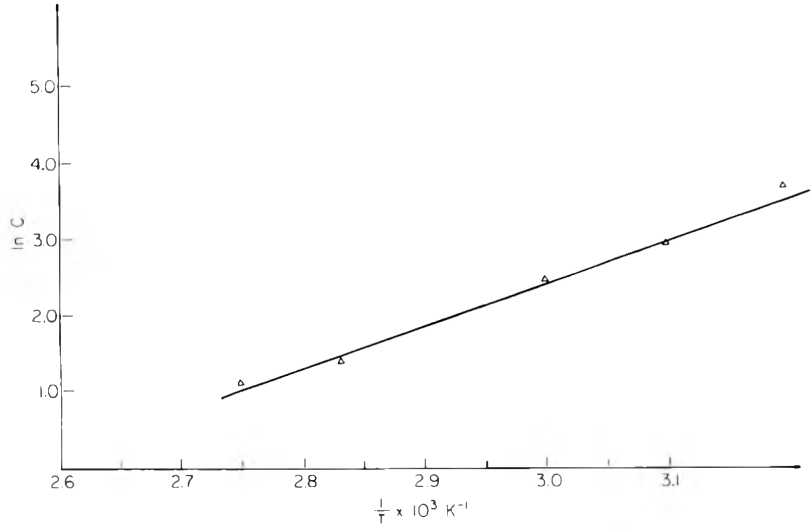

Figure 4. Arrhenius type plot for C constant of the BET equation.

According to the BET theory, desorption enthalpy can be obtained from a plot of ln C against $1/T$ (cf. Fig. 4). The resulting value of the enthalpy (ΔH_{des} = 5000 kJ/kg which corresponds to the first layer total sorption heat according to this theory) is very close to the corresponding value obtained from the Clausius-Clapeyron relation for X = 0.04 (cf. figure).

Conclusions

Water vapour desorption data for durum wheat pasta were measured between 40 and 90°C and for $0.10 < a_w < 0.90$. The desorption isotherms for pasta were below the corresponding values for durum wheat semolina of the same composition. Monolayer BET constants and net desorption heats deduced from these results are in agreement with literature values concerning durum wheat flour. In the humidity range corresponding to industrial drying (X > 13.6%), the heat of sorption can be neglected in the analysis of the drying process. In the investigated temperature and activity range, the sorption data can be fitted by an Oswin type equation $X = f(a_w, T)$ with a mean precision of 7%.

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(Received 25 January 1985)

Journal of Food Technology (1985) **20**, 659–667

Book Reviews

Flavour of Distilled Beverages: Origin and Development. Ed. by J.R. Piggott. Chichester: Ellis Horwood, 1983. Pp. 279. ISBN 085312 546 5. £18.50.

This book is effectively the proceedings of a symposium of similar title, which was organized by the Sensory Panel of the Food Group of the Society of Chemical Industry, and held in Stirling University between 1 and 4 June, 1983. There are twenty-two chapters covering the following topics: (1) flavours of malt and other cereals (R. Tressl, D. Bahri & B. Helak, 24 pp.); (2) fruit flavours and their relevance to the flavour of the final distilled beverage (P. Durr & H. Tanner, 16 pp.); (3) rum flavour (L. Nykanen & I. Nykanen, 15 pp.); (4) volatile and non-volatile compounds in the flavour of alcoholic beverages (M. Lehtonen & P. Jounela-Eriksson, 15 pp.); (5) factors influencing the congener composition of malt whisky new spirit (D.C. Watson, 14 pp.); (6) volatiles from grape and must fermentation (A. Bertrand, 17 pp.); (7) volatile phenols in wines (P. Dubois, 10 pp.); (8) the effect of distillation on grape flavour components (C.R. Strauss & P.J. Williams, 14 pp.); (9) computer modelling of aroma compound behaviour during batch distillation (L.A. Williams & W.P. Knuttel, 11 pp.); (10) descriptive sensory analysis of gin flavour (J.R. Piggott & A.M. Holm, 9 pp.); (11) appraisal of industrial continuous still products (G.D. Wilkin, M.A. Webber & E.A. Lafferty, 12 pp.); (12) the modification of certain constituents of flavourings after addition to alcoholic beverages (P.A.P. Liddle and A. Brossard, 17 pp.); (13) sensory aspects of whisky maturation (P.R. Canaway, 9 pp.); (14) flavour compounds in rum, cognac and whisky (R. de Rijke & R. ter Heide, 11 pp.); (15) a flavour company's contribution to distilled beverages (G.A. Hopkins, 3 pp.); (16) polysulphides and thiophenes in whisky (O. Leppanen, P. Ronkainen, J. Denslow, R. Laakso, A. Lindemann & I. Nykanen, 9 pp.); (17) automated data collection in sensory analysis (J.R. Piggott, 4 pp.); (18) a new approach to sensory profile analysis (A.A. Williams & S.P. Langron, 6 pp.); (19) chemical analysis of whisky maturation (G.H. Reazin, 16 pp.); (20) reactions of wood components during maturation (K. Nishimura, M. Ohnishi, M. Masuda, K. Koga & R. Matsuyama, 15 pp.); (21) blending for sale and consumption (J.W. Lang, 8 pp.); (22) the flavours of distilled beverages: what conclusions can be drawn from this symposium, and what can flavour research offer the spirit industry of the future? (A.A. Williams, 11 pp.).

Like most symposium proceedings, this text lacks continuity. However in this case, the problem is particularly acute and the reader has the impression of staggering, dare I say drunkenly, from one aspect of distilled beverages to another. Logically, the material covered in this book falls into three distinct categories: (i) chemical analysis of flavour components; (ii) sensory aspects; and (iii) the significance of ingredients and each stage of production to the flavour of the final product. The text would undoubtedly have been much more 'readable' had the editor grouped the chapters in this way. An introductory chapter, emphasizing the psychological nature of flavour would also have added a great deal.

Most of the papers are of a good scientific standard, although it is noticeable that many of the contributors stray from distilled to non-distilled beverages at the first possible opportunity. Some of the 'chemical' chapters lapse into mere catalogues of chemical compounds identified (e.g. chapter 1), with very little attention paid to their

likely contribution to the flavour of the product. These chapters will probably make rather dull reading for all but the specialist flavour/aroma chemist. The chapters dealing specifically with sensory aspects of beverage flavour are good. Of particular interest is the chapter by Williams & Langron (18) who describe a novel technique for profiling the sensory characteristics of beverages (equally applicable to foods in general) without the need for highly trained assessors or a specially derived vocabulary (so-called 'free-choice profiling').

The book is attractively presented, the print is bold and legible and there are very few typographical errors. Almost all of the chapters are well documented with up-to-date references: tables and diagrams are well produced and plentiful; and the index is adequate. In some of the chapters by overseas contributors, the English is rather stilted.

This is a specialist text. However, because it brings together many different aspects of the subject, it could be useful to the scientist or technologist who wishes to rapidly acquire an insight into the flavour of distilled beverages.

D.M.H. Thomson

Dairy Chemistry and Physics. By P. Walstra and R. Jenness.
New York: John Wiley, 1984. Pp. xviii + 467. ISBN 0 471 09779 9. £56.95.

The first two chapters form an introduction to dairy chemistry, dealing wth milk composition, structure, biosynthesis and secretion. The subsequent seven chapters deal with the components in more detail, i.e. carbohydrates, salts, proteins, enzymes and miscellaneous components plus a chapter on variability. These chapters summarize the present state of knowledge, which has been covered at greater length in recent more specialized publications.

These compositional aspects are followed by an extensive treatment of the physical characteristics of milk and milk products, especially the colloidal and surface phenomena. These chapters include discussions of the properties of casein micelles, milk fat globules and milk concentrates. The remaining chapters form a less intensive review, with chapters on nutritional aspects and the analysis of milk and dairy products. A separate chapter on flavours and off-flavours was contributed by Badings. The main text is complemented by a relatively large appendix of twenty-five tables (sixty-two pages) covering the structures of components, plus physical and other data.

The authors have produced an authoritative text on cow's milk in terms of physical and chemical properties, including specifically the surface phenomena that bridge the traditional approaches. A basic knowledge of biochemistry, chemistry and mathematics is assumed, making the book more suitable for students and workers at or approaching graduate level.

This publication differs from many of its contemporaries in not seeking to review comprehensively the enormous field of dairy publications. The authors have restricted their discussions of phenomena to what they consider to be the most likely explanations together with the greatest doubts about the evidence. This is reflected in the references which have been pruned severely and annotated critically as suggestions for further reading. The bibliography was based on the last 25 years to the date of publication.

The layout of the book is clear, with chapter titles and subtitles on alternate pages.

The latter include the chapter and subtitle numbers which aid cross-referencing. The standard of printing is good, with use of a bolder typeface for headings. The speed of cross-referencing would be improved however if the same numbering system were used for both the tables and figures. Typographical errors are few and generally of no significance.

The publication of this book will be welcomed by both students and practitioners of dairy science and technology. The cost however will be a constraint and I hope that a soft-bound edition becomes available.

R.A. Wilbey

Testing Methods in Food Microbiology. Ed. by István Kiss
Amsterdam: Elsevier, 1984. Pp. 447. ISBN 0 444 99648 6. US$75.00.

This is a multi-author book, originally published as two volumes in Hungary in 1977 and 1978, which has been translated and revised for publication in English under a title that I can read in three ways. The book is envisaged as a laboratory manual. It deals with: design and function of a microbiology laboratory; disinfection, sterilization, microscopy, media preparation, isolation of pure cultures, anaerobic growth, physiological tests; statistical principles of sampling and grading of foodstuffs; identification of bacteria, moulds and yeasts in foodstuffs; enumeration; examination of water, air, surfaces; testing of food, food ingredients and additives; culture media, stains and indicators. The book is well bound, clearly printed, has some clear diagrams, an extensive reference list (though, unfortunately, many media are not linked to this), and an index (though, unfortunately, there has been some transposition of the alphabetical order).

To me the book seems far from up-to-date. For example, the key for identifying bacteria published in the textbook of Sirockin & Cullimore (1969) is used, leading to the bizarre situation in which a 1984 book on food microbiology mentions *Beggiatoales*, *Caryophanales* and *Hyphomicrobiales* but apparently not *Arthrobacter* or *Brevibacterium*, *Campylobacter*, or *Yersinia*.

I was interested to find that the Einhorn saccharometer is 'indispensable to microbiological laboratory procedures', that a 2% agar solution is prepared by adding 200 g agar to 1000 ml distilled water, that Gram's staining method does not need the use of a counter stain, that one should wait for as long as 10 min for a positive oxidase reaction, that *Salmonella* is aerobic though *E. coli* is a facultative anaerobe; and to learn about the Csiszár and Demeter method, the Dreier-Korolev method, Polazaev's impinger, Losonczy's tube test and so on.

The Editor of the book hopes that the manual will be found extremely useful by all food microbiologists in industrial and research laboratories and claims that it is a book that has been needed for some time. Unfortunately I cannot agree. In fact I cannot deduce the reason for publication of an English version of this book, particularly in view of the availability of what the authors themselves describe as the 'excellent handbook' of Harrigan & McCance (*Laboratory Methods in Food and Dairy Microbiology*) and so I will detain the reader no longer.

R.W.A. Park

Food Microbiology. Ed. by A.H. Rose.
(Economic Microbiology, Volume 8)
London: Academic Press, 1983. Pp. xiii + 298. ISBN 0 12 596558 3. £34.00.

The Editor sees this collection of reviews of specific topics as dealing with the battle against unwanted microbial activity in foods, mainly from the point of view of application of preservation and processing methods. Treatment of foods by low or high temperature, chemicals, antibiotics, irradiation, or reduction of water activity are considered in two-thirds of the book; *Vibrio parahaemolyticus*, food-borne viruses, and spoilage by lactic acid bacteria being considered in the remainder. Authors have apparently been given considerable freedom to develop their subject; for example we read in chapter 3 about the modes of action of various antibiotics, only to read about the same topic in chapter 4, in greater detail. The book has good print and binding, and satisfactory subject and author index (though this constitutes more than 10% of the pages of the book). Extensive reference lists are given at the end of each chapter. Diagrams, the two photographs, and the tables (apart from an error in the title of Table 8) are satisfactory.

All the chapters have something for those concerned with research or development in food microbiology. Unfortunately the report of Smelt *et al.* on growth of *Clostridium botulinum* on certain foods as low as pH 4.0 apparently came too late for inclusion in the book and so one reads the traditional statements about the organism not growing below pH 4.5. A detailed discussion of membrane fluidity leads to the conclusion that there is a useful future in food preservation for amphiphatic molecules (molecules which partition into phospholipid bilayers and cause alterations to membrane structure and function). However it was surprising that the chemiosmotic hypothesis and its use to explain toxicity of weak acids was hardly mentioned. The potential usefulness of medically important antibiotics as food preservatives in developing countries was indicated by pointing out that an extension of storage life of fish by 3 days would facilitate transport of such food to people who might otherwise be undernourished. Some confusing statements in the otherwise good chapter on irradiation escaped the Editor: "The 12*D* value is defined as the dose required for 90% inactivation of one $\log_{10}$ decrease"; "Three log cycles in solid food will inactivate viruses in one minute at 71°C". It seems that use of low and intermediate levels of irradiation of foods to stop sprouting and to kill important portions of the microbial flora is likely to increase. It was interesting to read about lactic acid bacteria as rogues—spoiling milk and dairy products, packaged meats (when low temperature, presence of salts and high CO_2 concentration favour them), food from plants, alcoholic beverages, marinated herring, and salad dressing. Detailed consideration of pathogens is restricted to *Vibrio parahaemolyticus* and viruses; they form an interesting contrast. *V. parahaemolyticus*, which up to 1977 caused 50–70% of Japan's cases of bacterial food poisoning (more recent data, after discovery of *Campylobacter jejuni* as an important cause of enteritis, are not given), can grow in some foods with a generation time as low as 12 min at 30°C. Viruses, of course, cannot grow in foods and depend on contamination alone to facilitate their spread. One can see that the food industry has a lot of thinking to do about virology. Should foods be tested routinely for the presence of viruses—an expensive and difficult procedure (unless one eats the food)—or should things be left as they are, with epidemiology being the source of information?

If read as a review journal this book has value, but it does not provide the coverage to justify its title. One must hope that students are not attracted, thinking it is a treatise

on food microbiology. Presumably the *Economic Microbiology* series, of which this is said to be the final volume, has been a successful marketing venture. Neverthless, I imagine that few readers will find it necessary to have their own copy of this book.

R.W.A. Park

Coffee: Botany, Biochemistry and Production of Beans and Beverage. Ed. by M.N. Clifford and K.C. Willson.
Beckenham: Croom Helm, 1985. Pp. xiii + 457. ISBN 0 7099 0787 7. £35.00.

There can be little doubt that there has been need for an up-to-date comprehensive text on all aspects of coffee for many years. Our knowledge of many areas of the science of coffee has improved dramatically over the last decade and now is an appropriate time to bring this knowledge together. Clearly, it is a very ambitious task to attempt to cover all aspects of coffee in a single text and this may be seen as both the strength and weakness of the book. The first nine chapters, after a short historical introductory chapter, cover various topics which may be jointly considered as agronomy, that is to say botanical classification, breeding, effect of climate and soil, physiology of coffee crop, plant nutrition, cultural methods and pest and disease control. The general standard of these chapters, bearing in mind the limited space available, is excellent with the individual experts obviously well in control of their material. Some of the diagrams have been excessively reduced and I thought my son (age 5 years) had had a hand in at least one of them. Chapter 10 is concerned with green coffee processing, i.e. the conversion of coffee cherries to green coffee beans in the country of origin and together with chapter 14 (technology of converting green coffee into beverage) comprise the only coverage of coffee technology in the book. The main unit process operations, roasting, grinding extraction etc., are well described but the less familiar reader may well have appreciated a few diagrams, or even photographs as in other parts of the book. Chapter 11 describes the very complex organization of the World Coffee Trade and it is pleasing to see a readable account of how bodies, such as the International Coffee Organization, function. It is questionable whether this chapter would not have been better positioned as chapter 2. The topic of microscopy is elegantly covered in chapter 12 and I am sure it is not appreciated just how much information can be obtained by, for example, selective staining. The limitations of modern printing do not do justice to the photomicrographs, some of which (or at least some similar to these) were recently shown at the 11th ASIC Colloquium in Togo.

Chapter 13 tackles the daunting task of describing the chemical and physical aspects of coffee and its product in some fifty-four pages. The result must be a compromise, but all the major compounds are discussed in as much detail as space permits and the chapter is extensively referenced. The final chapter addresses the emotive topic of physiology and potential toxicity of coffee in a factual and objective manner, whilst stating the need for further *scientific* investigations, particularly into the effect of 'extra caffeinic substances'.

The book is finally 'rounded-off' with a glossary of the more complex words and abbreviations used. The editors have doubtless succeeded in bringing together a vast amount of data on many facets of coffee. The present reviewer would like to have seen

proportionately more space given to the chemistry and technology of coffee and possibly more mention of coffee substitutes. In fact the major readership for the book may well be amongst chemists and technologists who want a good comprehensive coverage of the *other facets* of the subject, e.g. agronomy. The standard of presentation is generally good and it is excellently referenced throughout; at £35 it is a book that most 'coffee scientists' will want to purchase.

R. Macrae

Food Analysis: Principles and Techniques. Volume 2. Physicochemical Techniques. Ed. by Dieter W. Gruenwedel and John R. Whitaker.
New York: Marcel Dekker, 1984. Pp. xvi+591. ISBN 0 8247 7128 6 8. US$107.50/ SFr. 244.00.

Physical chemistry, its theoretical and mathematical techniques are, to many food scientists and technologists, a source of mystery and fear and so one should voice approval for the efforts of the editors, in producing a series which tries to bring a little light into an area of darkness for so many.

The rationale for the treatise of eight volumes is to meet the need for a detailed treatment of the use of physical and biological theory and techniques in modern food analysis. As a reference for undergraduates and as a source of information for postgraduates, the series will certainly meet this criterion.

Volume 2 of the series entitled physicochemical techniques is written by contributors from the U.S.A., Germany and the U.K. covering widely divergent topics such as temperature measurement, solubility, viscosity, the light microscope in food analysis, ultra violet and visible spectrophotometry, optical activity and structure of biological molecules, fluorescence and phosphorescence, electron paramagnetic resonance spectroscopy, atomic absorption, infra red spectrophotometry in food technology, the application of Raman spectroscopy to the characterization of food and, finally, size exclusion chromatography with low angle laser light scattering detection.

To cover all these topics in a readable manner and to include a historical perspective, theoretical treatment and a clear mathematical layout, together with references up to 1981 and some 'in press' to 1985 in a book of such length, is a matter of praise for the Editors. The explanations and comments on interferences and interpretation are very useful and show the experience of the contributors, and will act as a guide to postgraduates who are entering the field of food analysis and, perhaps, sharpen their critical facilities in considering the use of a particular technique for determining a particular constituent or parameter.

I do object to the inclusion of tables comparing the prices of instruments, since both prices and models are changed so quickly as to make the information meaningless and rendering the table as if it originated from *Which* magazine.

In my opinion this volume would be an asset to students studying for an M.Sc in Food Analysis, since it is very clear with a no-nonsense, down-to-earth approach. The tables and diagrams are uncluttered and clear, giving information sought at a glance which, in a busy food laboratory, can save time and energy in literature searches.

The index is adequate and the layout and type easy to read but, in these 'hard times',

the price will preclude its purchase by students. Incidentally, although it may be exceptional, the reviewer's copy was bound back-to-front; perhaps a section on quality assurance would not come amiss.

S. Landsman

Glucose syrups: Science and Technology. Ed. by S.Z. Dziedzic and M.W. Kearsley.
Barking: Elsevier Applied Science, 1984. Pp. x+276. ISBN 0 85334 299 7. £35.00.

Although information on the science and technology of glucose syrups is scattered through the literature, to date no single text has been available which covers all aspects of glucose syrups. This book is therefore a welcome addition to the literature. The book is multi-authored and divided into seven chapters. The contents include the raw material, starch, as well as the production and physical, chemical and physiological properties of glucose syrups and hydrogenated glucose syrups. The analysis and use of glucose syrups is also described. Together the contents form a valuable source of information on the science and technology of glucose syrups which, in addition to being a useful reference book, will be of particular benefit to food scientists and technologists involved in the production, analysis and utilization of glucose syrups.

Where coverage of certain topics is brief the reader is referred to suitable reviews and publications for further details. This is particularly true for chapter 1 where starch composition, structure, biosynthesis and physico-chemical properties are mentioned briefly before a useful and detailed description is given of starch processing, both in general and with specific reference to starches from different sources. Although as the Editors suggest 'some overlap between chapters in the book is inevitable' there is generally no serious duplication of information. However, the section on enzyme catalysed degradation of starch in chapter 1, is explained far more thoroughly in chapter 2, where an excellent account is given of the enzymic production of glucose syrups together with a comparison of the acid and enzyme conversion processes.

Chapter 3 discusses the production, properties and uses of hydrogenated glucose syrups and points out the advantages offered by these syrups over normal glucose syrups. The physico-chemical properties of glucose syrups are discussed in chapter 4 together with some examples of how these properties may differ in hydrogenated glucose syrups.

Chapter 5 on the physiological aspects of glucose syrups and related carbohydrates is not always clear, particularly with reference to the diagrams. For example Fig. 2(b) shows the decrease in blood maltitol although the text suggests a reference to no sorbitol being detected; Table 3 does not show blood glucose levels after 90, 120 and 150 min despite the text suggesting so, and Fig. 8 shows a decrease in serum triglycerides but the text suggests an increase in the serum triglycerides for the maltitol syrup diet. Figures 9 and 10 would appear to be the wrong way round. Stricter editing of this chapter would have been of benefit.

In contrast, chapter 6 on the analysis and characterization of glucose syrups was a delight to read. Traditional analyses of glucose syrups for moisture, dextrose equivalent and trace components are covered, followed by a detailed discussion on the use of GC and LC for determining the distribution of saccharides in glucose syrups. The

final chapter describing the uses of glucose syrups in the food industry is disappointing in that the author has relied heavily on a previous publication by Howling and skimps over the possible uses of high fructose corn syrups. Nevertheless this chapter does illustrate the diverse application of glucose syrups in the food industry.

The book is well laid out and clearly printed but contains several typographical errors. Each chapter has its own set of up-to-date references. The index is comprehensive. No tables on the physical properties of glucose syrups have been included as such information is readily available elsewhere. In conclusion this book provides a valuable collecton of up-to-date information and references on the science and technology of glucose syrups.

M.J. Saunders

PORIM Technology ISSN 0127 0257. No. 9. **The Practice of Frying.** By Kurt G. Berger. Pp. iv + 34. No. 10. **Hydrogenation.** By M.S.A. Kheiri. Pp. v + 52.
No. 11. **Citric Acid in the Processing of Oils and Fats.** By K.S. Law and K.G. Berger. Pp. iv + 32.
Each M$2.50 from the Palm Oil Research Institute of Malaysia, P.O. Box 10620, Kuala Lumpur, Malaysia.

These publications represent practical guides for food processors. They present outlines of the underlying principles, but they pay particular attention to the practical details and problems likely to be encountered in the processes. The booklets have been prepared and published by PORIM (Palm Oil Research Institute of Malaysia) which has acquired considerable expertise in the applications of oils and fats. The use of palm oil and fats derived from palm oil is emphasized.

Publication No. 9, entitled 'The Practice of Frying' gives useful information about the equipment used in deep fat frying and practical aspects of the frying operation. Oil deterioration is discussed, and possible analytical tests for monitoring oil quality are described. Guidance is given for the selection of oils for frying and antioxidants to stabilize the oils. The booklet is generally accurate and provides a useful introduction to the frying of foods.

Publication No. 10 entitled 'Hydration' covers the principles, equipment used, factors affecting the process, process control, products formed by the hydrogenation of palm oil and the nutritional value of partially hydrogenated products. Much useful information is contained within the booklet, but it is the least satisfactory of the three publications. The presentation is generally not as clear as it should be, and in particular several diagrams are used without sufficient explanation of the information they convey. The hydrogenation mechanism quoted is rather uninformative, and it would have been preferable if the author had described the Horiuti-Polyanyi mechanism which can explain the formation of trans fatty acids and positional isomers.

There are also rather too many errors in this booklet. Thus Fig. 23 is entitled 'Effect of Temperature on Selectivity Ratio' but it consists of a plot of selectivity ratio against pressure at a fixed temperature. On page 3, the fatty acids are numbered in an unconventional fashion starting at the methyl end of the molecule, and indeed one fatty

acid does not have the correct number of carbon atoms shown. (However, it is understood that a revised version of No. 10 is in preparation.)

Publication No. 11, entitled 'Citric Acid in the Processing of Oils and Fats', covers a narrow area compared with the other two booklets. The properties of citric acid are described and this is followed by the mechanism for the autoxidation of oils and fats. The role of citric acid in retarding oxidation is discussed and practical details concerning the addition of citric acid during refined are included. Several examples of the effectiveness of citric acid both in the presence and absence of TBHQ are described. This booklet represents a useful discussion of this important aspect of the stabilization of oils and fats.

The publications are relatively low in cost, and all individuals interested in the processing of oils and fats would be well advised to purchase a copy of each of the three booklets.

M.H. Gordon

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SI UNITS

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

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Printed by Adlard and Son Ltd, Bartholomew Press, Dorking