Volume 12 Number 1 February 1977

Journal of Food Technology

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

// JOURNAL OF FOOD TECHNOLOGY
Institute of Food Science and Technology (U.K.)

Editor
H. LIEBMANN

Associate Editors
E. C. BATE-SMITH G. H. O. BURGESS J. G. DAVIS
D. PEARSON H. S. YOUNG

Publications Committee
D. A. HERBERT (Chairman)
J. F. HEARNE (Vice-Chairman)
P. WIX (Secretary)
JUDITH V. RUSSO (Editor of the Proceedings)
A. S. ALISON
A. E. BENDER
W. B. CHAPMAN
R. HARPER
S. M. HERSCHDOERFER
BETTY C. HOBBS
S. D. HOLDSWORTH
H. JASPERSON
T. L. PARKINSON
P. SHERMAN

Contributions and editorial correspondence should be sent to Dr H. Liebmann, c/o Research and Development Department, Metal Box Ltd, Twyford Abbey Road, London NW10 7XQ.

General correspondence should be sent to Dr P. Wix, Polytechnic of the South Bank, Borough Road, London S.E.1 and items for the Proceedings to Mrs J. V. Russo, 2 Hexham Gardens, Isleworth, Middlesex TW7 5JR.

Objects of the Journal. The Journal covers a wide field ranging from pure research in the various sciences associated with food to practical experiments designed to improve technical processes. While the main object is to provide a forum for papers describing the results of original research, review articles are also included. The Editor welcomes papers from food scientists and technologists. These must be of a high standard of original research or comprehensive reviews of specialized sections of food science or technology.

Business matters, including correspondence and remittances relating to subscriptions, back numbers, advertising and offprints, should be sent to the publishers: Blackwell Scientific Publications Ltd, Osney Mead, Oxford OX2 0EL.

The Journal of Food Technology is published bimonthly, each issue consisting of 90–120 pages; six issues form one volume. The annual subscription is £35.00 (U.K.), £42.00 (Overseas), $110.00 (N. America) post free. Back volumes are still available.

The Institute of Food Science and Technology of the United Kingdom was established in 1964. It is a professional qualifying organization having the following grades of membership; Fellows, Associates, Licentiates and Students. Application forms and information about the Institute can be obtained from the Honorary Secretary, Mr N. D. Cowell, National College of Food Technology, St George's Avenue, Weybridge, Surrey.

J. Fd Technol. (1977) 12, 1–8

Volatile constituents of white bread crust

D. J. FOLKES* AND J. W. GRAMSHAW†

Summary

A total of 190 volatile components were identified in essences prepared from white bread crust (in twenty cases the identification is provisional only); of these compounds, ninety-seven have not previously been reported as constituents of white bread. A limited number only, of the compounds now reported, may be partially responsible for the crusty notes which are present in bread aroma.

Introduction

In view of continuing interest in the volatile constituents of foodstuffs, we wish to record the preliminary results of our investigations into bread aroma. A detailed report, together with an assessment of the aroma of bread crust essences and the contribution which identified components make to the collective aroma, will appear later.

Freshly baked bread derives its characteristic aroma and, to a lesser extent, its flavour from volatile compounds formed during fermentation and baking. Indeed as Baker, Parker & Fortmann (1953) emphasized, fermentation followed by the formation of a brown crust is essential to the development of a full aroma and flavour. Thus, a conventionally fermented dough may be 'baked', by internal electrical resistance heating, without formation of a crust, but the product has a mild yeasty flavour, deficient in aroma (Baker & Mize, 1939). Similarly, chemically leavened doughs, when subjected to normal oven baking, have been reported to yield breads having very bland flavours (Miller, McWilliams & Matz, 1959; Wiseblatt & Kohn, 1960; Jackel & Ersoy, 1961) or no flavour (Chichester, Sharrah & Simone, 1960; Simone, Sharrah & Chichester, 1962). These, and other aspects of bread flavour formation are fully described in a comprehensive review by Collyer (1964).

Authors' addresses: * The Lord Rank Research Centre, High Wycombe, Bucks HP12 3QR and † Procter Department of Food and Leather Science, The University of Leeds, Leeds LS2 9JT.

ห้องสมุด กรมวิทยาศาสตร์
-4.ค.ค. 2520

Compounds formed during fermentation and by Maillard-type reactions in the crust during baking doubtless contribute directly to the overall aroma of bread. However, it seems almost certain that organoleptically important constituents are formed by reactions which occur in the browning crust and involve precursors derived by fermentation. Experiments involving model systems (S.K. Berry & J.W. Gramshaw unpublished) support this view and have indicated that both acidic and non-acidic constituents of dough are important in the generation of crusty aromas.

Modern plant-produced bread is often considered to be somewhat deficient in flavour and aroma, this probably being a reflection of the short baking period normally employed which is insufficient for full flavour development, although adequate quantities of the relevant precursors may well be present. This investigation was therefore undertaken in an attempt to identify volatile compounds present in bread crust and, in particular, those responsible for crusty bread-like aroma notes. Such knowledge is essential before the formation of bread aroma can properly be understood and any logical attempt made to increase the flavour of bread produced in plant bakeries. It was thus appropriate to choose baking conditions as close as possible to those used for a traditional loaf.

Crust rather than whole bread was chosen for the preparation of essences, because, as the site at which organoleptically important constituents of the aroma complex form during baking, it contains these materials in highest initial concentration and thus much less material needs to be processed. It is necessary, however, to separate the crust before an appreciable proportion of these volatiles have an opportunity to migrate into the crumb. Use of crust alone gives a further advantage in that it produces essences which contain a much smaller proportion of the major fermentation volatiles, although ethanol and amyl alcohol nevertheless remain very prominent constituents of crust essences.

Materials and methods

Preparation of bread crust essences

Bread was produced using a standard formulation specifying flour, water, yeast and salt only. Initially, bleached flour was used, but in later experiments the flour was unbleached. The dough was bulk fermented, split, proved and baked as Coburgs (which are large round crusty loaves, normally baked on the oven sole or on a flat metal tray), rather than in a deep metal baking container, so that the maximum proportion of crust was formed. Baking time, in steam, was 40–45 min, i.e. well beyond the 25–35 min of commercial practice, in order to provide a thick dark brown crust of optimum flavour. Volatile constituents were isolated in a number of ways, of which the following was strongly preferred. As soon as possible after baking, the crust was separated from the crumb, finely comminuted and extracted with purified diethyl ether. Acidic

components, which otherwise complicate the GLC pattern and interfere in the subsequent mass spectrometric examination, were removed from the ethereal extract and examined separately. Removal of acids and water was achieved simultaneously by stirring with a mixture of anhydrous sodium carbonate and anhydrous sodium sulphate. Solids were removed by filtration and the extract was freed of ether and subjected to vacuum distillation in a closed system at a pressure of 0·01 Torr using the apparatus described by Lea, Swoboda & Hobson-Frohock (1967).

The distillate thus obtained was a pale yellow oil which retained its powerful crusty, bread-like aroma for considerable periods when stored at −20°C.

Absence of acids from the essence produced only a minor change in the overall aroma, although the isolated acidic fraction possessed a rich burnt note which probably contributes to a small extent to the total aroma of bread.

Instrumental examination of bread crust essences

In the early stages of the investigation, bread essences were subjected to gas-liquid chromatography (GLC), using a variety of stationary phases. A linearly temperature programmed oven and either a flame ionization detector (FID) or an electron capture detector (ECD) were employed and identification of constituents was made by comparison of their retention data with those of reference compounds. Certain major components were recovered from column effluents by trapping and the identities of these were confirmed from infrared spectra. A double beam grating instrument (Perkin-Elmer 237) was used and each sample was presented in solution (microcell) or as a film on a sodium chloride plate.

Later, essences were examined by linked gas-liquid chromatography–mass spectrometry (GLC–MS), using a fast scanning low-resolution mass spectrometer (Cronin, Nursten & Woolfe, 1972–73) and the identities of a considerable number of components were thus established (or confirmed) by comparison of their mass spectra and retention data with those of reference compounds. In this connection, porous layer open tubular (PLOT) columns (Cronin, 1970) and wide bore capillary columns, coated with a selection of stationary phases, were extensively employed, usually in conjunction with a FID. Further valuable information was gained by the use of element-specific chromatographic detectors. A nitrogen-specific alkali FID (AFID) and a flame photometric detector (FPD), used in the sulphur mode, were separately employed in conjunction with GLC–MS. GLC was also performed using both detectors simultaneously by splitting the effluent between them.

Sensory examination of bread crust essences

In addition to instrumental examination, effluents from the columns most frequently used were submitted to 'nasal appraisal'. This was performed by

splitting the effluent between the instrumental detector and an odour port and writing descriptions of odour notes, as they emerged, on the chromatogram. In this way, particular odour notes were associated with the appropriate regions of the chromatogram and the most important regions were selected for more intensive study.

Nasal appraisal was also carried out in conjunction with the AFID and the FPD in order to correlate the elution of compounds containing nitrogen and/or sulphur with regions of bread-like aroma.

Results

Constituents identified in bread crust essences

The compounds which were detected in bread crust essences are listed in Table 1. Identities were assigned on the basis of retention data, positive responses (where relevant) to element-specific detectors and, in almost all cases except carboxylic acids and phenol, by mass spectra. A few major components (19, 23–25, 68, 70, 75, 77, 83, 112, 119, 120, 122) were also recognized on the basis of their infrared spectra. In a few cases identification is tentative, either because authentic compounds were not available for comparison or because the constituents were present at levels too low to give an unequivocal mass spectrum. Such exceptions are indicated in Table 1.

Sensory evaluation of essences

When the essences were subjected to nasal appraisal, a very large number of distinct aroma notes were perceived, very many of which had no obvious relationship to bread aroma although in total they may exert a minor influence on the overall aroma. However, twelve regions of the chromatograms were clearly associated with distinct and strong bread-like aroma notes. These, and the compounds associated with them, will be described in a later publication.

Discussion and conclusion

Several isolation techniques were examined in addition to that described above. This method was selected because it gave the optimum recovery of volatiles important to bread crust aroma, as judged on the basis of the following criteria:

(a) the resulting essence had the strongest overall crusty aroma;

(b) the resulting essence revealed the greatest number of bread-like aroma notes upon nasal appraisal and these notes were also more intense than those from essences prepared in other ways;

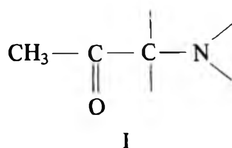
(c) the extracted crust had the weakest residual bread-like odour.

It also gave an essence free of non-volatile material, thus reducing the possibility of artefact information upon injection as well as permitting a greater degree of concentration.

A total of 170 compounds were identified in bread crust essences and a further twenty were provisionally identified. Of these, twenty-four have already been found in rye bread or crispbread, but not in white bread, and seventy-three have not previously been reported in wheat or rye breads (Table 1). Previously recorded volatile constituents of breads have been summarized by Maga (1974) and by Rothe (1974). The intricacy of gas chromatographic traces derived from the essences and the complexity of many of the mass spectra recorded during the investigation indicate, however, that many more compounds remain to be identified.

Nasal appraisal of bread crust essences during gas chromatography indicated twelve distinct, well separated, regions of the chromatogram to be associated with crusty or bread-like aroma notes. Hunter *et al.* (1969) detected only two regions of biscuit-like aroma during chromatography of white bread volatiles isolated via bisulphite complexes and Mulders (1973) found only one region of the gas chromatographic effluent to contain crusty or bread-like aroma notes. Bread aroma, therefore, appears to be a much more complex problem than may previously have been supposed.

The structural feature,



I

where the nitrogen atom (and in most cases, the adjacent carbon atom) forms part of a heterocycle is present in a number of compounds, many of which exhibit sponge cake-, biscuit- or cracker-like aromas in dilution. One of these compounds, 1,4,5,6-tetrahydro-2-acetylpyridine, has been claimed (Hunter *et al.*, 1969) to occur in and contribute to the aroma of American white pan bread. However, 1,4,5,6-tetrahydro-2-acetylpyridine was not detected in bread crust during the present investigation, although an isomer, tetrahydro-3-acetylpyridine, was tentatively identified and may contribute to the aroma of bread crust. Slight evidence only was also obtained for the presence of 2-acetylpyridine, which is known (Buttery *et al.*, 1971) as a breakdown product of 1,4,5,6-tetrahydro-2-acetopyridine and has a similar, although weaker, aroma. The aroma of 2-acetylthiazoline, a component of beef broth, has been described as bread-like (Tonsbeek, Copier & Plancken, 1971). The present authors, however, consider that it is an aroma typical of compounds possessing the partial structure I, rather than truly bread-like in character. Inconclusive evidence only was obtained relating to the presence of 2-acetylthiazoline in crust essences.

Table 1. Compounds identified in bread crust essences

No.	Compound
	Hydrocarbons
1	octane (*)
2	limonene
3	toluene
4	*o*-xylene (*)
5	*o*-ethyltoluene (*)
6	*m*- and/or *p*-ethyltoluene (*)
7	mesitylene (*)
8	ψ -cumene (*)
9	hemimellitene (*)
10	*o*-diethylbenzene (*)
	Alcohols
11	ethanol
12	1-propanol
13	1-butanol
14	1-pentanol
15	1-hexanol
16	1-octanol (+)
17	1-nonanol (*)
18	2-methyl-1-propanol
19	3-methyl-1-butanol (f)
20	2-ethyl-1-hexanol
21	2-pentanol
22	3-pentanol
23	2-phenethanol (f)
24	2,3-butanediol (meso) (f)
25	2,3-butanediol (laevo) (f)
26	benzyl alcohol (c)
	Ketones
27	2-propanone
28	2-butanone
29	2-pentanone
30	2-hexanone
31	2-heptanone
32	2-octanone (+)
33	2-nonanone

No.	Compound
55	2-ethylhexanal
56	benzaldehyde
57	2-phenylacetaldchydc
58	but-2-enal
59	pent-2-enal
60	non-2-enal
61	2-methylbut-2-enal (*)
62	3-methylbut-2-enal (*)
63	hexa-2,4-dienal
64	hepta-2,4-dienal (+)
65	deca-2,4-dienal
	Acids and Phenol
66	acetic
67	propionic
68	butanoic (f)
69	pentanoic
70	hexanoic (f)
71	heptanoic (*)
72	octanoic
73	nonanoic (*)
74	decanoic
75	2-methylpropanoic(f)
76	2-methylbutanoic (*)
77	3-methylbutanoic (f)
78	2-ethylbutanoic (*)
79	4-methylpentanoic (*)
80	propenoic (*)
81	3-methylbut-2-enoic (*)
82	4-oxopentanoic
83	benzoic (f)
84	phenol (+)
	Esters, lactones and ethers
85	methyl formate (*)
86	ethyl formate
87	ethyl acetate

No.	Compound
	Furanoid and pyranoid compounds
110	2-methylfuran
111	2-n-pentylfuran
112	2-furfuryl alcohol (f)
113	2-acetylfuran
114	2-propionylfuran (+)
115	dihydro-2-methyl-3-(2H)-furanone
116	1-(2′-furyl)-propan-1, 2-dione
117	1-(2′-methyl-5′-furyl) -propan-1,2-dione (+)
118	2-acetyl-3-hydroxyfuran (isomaltol)
119	3-hydroxy-2-methyl-1,4-pyrone (maltol) (f)
120	2-furfural (f)
121	5-methyl-2-furfural
122	5-hydroxymethyl-2-furfural (f)
123	2-furoic acid (*)
124	2-furfuryl formate
125	ethyl-2-furfuryl ether
126	2,2′-difurfuryl ether (+)
	Heterocyclic nitrogen compounds
127	pyrazine (+)
128	2-methylpyrazine
129	2,3-dimethylpyrazine
130	2,5-dimethylpyrazine
131	2,6-dimethylpyrazine (*)
132	2-ethylpyrazine
133	2,3,5-trimethylpyrazine (+)
134	2-ethyl-3-methylpyrazine

No.	Compound
151	2-methyl-6,7-dihydro-(5H)-cyclopenta-pyrazinc (*) (b)
152	5-methyl-6,7-dihydro-(5H)-cyclopenta-pyrazine (*)
153	5,6,7,8-tetrahydroquin-oxaline (*)
154	2-(2′-furyl)-pyrazine (*)
155	2-methyl-5(or 6)-(2′ furyl)-pyrazine
156	2-acetylpyrazine (*)
157	pyrrole
158	2-methylpyrrole (*)
159	"N"-methylpyrrole
160	"N"-*n*-propylpyrrole (*) (b)
161	"N"-furfurylpyrrole
162	2-acetylpyrrole
163	"N"-acetonylpyrrole (*) (b)
164	2-formylpyrrole
165	2-formyl-"N"-methyl-pyrrole (+)
166	2-formyl-5-methylpyrrole (+)
167	2-formyl-"N"-furfuryl-pyrrole (+)
168	1-pyrroline (*)
169	2-pyrrolidinone (γ-butyrolactam) (*)
170	pyridine
171	2-methylpyridine
172	2-acetylpyridine (*) (e)
173	3-acetylpyridine (*)
174	2-formylpyridine (*)
175	tetrahydro-3-acetyl-pyridine (*) (b)

No.	Compound
34	2-dodecanone (*)
35	3-methylbutan-2-one (a)
36	cyclopentanone (*) (a)
37	2,3-butanedione (diacetyl)
38	2,3-pentandione
39	1-hydroxy-2-propanone (acetol) (*)
40	2-hydroxy-3-butanone (acetoin)
41	3-penten-2-one
42	2-cyclopenten-1-one
43	3-methylcyclopent-2-en-2-ol-1-one (*)
	Aldehydes
44	propanal
45	butanal
46	pentanal
47	hexanal
48	heptanal
49	octanal
50	nonanal
51	dodecanal (+)
52	2-methylpropanal
53	3-methylbutanal (+2-methylbutanal)
54	2-ethylbutanal (*)
88	ethyl octanoate (+)
89	ethyl nonanoate (*)
90	ethyl decanoate (*)
91	ethyl undecanoate (*)
92	ethyl dodecanoate (+)
93	2-methyl-2-propyl acetate (*)
94	ethyl phenylacetate
95	ethyl 4-oxopentanoate
96	2-phenethyl formate (+)
97	2-phenethyl acetate (+)
98	1-acetoxy-2-propanone (acetonyl acetate)
99	1,2-diacetoxyethane (+)
100	γ-butyrolactone
101	γ-hexalactone
102	γ-nonalactone (+)
103	but-2-enoic acid-γ-lactone
104	4-methylbut-2-enoic acid-γ-lactone (*)
105	1,1-diethoxyethane
106	1,1-diethoxynonane (*)
107	1,1-diethoxyundecane (*)
108	1,1-diethoxy-3-methylbutane (*)
109	2,4,5-trimethyl-1,3-dioxolane (*) (b)
135	2-ethyl-5-methylpyrazine (*)
136	2-ethyl-6-methylpyrazine (+)
137	2-propylpyrazine (+)
138	tetramethylpyrazine (*)
139	2,3-diethylpyrazine (*) (b)
140	2,5-diethylpyrazine (*) (b)
141	2,6-diethylpyrazine (*) (b)
142	2,5-dimethyl-2-ethylpyrazine (+)
143	2,6-dimethyl-3-ethylpyrazine (*) (b)
144	2-methyl-6(and/or 5)-propylpyrazine (*) (b)
145	2,5-dimethyl-3-isopropylpyrazine (*) (b)
146	vinylpyrazine (+)
147	propenylpyrazine (*) (b)
148	2-methyl-6(and/or 5)-vinylpyrazine (+) (b)
149	2-methyl-6(and/or 5)-propenylpyrazine (*) (b)
150	6,7-dihydro-(5H)-cyclopentapyrazine (*) (b)
	Sulphur compounds
176	dimethyl sulphide
177	dimethyl disulphide
178	thiophene (*)
179	2-methylthiophene (*)
180	3-methylthiopropanal (*) (d)
181	2,5-dimethylthiophene (*)
182	2-acetylthiophene (*)
183	2-formylthiophene
184	2-formyl-5-methylthiophene (*)
185	2-thiophenemethanol (*) (d)
186	benzthiophene (*)
	Heterocyclic compounds containing nitrogen and sulphur
187	benzthiazole (*)
188	2-methylbenzthiazole (*)
189	2-acetylthiazoline (*) (e)
190	5,7-dihydrothieno-[3,4b]-pyrazine (*) (b)

(*) Not previously identified in wheat or rye breads;
(+) previously only identified in rye bread or rye crispbread.
(a) Previously identified as a wheat flour constituent;
(b) tentative; authentic sample not available for comparison;
(c) tentative; insufficient material present to give unambiguous mass spectrum;
(d) tentative; mass spectra of bread constituent and authentic compound could not be obtained due to losses in spectrometer inlet system;
(e) very tentative; insufficient material present to penetrate into spectrometer (authentic compound gave adequate spectrum at concentrations much higher than those possibly present in bread crust essences);
(f) also identified via ir spectrum.

Compounds of the type represented by structure I do occur in bread crust and, bearing in mind that other compounds present may exert a marked synergistic effect, they may well account for a part of the characteristic bread aroma complex. It seems reasonable, however, to suppose at this stage that much of bread aroma, particularly the crusty notes, may be due to compounds other than those which possess the partial structure I.

Acknowledgments

The authors wish to thank Professor A. Spicer for initiating this project and for his encouragement, Dr H. E. Nursten for his advice and encouragement, Dr J. Edelman, Director of Research, for his approval of the publication of this paper and R.H.M. (Research) Ltd, for generous financial support.

References

Baker, J.C. & Mize, M.D. (1939) *Cereal Chem.* **16**, 295.
Baker, J.C., Parker, M.K. & Fortmann, K.L. (1953) *Cereal Chem.* **30**, 22.
Buttery, R.G., Seifert, R.M., Guadagni, D.G. & Ling, L.C. (1971) *J. Agric. Fd Chem.* **19**, 969.
Chichester, C.O., Sharrah, N. & Simone, M. (1960) *Fd Technol., Champaign,* **14**, 653.
Collyer, D.M. (1964) *Bakers' Dig.* **38**, (1), 43.
Cronin, D.A. (1970) *J. Chromat.* **48**, 496.
Cronin, D.A., Nursten, H.E. & Woolfe, M.L. (1972–73) *Int. J. Mass Spectrom., Ion Phys.* **10**, 47.
Hunter, I.R., Walden, M.K., Scherer, J.R. & Lundin, R.F. (1969) *Cereal Chem.* **46**, 189.
Jackel, S.S. & Ersoy, E. (1961) *Bakers' Dig.* **35**, (4), 46.
Lea, C.H., Swoboda, P.A.T. & Hobson-Frohock, A. (1967) *J. Sci. Fd Agric.* **18**, 245.
Maga, J.A. (1974) *Crit. Rev. Fd Technol.* **5**, 55.
Miller, J.A., McWilliams, G.S. & Matz, S.A. (1959) *Cereal Chem.* **36**, 487.
Mulders, E.J. (1973) *Z. Lebensmittelunters. u.-Forsch.* **151**, 310.
Rothe, M. (1974) *Handbuch der Aroma Forschung : Aroma von Brot,* pp. 10-14. Akademie-Verlag, Berlin.
Simone, M., Sharrah, N. & Chichester, C.O. (1962) *Fd Technol., Champaign,* **16**, 93.
Tonsbeek, C.H. Th., Copier, H. & Plancken, A.J. (1971) *J. Agric. Fd Chem.* **19**, 1014.
Wiseblatt, L. & Kohn, F.E. (1960) *Cereal Chem.* **37**, 55.

(Received 12 July 1976)

J. Fd Technol. (1977) 12, 9–26

Methylated amino acids as indices in meat products

II. Further examination of protein sources and the practical application of methylamino acid titres in predicting meat content

W. R. D. RANGELEY AND R. A. LAWRIE

Summary

The content of protein-bound 3-methylhistidine (in relation to total nitrogen) was determined in a number of proteins. The titre was higher in chicken and turkey, lower in clupeine and elasmobranch fish, than the value hitherto found to be characteristic of mammalian flesh. 3-methylhistidine was present in offal in which muscular tissue was a minor constituent. The amino acid was absent from products fabricated from non-meat proteins; but N^{ϵ}-methyllysine was found in some of these.

Certain meat products contain substances which interfered with the determination of 3-methylhistidine. This difficulty was overcome by prior dialysis and solvent extraction, permitting the use of the 3-methylhistidine titre to evaluate lean meat content.

Introduction

In the previous paper (Rangeley & Lawrie, 1976), titres of *protein-bound* 3-methylhistidine were found to be similar for all common meat species examined Additionally, *soluble* 3-methylhistidine, possibly in the form of a dipeptide (in conjunction with β-alanine), was identified in samples of pork and whale. This was readily removed by a prior washing procedure, to leave residual levels characteristic of *protein-bound* 3-methylhistidine. The titre of N^{ϵ}-methyllysine showed a greater variation within a species. Before either or both methylamino acids could be recommended as unequivocal indices of meat content, other muscle sources require examination.

It is thought that, although the 3-methylhistidine content of actin is constant (Johnson, Harris & Perry, 1967; Asatoor & Armstrong, 1967), that of myosin is rather more dependent on muscle type, and indeed higher 3-methyl-

Authors' address: Food Science Laboratories, Department of Applied Biochemistry and Nutrition, University of Nottingham School of Agriculture, Sutton Bonington, Loughborough, Leics. LE12 5RD.

histidine levels are found in white than red skeletal muscle (Johnson & Perry, 1970), whilst 3-methylhistidine is absent from cardiac and foetal myosins (Kuehl & Adelstein, 1970). Furthermore, since the basic premise demands the absence of methylamino acids from non-meat proteins, and although their presence has not yet been established in such sources (Rangeley & Lawrie, 1976; Hibbert & Lawrie, 1972), extended examination of products fabricated from non-meat protein to simulate meat is clearly important.

Such considerations form the basis of the present paper.

Materials

All meat samples were obtained fresh from the local butcher. Chicken and turkey samples were obtained frozen. Products fabricated from non-meat protein and meat products were donated privately.

Methods

The identification and determination of methylamino acids in miscellaneous animal sources

Samples of rabbit, chicken, turkey, fish species and meat offals were prepared for analysis by ion exchange chromatography for methylamino acid content by the method of Rangeley & Lawrie (1976).

The identification and determination of methylamino acids in vegetable protein products

The examination of such products was carried out on the dehydrated form. The meat-like chunks were first crushed to a powder, and methylamino acid analysis carried out on both the intact material and its protein fraction isolated by precipitation. The latter step was performed by solubilizing the crushed powder at pH 12 using conc. NaOH, and subsequently reprecipitating and isolating the protein residue by the addition of conc. HCl to pH 4, followed by centrifugation.

Portions of both the crushed whole powder and the isolated protein residue were then hydrolysed as for meat samples (Rangeley & Lawrie, 1976). The hydrolysate was reduced in volume to 5 ml, using a rotary evaporator, and a 2 ml aliquot taken for ion exchange chromatography. The eluant fractions corresponding to the suspected methylamino acid peaks eluting after lysine and histidine were collected and reduced in volume. Analysis of these eluant fractions by ion exchange chromatography was carried out, both on the fraction alone and in the presence of a known concentration of the respective methylamino acid. In order to lessen interference with the methylamino acid determinations, an alternative extraction procedure was employed for the further examination of samples C, D and H (Table 5).

A 20-g powdered portion was homogenized with 60 ml distilled water for

1 min. The solution was centrifuged at 30 000 g for 30 min. After discarding the supernatant the residue was homogenized with 4 vol. 75% ethyl alcohol in 0.1 N HCl for 1 min, and recentrifuged. The residue was suspended in 5 vol. acetone for 3 hr, centrifuged and homogenized in 5 vol. acetone for 1 min. This latter operation was repeated twice, and after centrifugation, the powder was dried at 45–50°C. Aliquots of the powder were then taken for hydrolysis and nitrogen determination. A 0.5-g sample was hydrolysed (200 ml 6 N HCl for 72 hr), and a 2-ml aliquot of the volume reduced hydrolysate (6 ml) analysed by ion exchange chromatography.

The identification and determination of methylamino acids in retail meat products

From previous experiments (Rangeley & Lawrie, 1976) and from preliminary investigations carried out in the present study using a pork sausage sample, the need for prior dialysis and washing treatments with certain meat products of unknown composition was evident.

Such a treatment was initially employed for the examination of sausage samples (A–F), and pie samples (A–F). A 20-g portion, taken from the sum contents of six similar samples, was homogenized in 3 vol. distilled water for 2 min, and the resulting suspension dialysed against distilled water for 24 hr. After centrifugation the residue was again placed in 3 vol. distilled water and homogenized. This procedure was repeated twice more, and aliquots of the residue taken for hydrolysis and the determination of collagen and nitrogen.

When a 2-ml aliquot of the total hydrolysate (6 ml) for each sausage sample was analysed by ion exchange chromatography, only the presence of a N^{ϵ}-methyllysine peak was noted: most surprisingly no 3-methylhistidine peak was evident. The addition of known amounts of both methylamino acids confirmed this observation. In contrast, pie samples (A–F) (Table 7), given a similar washing procedure (the dialysis stage was omitted), contained both N^{ϵ}-methyllysine and 3-methylhistidine.

Since it was evident that a pre-dialysis and washing procedure did not permit a determination of 3-methylhistidine content in sausages, an alternative extraction procedure, as used for the previous examination of non-meat protein products, was considered. The procedure was similar to that previously described, except that the pre-dialysis treatment was still employed. Aliquots of the resultant protein powder were taken for hydrolysis, collagen determination and nitrogen determination. All sausage samples (A–F) were examined by this method, as were beefburger and rissole samples.

Results

The 3-methylhistidine and N^{ϵ}-methyllysine content of samples from animal sources

From the results in Tables 1 and 3, the 3-methylhistidine titres of rabbit and gadoid fish species are seen to be similar to those of beef and lamb (i.e.

Table 1. 3-methylhistidine and N^{ϵ}-methyllysine in rabbit, chicken and turkey muscles

Sample	3-methyl-histidine (mg/g N)	N^{ϵ}-methyl-lysine (mg/g N)	Ratio 3-mehis.: N^{ϵ}-melys.
Rabbit (thigh) A	6.35	2.81 4.19	2.26 1.52
Rabbit (thigh) B	4.82	2.86	1.68
Chicken (leg) A	10.28	1.86 1.29	5.53 7.97
Chicken (leg) A washed x3 sarcoplasmic	9.46	–	–
Chicken (leg) B	6.15	3.87	1.62
Chicken (breast)	8.44	3.16	2.67
Turkey (breast) A	7.44	2.15	3.46
Turkey (breast) B	8.15	2.43	3.35

All titres are mean values calculated from duplicate determinations.

5–6 mg/g N). Those of clupeine and elasmobranch species have a lower 3-methylhistidine content (3.8–4.8 mg/g N), both before and after fat extraction. However, after the extraction of fat by hexane, titres of 3-methylhistidine increased by a similar order in one sample of fatty and non-fatty species examined (Table 3).

The 3-methylhistidine contents of chicken and turkey, however, are markedly higher than those of beef and lamb, varying from 6 to 10 mg/g N. Preliminary washing did not lower these values, suggesting that, unlike pig and whale muscles, this is not due to the presence of non-protein bound 3-methylhistidine. Further studies with hot water extracts taken from rabbit, chicken and turkey also did not indicate the presence of a 3-methylhistidine dipeptide (Tables 2b, 2c and 2d). In all three samples the only histidine dipeptides identified were carnosine and anserine (β-alanyl-1-methylhistidine). 3-methylhistidine, as expected, was not present in the hot water extracts of chicken and rabbit when examined by ion exchange chromatography (Table 2a). However, soluble 3-methylhistidine was indicated in the hot water extract of turkey, prior to hydrolysis, but not after. This finding is difficult to explain since 3-methylhistidine is stable during hydrolysis. Its presence in the hot water extract, however, together with a subsequent decrease in the 3-methylhistidine titre of the whole sample (Table 2a), suggests some of the free amino acid may have been present.

Table 2a. The occurrence and identification of soluble 3-methylhistidine in chicken, rabbit and turkey using ion exchange chromatography

Sample	Hot water extract (μmol/g)	3-methylhistidine Hot water extract (hydrolysed) (μmol/g)	Whole hydrolysate (mg/g N)
Rabbit (thigh) B	None detected	None detected	4.82
Chicken (leg) A	None detected	None detected	10.28
Turkey (breast) A	0.19	None detected	7.44

Table 2b. The Rf values and colour of known dipeptides and their constituent amino acids using paper chromatography

Standard	Rf value	Colour
Carnosine	0.41	Green
Anserine	0.75	Green
Histidine	0.17	Purple
β-alanine	0.40	Blue-purple
1-methylhistidine	0.54	Green
3-methylhistidine	0.75	Grey-purple

The 3-methylhistidine content of various offals are shown in Table 4. It is absent from kidney, lung and blood serum protein (in the form of spun fibres). A small amount was detected in liver, which possibly reflects soluble 3-methylhistidine formed from the detoxification of histidine. The titres for heart and stomach were lower than the value characteristic of lean meat. In the case of heart the titre reflects the probable absence of 3-methylhistidine from cardiac myosin (it is present in cardiac actin). The low value for stomach (not corrected for connective tissue) is not unexpected since there is muscular tissue in its constitution. Cleansed stomach (boiled tripe) in which the concentration of muscular tissue is higher, reflected this in the markedly high 3-methylhistidine titre.

Table 2c. The identification of histidine dipeptides present in chicken, rabbit and turkey using paper chromatography

Sample	Spot 1 Rf colour	Spot 2 Rf colour	Suggested dipeptides present
Rabbit (thigh) B	0.43 Green	0.74 Green	Spot 1 Carnosine Spot 2 Anserine
Chicken (leg) A	0.35 Green	0.73 Green	Spot 1 Carnosine Spot 2 Anserine
Turkey (breast) A	0.41 Green	0.70 Green	Spot 1 Carnosine Spot 2 Anserine

Table 2d. The analysis of histidine dipeptides from rabbit, chicken and turkey using paper chromatography

Sample	Amino acid spots obtained from the hydrolysis of spot 2 in Table 2c		Suspected amino acids present
	Spot 2a Rf colour	Spot 2b Rf colour	
Rabbit (thigh) B	0.49 Purple	0.56 Green	Spot 2a β-alanine Spot 2b 1-methylhistidine
Chicken (leg) A	0.29 Purple	0.47 Green	Spot 2a β-alanine Spot 2b 1-methylhistidine
Turkey (breast) A	0.33 Purple	0.51 Green	Spot 2a ρ-alanine Spot 2b 1-methylhistidine

Titres for N^{ϵ}-methyllysine in rabbit, chicken, turkey and fish species (Tables 1 and 3) are similar to those found for mammalian muscle (Rangeley & Lawrie, 1976). Titres varied between different offals. None was found in kidney. Values were low in stomach (<1 mg/g N), whilst high in tripe (>5 mg/g N). Two types of N^{ϵ}-methyllysine were detected in offals, chicken and species of fish.

The 3-methylhistidine and N^{ϵ}-methyllysine content of vegetable protein products

The results for the analysis of nine non-meat protein products (all of which were soya based except A, which was a field bean product) are shown in Table

Table 3. 3-methylhistidine and N^{ϵ}-methyllysine in fish muscles

Sample	3-methyl-histidine (mg/g N)	N^{ϵ}-methyl-lysine (mg/g N)	Ratio 3-mehis.: N^{ϵ}-melys.
Cod (gadoid) White, non-fatty No fat extracted	6.11	2.37 2.90	2.58 2.11
Lemon sole (gadoid) Non-fatty Fat extracted	5.85	3.18	1.83
Plaice (gadoid) Non-fatty Fat extracted	6.31	4.17	1.51
Plaice (gadoid) Non-fatty No fat extracted	5.79	3.82 4 95	1.51 1.17
Mackerel (clupeine) Fatty No fat extracted	4.41	3.17	1.39
Mackerel (clupeine) Fat extracted	4.81	3.96	1.22
Herring (clupeine) Fatty Fat extracted	3.80	2.34	1.62
Skate (elasmobranch) Fat extracted	3.90	3.37	1.16

All titres are mean values calculated from duplicate determinations.

5. A peak eluting after histidine was found only in samples C, D, F, G and H, whilst one eluting after lysine was found in all samples except B and G. When the non-meat protein was further isolated by re-precipitation, the results showed (Table 5) that the peak eluting after histidine was present only in Samples C and D. However, the peak after lysine eluted in all samples except G, H and I.

When known concentrations of the authentic methylamino acids were added to hydrolysates from both whole and re-precipitated samples, only in the case of N^{ϵ}-methyllysine was the elution position of the presumed and authentic peak similar. Thus it would appear that, although N^{ϵ}-methyllysine may be present in certain non-meat protein samples, 3-methylhistidine is absent. Nevertheless a peak did appear after histidine, and also after 3-methylhistidine, in samples C, D, F, G and H; and as such warranted closer investigation. All results

หอสมุด กรมวิทยาศาสตร์

Table 4. 3-methylhistidine and N^{ϵ}-methyllysine in meat offals

Sample	3-methyl-histidine mg/g N	N^{ϵ}-methyl-lysine mg/g N	Ratio 3-mehis.: N^{ϵ}-meyls.
Kidney (lamb)	None detected	None detected	–
Liver (pig)	0.80	3.52	0.23
Lung (pig)*	None detected	2.21 3.83	–
Stomach (pig)*	2.00	0.91	2.20
Tripe (ox) A	4.77	–	–
Tripe (ox) B washed ×2	4.12	5.42 8.07	0.76 0.51
Stomach (sheep)*	2.73	0.81 1.38	3.37 1.98
Heart (ox)	2.83	2.70	1.05
Blood serum proteins (fibres)	None detected	2.77	–

*Nitrogen content not corrected for connective tissue (Collagen).
All titres are mean values calculated from duplicate determinations.

shown in Table 5 were calculated on the assumption that the suspected peaks are N^{ϵ}-methyllysine and 3-methylhistidine.

From the analysis of the fractions containing the suspected methylamino acid peaks, the absence of a significant 3-methylhistidine peak was confirmed in samples C and D (Table 6): only in sample H did a noticeable peak remain. However, on the addition of authentic 3-methylhistidine to the fraction concerned, no increase in concentration above that added occurred (Table 6), thus suggesting that the unknown peak is indeed not 3-methylhistidine. The presence of N^{ϵ}-methyllysine was confirmed in samples C and H; however, a peak was not detected in sample D, and no increase in its concentration occurred when authentic N^{ϵ}-methyllysine was added.

In view of the previous results regarding the presence of methylamino acids

Table 5. The analysis of products fabricated from non-meat protein

Sample	Whole sample		After re-precipitation	
	Presumed '3-methyl-histidine' (mg/g N)	Presumed 'N^{ϵ}-methyl-lysine' (mg/g N)	Presumed '3-methyl-histidine' (mg/g N)	Presumed 'N^{ϵ}-methyl-lysine' (mg/g N)
Spun protein A	None detected	2.54	None detected	1.34
Spun soya fibres B	None detected	None detected	–	–
Textured veg. protein C	1.71	0.64	0.65	1.47
Soya protein powder D	0.96	0.56	2.93	1.33
Soya chiplets E	None detected	1.71	None detected	1.92
Textured veg. protein F	1.10	1.70	None detected	0.98
Textured veg. protein G	0.37	None detected	None detected	None detected
Textured veg. protein H	1.96	2.19	None detected	None detected
Textured veg. protein I	None detected	1.11	None detected	None detected

in vegetable protein products, an alternative extraction procedure was chosen in the hope of eliminating possible interfering compounds and thus of providing a firmer basis for future studies. The results shown in Table 6 again suggest the presence of N^{ϵ}-methyllysine in samples C, D and H, which would thus appear to be an integral part of their (non-meat) proteins. Concentrations, however, were lower than found in mammalian muscle and only one type appears to be present. 3-methylhistidine was again absent from samples D and H; however, a small amount was found in sample C (<0.5 mg/g N). Thus even if this

Table 6. The identification of the suspected 3-methylhistidine and N^{ϵ}-methyllysine peaks in products fabricated from non-meat protein

Sample (vegetable protein product)	Peak found to elute after histidine (suspected 3-mehis.)				Peak found to elute after lysine (suspected N^{ϵ}-melys.)			
	Fraction collected (ml)	Concentration (μmol)		After acetone – alcohol extraction (mg/g N)	Fraction collected (ml)	Concentration (μmol)		After acetone alcohol extraction (mg/g N)
		Fraction alone	Fraction added 3-mehis (0.5 μmol)			Fraction alone	Fraction + added N^{ϵ}-melys (0.3 μmol)	
Textured veg. protein C	205–230	0.005	0.52	0.31	130–148	0.06	0.41	1.33
Soya protein powder D	210–235	0.007	0.51	None detected	134–150	None detected	0.33	1.66
Textured veg. protein H	212–238	0.113	0.47	None detected	133–146	0.22	0.49	1.33

peak were 3-methylhistidine, which in light of previous evidence would appear doubtful, it is not present in significant amounts. Moreover, it is always possible that some meat protein, could have been added to the so-called vegetable product.

The determination of 3-methylhistidine and N^{ϵ}-methyllysine in various products and their practical application in predicting meat content

When sausage and meat pie samples were given a pre-dialysis and water washing treatment, only in samples of the latter were both 3-methylhistidine and N^{ϵ}-methyllysine titres found (Table 7). Titres for 3-methylhistidine varied between the different pies, and as expected in Pie E (which was known to contain solely non-meat protein), no 3-methylhistidine was detected. The N^{ϵ}-methyllysine titre in this sample was higher than previously found for non-meat protein products. A decrease in the 3-methylhistidine content was not always accompanied by a decrease in the N^{ϵ}-methyllysine content.

Since, in the initial investigations, only a N^{ϵ}-methyllysine determination proved to be possible with sausage samples, it was necessary to assume either that 3-methylhistidine had become unavailable during the preparation of samples for hydrolysis, or during hydrolysis itself. Thus additional experiments were carried out to establish how possible losses of 3-methylhistidine might have occurred.

(i) *Losses of methylamino acids during hydrolysis of a commercial sausage sample.* The chromatographic analysis of sausage sample H, prepared by pre-dialysis and washing, did not show the presence of a 3-methylhistidine peak (Table 8). Examination of a similar sample, containing a known amount of 3-methylhistidine (1.25 μmol) (added prior to hydrolysis), revealed that only the amount added was found. Furthermore the elution position of the hydrolysed authentic 3-methylhistidine was similar to that of added authentic 3-methylhistidine.

(ii) *Interaction of carbohydrate sources and methylamino acids in sausages.* When known amounts of authentic 3-methylhistidine and N^{ϵ}-methyllysine were hydrolysed with different carbohydrate sources used in sausage manufacturing, the following results were obtained.

(a) *Sausage starch.* Neither methylamino acid was found to react with sausage starch recovered during the washing procedure (Table 9).

(b) *Native wheat starch (12% solution).* Only 50% of the original concentration of 3-methylhistidine was recovered (Table 9), thus suggesting that a reaction does take place between native wheat starch and 3-methylhistidine. When the latter was added to the mixture *after* hydrolysis, however, it was recovered quantitatively.

(c) *Wheat rusk.* (As used as an ingredient in sausage samples A–F.) No reaction between 3-methylhistidine and wheat rusk appeared to take place, although the concentration of N^{ϵ}-methyllysine did decrease (Table 9).

Table 7. 3-methylhistidine and N^{ϵ}-methyllysine in commercial meat pies

Sample	3-methyl histidine (mg/g N)	N^{ϵ}-methyl lysine (mg/g N)	% lean meat* (based on 3-mehis. value 6 mg/g N)
Pie A steak and kidney	5.30	3.25	88
Pie B minced beef	4.74	3.79	79
Pie C steak and kidney	4.09	4.80	68
Pie D steak and kidney	5.21	3.91	87
Pie E non-meat protein	None detected	5.57	–
Pie F steak and kidney	3.23	1.54	54

* This value refers to that proportion of the nitrogen content derived from lean meat, but does not represent the lean meat content of the whole product.

It would thus appear that 3-methylhistidine preferentially reacts with pure wheat starch, and that such a reaction must occur during the sample preparation procedure.

Determination of 3-methylhistidine and N^{ϵ}-methyllysine content in sausage samples after alcohol-acetone extraction

Sausages (Table 10) and samples of beefburger and rissole (Table 11) were successfully analysed using this procedure. For sausage samples A-F, the 3-methylhistidine titres were found to vary as expected, since these examples contain meat and non-meat protein in various proportions. However, sample G, which is an unknown commercial sample, gave a very low 3-methylhistidine titre, suggesting that little meat protein was present. 3-methylhistidine titres for both the beefburger and the rissole samples were higher than found in sausages.

Titres for N^{ϵ}-methyllysine were more varied, and a low 3-methylhistidine

Table 8. The loss of methylamino acids during hydrolysis of a commercial sausage sample (pork)

Treatment	3-methylhistidine		N^{ϵ}-methyllysine		
	Total concentration detected in sample (μmol)	Concentration of authentic acid present in sample (μmol)	Total concentration detected in sample (μmol) mono	tri	Concentration of authentic acid present in sample (μmol)
Sausage sample H (washed ×3)	None detected	None present	0.13	0.21	None present
Sausage sample H (washed ×3). Authentic 3-mehis. added prior to hydrolysis (1.25 μmol)	0.52	0.50	0.16	0.28	None present
Sausage sample H (washed ×3) Authentic 3-mehis. added prior to hydrolysis (1.25 μmol) Authentic 3-mehis. (0.3 μmol) and N^{ϵ}-melys. (0.3 μmol) added to 2 ml hydrolysate	0.81	0.80	0.48	0.35	0.30 (assumed to be N^{ϵ}-mono-methyllysine)

All determinations were made from analysis of a 2-ml sample taken from the total hydrolysate (5 ml).

Table 9. The effect of carbohydrates (as found in pork sausage) on the determination of 3-methylhistidine and N^{ϵ}-methyllysine

Carbohydrate and hydrolysis components	3-methylhistidine		N^{ϵ}-methyllysine	
	Amount detected in sample (μmol)	Authentic present in sample (μmol)	Amount detected in sample (μmol)	Authentic present in sample (μmol)
Sausage starch and added 3-mehis. and N^{ϵ}-melys.	0.50	0.50	0.31	0.30
Wheat starch (12%) and added 3-mehis. and N^{ϵ}-melys.	0.15	0.33	0.18	0.20
Wheat starch (12%): 3-mehis. and N^{ϵ}-melys. added before *and* after hydrolysis	0.61	0.83	0.33	0.55
Wheat rusk (alone)	None detected	—	None detected	—
Wheat rusk + added 3-mehis. and N^{ϵ}-melys.	0.84	0.83	0.52	0.60

All titres are mean values calculated from duplicate determinations.

Table 10. 3-methylhistidine and N^{ϵ}-methyllysine in sausage samples prepared after acetone-alcohol extractions

Sample	3-methyl-histidine (mg/g N)	N^{ϵ}-methyl lysine (mg/g N)	% lean meat* (based on 3-mehis. value 5 mg/g N)
Sausage A	4.69	1.76 2.06	78
Sausage B	4.81	1.68	80
Sausage C	3.91	2.12	65
Sausage D	3.71	2.39	62
Sausage E	4.20	1.74 2.05	70
Sausage F	4.31	2.51 2.65	72
Sausage G	1.78	0.81 1.21	30

* This value refers to that proportion of the nitrogen content of the meat product derived from lean meat, but does not represent the lean meat content of the whole product.

Table 11. 3-methylhistidine and N^{ϵ}-methyllysine in beefburger and rissole

Sample	3-methyl-histidine (mg/g N)	N^{ϵ}-methyl-lysine (mg/g N)	% lean meat* (based on 3-mehis. value 5 mg/g N)
Beefburger	5.61	3.08	93
Rissole	4.79	2.94 4.77	80

* This value refers to that proportion of the nitrogen content derived from lean meat, but does not represent the lean meat content of the whole product.

titre was not always accompanied by a corresponding decrease in the N^{ϵ}-methyllysine content. N^{ϵ}-methyllysine titres, however, are complicated by the presence of both N^{ϵ}-mono and N^{ϵ}-trimethyllysines.

The practical application of the 3-methylhistidine index in predicting meat content

From the results previously presented (Rangeley & Lawrie, 1976), and from those presented in this paper, it would appear that 3-methylhistidine provides a more reliable index of meat content than does N^{ϵ}-methyllysine, and

that a concentration of 6 mg of protein-bound 3-methylhistidine/g N is characteristic for samples consisting entirely of beef, lamb, pork, whale and rabbit species. On this basis, results for the apparent meat content of a number of commercial sausages, meat pies and other products, which were previously examined, are presented in Tables 7, 10 and 11 respectively. It is emphasized that the percentages shown are indicative of lean meat content (not including intramuscular fat and connective tissue) expressed in terms of the protein content and not in terms of the whole product. Low titres, due to the presence of large amounts of connective tissue, have been avoided by applying a suitable correction to the nitrogen value.

The estimated meat contents of the sausage samples are shown in Table 10, and none of the sausages examined show a lean meat content greater than 80%. Since protein binders (e.g. rusk) form an acceptable constituent of the sausage, not all protein present would be reflected as that of lean meat. Similar considerations would apply to beefburger and rissole samples (Table 11), where, although meat contents are higher than found in the sausages, rusk and additional sources of non-meat protein (e.g. breadcrumbs and wheat flour) are accepted ingredients of the product.

The results in Table 10, however, do suggest that in certain samples (i.e. sample G), some replacement of lean meat by non-meat protein had taken place.

From the examination of the pie samples (Table 7), higher meat contents were found than in sausages. The presence of acceptable protein sources, such as kidney (which contains no 3-methylhistidine), however, leads to estimates of lean meat contents of less than 100%.

Discussion

The 3-methylhistidine titres determined in the various meat products have been shown to serve as a predictive index of meat content, when related to the 3-methylhistidine content characteristic of mammalian muscle. They appear more suited to this purpose than do titres of N^{ϵ}-methyllysine, of which the content not only varies both within and between species, but whose determination is complicated by the presence of more than one type. Moreover, N^{ϵ}-methyllysine, unlike 3-methylhistidine, was found to be present in certain vegetable protein powders examined.

The results presented in this paper indicate that the procedure previously suggested for the examination of meat products (Rangeley & Lawrie, 1976) is not suitable when applied directly to products which contain large amounts of cereal fillers and binders (e.g. sausages). When, however, steps are taken to remove all possible interfering compounds using an alcohol-acetone extraction method, such products can be analysed accurately for 3-methylhistidine content. The amended procedure, by ensuring the removal of all soluble nitrogen sources (and thus soluble 3-methylhistidine), to leave a sample

containing protein, would appear to be desirable in future work since it could be used when examining all types of meat products. It would also enable 3-methylhistidine factors for different species to be expressed as mg/g protein N, rather than mg/g total N, as was previously the case.

The values for protein-bound 3-methylhistidine appear to be markedly higher in avian muscle than in those of meat. This may represent an increased degree of methylation of the myosin in birds. On the other hand, Johnson & Perry (1970) suggest that the content of 3-methylhistidine is generally higher in white, than in red, skeletal muscle. The fact that not all chicken samples contain high 3-methylhistidine contents (Table 1), suggests that the phenomenon is not consistent, and as such warrants further investigation.

Further deviations from the characteristic protein-bound 3-methylhistidine content were found on the examination of different fish species (Table 3). Although titres for gadoid species are similar to those for mammalian muscle (i.e. 6 mg/g N), those of clupeine and elasmobranch species are lower (i.e. 4 mg/g N). This variation could well reflect a difference in the methylating system. Recently Shenouda & Pigott (1975) stated that 3-methylhistidine was absent from actin in rockfish (an elasmobranch). Such might also be the case for clupeine species.

It has now been confirmed that 3-methylhistidine has a practical application in predicting meat contents in meat products. In order to enhance the significance of such an application, further work needs to be undertaken to develop the method, whereby the characteristic concentration of protein-bound 3-methylhistidine in lean meat can be more firmly established, and hence this index can be applied with confidence to a wide variety of processed and unprocessed meat products. Such investigations will form the basis for subsequent publications.

Acknowledgment

This work was supported from the J. Sainsbury Centenary Grant and this assistance is gratefully acknowledged.

References

Asatoor, A.M. & Armstrong, M.D. (1967) *Biochem. Biophys. Res. Commun.* **26**, 168.
Hibbert, I. & Lawrie, R.A. (1972) *J. Fd Technol.* **7**, 333.
Johnson, P., Harris, C.I. & Perry, S.V. (1967) *Biochem. J.* **105**, 361.
Johnson, P. & Perry, S.V. (1970) *Biochem. J.* **119**, 293.
Kuehl, W.W. & Adelstein, R.S. (1970) *Biochem. biophys. Res. Commun.* **39**, 956.
Rangeley, W.R.D. & Lawrie, R.A. (1976) *J. Fd Technol.* **11**, 143.
Shenouda, S.J.K. & Pigott, G.M. (1975) *J. Fd Sci.* **40**, 520.

(Received 14 May 1976)

J. Fd Technol. (1977) 12, 27–35

A study of the composition of three popular varieties of fish in Israel, with a view towards further processing

S. ANGEL* AND R. C. BAKER†

Summary

The weight proportions and proximate composition of fillets and remains after filleting of carp, silver carp and hake were investigated with a view to further processing of these fish, which are usually sold whole or gutted in the fresh or frozen state.

From the percentage of fillets obtainable, the proximate composition of the fillets, the remains after filleting, and the organoleptic qualities of the flesh, it is concluded that hake and silver carp fillets, and a combination of their deboned flesh, offer good possibilities for producing high-quality convenience products that could lead to increased consumption of these fish in Israel.

Introduction

The Israel fish processing industry produces almost exclusively several canned products: sardines, tuna, gefilte fish and silvercarp slices. Products such as ready-to-heat fish fingers or breaded sticks, steaks, fillets, scallops, chowders, etc., which are common fare in Europe and the U.S.A. are not produced.

In order to promote increased fish consumption and thereby replace some of the costly imported meat, high quality convenience products should be produced. The A.R.O. Division of Food Technology recently embarked on a research programme to develop convenience products from locally available pond, lake and sea fish, which are normally purchased live, iced or frozen on the local market. A knowledge of the chemical and physical composition and such characteristics as water holding capacity and emulsification capacity of the proteins is essential for decision making on the types of products that could best be produced and the best means of processing the various types of fish.

In this, the first of a series of papers on the research undertaken, the pro-

Authors' addresses: *Division of Food Technology, Agricultural Research Organization, The Volcani Center, P.O.B. 6, Bet Dagan, Israel (contribution no. 182-E, 1976 series) and †Food Science and Marketing Institute, 100 Rice Hall, Cornell University, Ithaca, New York, U.S.A.

portions by weight of the various parts of the fish are compared. Such information should be of economic interest to the processor. The proximate chemical compositions are needed in order to formulate comminuted and emulsified products, and for comparing and controlling the nutritive quality of products from various fish species.

Materials and methods

Carp (*Cyprinus carpio*) is the most popular pond fish. It is retailed live at between 500 g and approximately 1.5 kg. Silver carp (*Hypophthalmichthys molitrix*) was introduced from south-east Asia over five years ago and is a pond and lake fish in Israel. It is retailed iced at weights ranging from 1 to 2 kg. The meat has a bland taste. Hake (*Merluccius merluccius*), or bakala in Hebrew, is caught in the south-east Atlantic and gutted, beheaded and frozen at sea. It is retailed frozen at 250 to 500 g as single fish or frozen slices.

All the fish were purchased on the local market in sizes representative of marketable fish and gutted, headed, filleted and skinned in the laboratory. Each part of the fish – head, viscera, fillets, skin and remains after filleting – was weighed to the nearest 0.5 g. There were liquid and slight trimming losses during the cutting of the parts. Skins were pulled from the fillets. Adhering traces of flesh were removed from the skin and discarded. Following skinning and weighing the fillets and remains were chopped in a Moulinette meat chopper (Moulinex, France) in the following manner: 150 g of –3°C meat was placed in the chopper and chopped for 3 sec, three times, at 5- to 10-sec intervals. Following chopping, aliquots were taken for chemical analysis and the remaining chopped sample was placed in a tightly covered sample jar and either frozen or kept at 0°C. Where necessary, further aliquots were taken for analysis from these jars in the following manner: half the meat in the jar was emptied into the Moulinette chopper, and the other half was used to wipe up the drip in the jar before being placed in the chopper. The entire sample was rechopped three times for 3 secs as done originally.

The water, fat, ash and protein contents of the sample were determined by the rapid analysis Ultra X-70 apparatus (Gronert, West Germany). This is an infrared moisture tester and rapid ashing device. Random samples were also tested by conventional methods according to the A.M.I. (1950). All tests were carried out in duplicate. With the Ultra X-70 method the sample was dried in a quartz dish by infrared radiation for determination of water content. Following this, the dried material was quantitatively scraped from the quartz dish and pulverized. Upon being replaced in the dish the pulverized dry material was washed a number of times with ether to extract the fat. After an additional drying by infrared, the fat content was calculated. The remaining material was ashed and the ash was weighed. The difference in weight was considered to be crude protein. This agreed with the Kjeldahl determination.

The significance of the results was determined by analysis of variance

Table 1. Weights of the parts used to prepare fillets of carp and silver carp

Date (1975)	Fish species and identification number	Whole fish (g)	Weights of parts (% of whole fish)					
			Head	Viscera	Remains after filleting	Fillets with skin	Skin	Fillets without skin
	Carp							
17.IX	I	625	15.0	9.4	32.0	40.4	4.7	32.0
17.IX	II	748	19.4	11.9	32.5	34.4	5.2	27.3
1.X	I	1678	17.4	12.1	27.0	41.7	5.6	35.7
1.X	II	1472	.20.1	11.7	24.2	43.0	6.8	34.9
1.X	III	1738	20.1	17.4	20.1	38.9	5.6	32.8
1.X	IV	1442	21.4	13.6	23.6	40.0	7.0	32.7
Mean (%)			18.9	12.7	26.6	39.7	5.8	32.6
Standard deviation			2.31	2.99	4.92	2.99	0.90	2.94
	Silver carp							
17.IX	I	1197	26.0	7.4	25.7	40.2	4.2	35.2
18.IX	II	1090	24.1	10.0	24.9	39.3	4.6	34.5
1.X	I	2187	23.4	13.1	25.0	37.8	4.0	33.8
1.X	II	1387	25.6	11.3	22.8	40.0	4.8	35.4
1.X	III	1405	25.8	8.4	29.0	39.0	5.0	33.6
1.X	IV	1630	24.4	9.0	23.2	43.6	5.9	38.4
1.X	V	1347	29.8	10.8	24.0	35.4	3.9	31.1
Mean (%)			25.6	10.0	24.9	39.3	4.6	34.6
Standard deviation			2.10	1.93	2.06	2.49	0.69	2.21

(Alder & Roessler, 1964). Comparisons were made between the weights of the various parts of the fish for the three species, proximate composition of the fillets and the remains after filleting between the species, and the proximate composition between the fillets and the remains after filleting within each species of fish.

Results

Table 1 shows the percentage by weight of various parts of the whole fish from which fillets of carp and silver carp were made. Table 2 shows the percentage by weight of fillets and remains after filleting of the gutted and beheaded carp, silver carp and hake. Due to liquid and slight trimming losses the weights of the parts do not necessarily total 100%.

In Table 1 the weights of silver carp heads averaged 25.5% of the whole weight and that of carp averaged almost 19%, the differences were significant. The differences in the weights of the viscera for the two species were not significant.

Fillets with skin accounted for approximately 40% of the weights of the whole carp and silver carp, and fillets without skin averaged approximately 33% of the whole weight (Table 1). Skin was approximately 5% of the weight of the whole fish for both species. The weight of the fillets without skin are not exactly the weight of the fillet with skin less the weight of the skin due to the discarding of adhering traces of flesh.

There was no significant difference in the weight percentage of the fillets of carp and silver carp, based on the whole fish weight. The percentage of bones and meat remains averaged c. 25% after filleting for carp and silver carp, based on the weight of the whole fish. There were no significant differences between the two species.

The percentage weights of the skinned fillets for the gutted, beheaded fish averaged 45% for carp, 54% for silver carp, and 61% for hake (Table 2). The differences between the three species were highly significant.

The percentage remains after filleting of the gutted and beheaded fish averaged 38.5% for carp and silver carp and 33% for hake, with no significant difference between carp and silver carp or between carp and hake, but the difference between silver carp and hake was significant.

The proximate compositions are presented in Table 3 for the fillets without skin and the remains after filleting, for the three species of fish in this study.

The protein content of the fillets averaged from 16.7 to 18.9%; by analysis of variance, the differences between the three species were not significant.

The protein content of the remains after filleting averaged from 12.7 to 19.6% for the three species. That of silver carp was significantly higher than of carp, but there was no significant difference between carp or silver carp and hake.

Table 2. Weights of the parts, used to prepare filleted carp, silver carp and hake

Date (1975)	Fish species and identification number	Weight of parts (% of gutted, beheaded fish)				
		Gutted and beheaded weight (g)	Remains after filleting	Fillets with skin	Fillets without skin	Skin
	Carp					
19.IX	I	450.0	44.4	55.6	44.4	6.5
17.IX	II	500.4	48.6	51.3	40.8	7.8
1.X	I	1152.7	39.3	60.6	52.0	8.1
1.X	II	989.1	36.0	64.0	51.9	10.1
1.X	III	1025.0	34.0	65.9	55.6	9.5
1.X	IV	917.1	37.1	62.8	51.2	8.5
Mean (%)			39.9	60.0	49.3	8.4
Standard deviation			5.55	5.54	5.54	1.27
	Silver carp					
17.IX	I	784.0	39.2	61.3	53.7	6.4
18.IX	II	751.0	38.2	63.3	55.7	7.3
1.X	III	1373.0	39.8	60.2	53.8	6.4
1.X	II	871.0	36.3	63.6	56.0	7.6
1.X	III	927.3	40.9	59.1	50.9	7.9
1.X	IV	1088.8	32.9	66.2	57.5	8.8
1.X	V	800.1	40.4	59.6	52.3	6.6
Mean (%)			38.2	61.9	54.3	7.3
Standard deviation			2.81	2.57	2.92	0.89
	Hake					
17.IX	I	340	29.4	–	65.0	6.0
17.IX	II	312	34.6	–	60.9	4.6
17.IX	III	314	36.3	–	58.0	4.4
17.IX	IV	278	33.1	–	62.2	4.6
Mean (%)			33.3		61.5	4.9
Standard deviation			2.93		2.91	0.74

Table 3. Proximate composition (in percentage) of fillets (without skin) and bone and flesh remains after filleting of carp, silver carp and hake

Date (1975)	Fish species and identification number	Fillets (%)				Bone and flesh remains after filleting (%)			
		Water	Fat	Protein	Ash	Water	Fat	Protein	Ash
	Carp								
17.IX	I	73.8	7.8	16.7	1.6	60.3	24.7	12.7	2.3
17.IX	II	75.6	6.6	17.2	0.6	–	–	–	–
1.X	I	74.2	8.3	16.7	0.8	–	–	–	–
1.X	II	71.8	12.1	15.2	0.7	55.2	28.6	12.4	3.8
1.X	III	69.1	13.1	16.8	1.6	59.2	25.2	13.6	2.0
1.X	IV	74.2	7.4	17.8	1.0	–	–	–	–
Mean (%)		73.1	9.2	16.7	1.0	58.2	26.1	12.9	2.7
Standard deviation		2.30	2.69	0.86	0.44	2.68	2.12	0.62	0.96
	Silver carp								
17.IX	I	72.8	8.4	17.2	1.6	50.4	9.2	21.6	18.8
1.X	I	76.8	5.8	16.3	1.0	62.8	9.2	16.4	11.6
1.X	II	76.0	6.4	16.6	1.0	58.8	8.8	20.8	11.8
1.X	III	78.0	4.5	16.5	1.0	–	–	–	–
1.X	IV	74.0	6.8	18.2	1.0	–	–	–	–
1.X	V	77.1	6.3	15.6	0.9	–	–	–	–
Mean (%)		75.8	6.4	16.7	1.1	57.3	9.1	19.6	14.1
Standard deviation		2.56	1.27	0.78	0.25	6.44	0.23	2.80	4.10
	Hake								
17.IX	I	79.2	0.86	18.6	1.3	74.7	0.59	17.0	2.7
17.IX	II	78.9	1.22	18.4	1.4	74.0	0.60	15.2	3.2
17.IX	III	80.0	0.80	18.5	1.3	79.3	0.98	15.5	4.2
17.IX	IV	77.3	0.84	20.4	1.3	76.7	0.75	20.2	2.3
Mean (%)		78.8	0.93	18.9	1.3	76.2	0.73	16.9	3.1
Standard deviation		1.13	0.20	0.95	0.06	2.38	0.18	2.28	0.82

An analysis of variance showed that the protein content of fillets of carp and hake were significantly higher than that of the remains after filleting. There was no significant difference in silver carp between the protein contents of the fillet and of the remains after filleting.

The average fat content of the fillets for the three species ranged from 0.93 to 9.2%. Analysis of variance showed that silver carp fillets had a significantly lower fat content than carp and that hake had a significantly lower fat content than carp or silver carp. The bone and flesh remains of silver carp had a lower fat content than that of carp and those of hake were significantly lower than carp or silver carp.

Analysis of variance showed that the fat content of carp and silver carp fillets was significantly lower than that of the remains after filleting. There was no such significant difference in hake.

The average water content of the fillets ranged between 73.1 and 78.8% in the three species. There were no significant differences in water content between carp and silver carp, but the water content of hake fillets was significantly higher than that of carp or silver carp. The relationships between the water content of the remains after filleting were similar to that of the fillets. Analysis of variance showed a significantly higher water content in the fillets than in the remains after filleting within each of the three species tested.

Discussion and conclusions

From the results it appears that, based on the whole fish, 34% by weight of fillets could be obtained from carp and silver carp fish. The weights of the hake fillets could only be compared with the gutted and beheaded fish, since this is the only kind available. On this basis 61.5% fillets could be obtained from hake, compared with 47% for carp and 53.5% for silver carp.

The fillets of hake are essentially boneless, while those of carp and silver carp contain bones. However, the two latter fish fillets can be processed so that the bones cease to be a problem. The methods will be reported in subsequent papers. Excellent fillets can therefore be prepared from all three fish species tested. They would be higher priced than the whole fresh, iced or frozen fish, but would be very convenient to prepare.

However, two-thirds of the carp and over one-third of the weight of the gutted and beheaded hake would be left. The parts of the fish not used for filleting can be utilized to produce various processed products. Entrails of carp and silver carp, which averaged 11% of the whole weight, could be used to produce animal food. Heads of the carp and silver carp which averaged 19 and 25%, respectively, of the whole weight have been used by the author to prepare soups and chowders or added to the viscera for production of animal foods.

The bones and flesh remains, after filleting, accounted for the largest percentage of leftover weight from the filleting operation. Based on the whole fish weight this amounted to 25.7% for carp and silver carp and to 35% of the gut-

ted and beheaded hake. Such remains can be mechanically deboned and have yielded between 30 and 40% meat, in preliminary trials. Paoli (Stephen Paoli, Rockford, Illinois, U.S.A.) and other manufacturers of deboners claim over 50% edible meat yield from the remains after filleting. The meat obtained resembles a soft ground meat and it can be processed in several ways, e.g. as sausages. A description of such products being developed will appear in subsequent papers. The separated bones can be added to the entrails for animal food production or used for producing fish meal.

The proximate composition aids in deciding on the type of products that could be processed from the fish. The protein content of the fillets averaged 17.4% for all three species. This is similar to that of red meat (Watt & Merrill, 1963). While the protein contents of the three species did not differ significantly, the fat contents did (carp = 9.2%, silver carp = 6.4%, hake = 0.9% fat). The water content of carp and silver carp averaged 73 and 76%, respectively (no significant difference), while that of hake was 79% (significantly different from carp and silver carp). The protein and water contents of the remains after filleting were lower than those of the fillets, while the fat contents were higher.

According to the proximate composition, hake and silver carp would be a source of low fat, high protein fillets. This cannot be claimed for carp. The percentage carp fillet obtained, based on either the whole (32%) or the gutted (49%) carcass, was also lower than for silver carp (34% whole and 54% gutted) or hake (61% gutted).

The protein content of hake fillets was significantly higher than that of the remains after filleting. However, silver carp remains averaged 19% protein which was almost significantly higher than that of the fillets. Hake meat is very flavourful, while silver carp is bland. The combination of mechanically deboned hake meat from flesh and bone remains after filleting, which is white and rich in flavour, with that of silver carp, bland tasting but having an attractive dark pink colour, would be complementary. The lower fat content of the hake would balance out the fat content of the silver carp meat, while the higher protein content of the silver carp deboned meat would counterbalance the lower protein of hake deboned meat flesh.

The combination of local silver carp and hake deboned fish meat could be the basic raw material for a number of sophisticated formed fish meat and sausage products.

Acknowledgments

This research was supported by a grant from the United States–Israel Binational Science Foundation (BSF), Jerusalem, Israel.

References

Alder. H.A. & Roessler, E.G. (1964) *Introduction to Probability and Statistics.* Freeman, San Francisco.

A.M.I. (1950) *Laboratory Methods of the Meat Industry*, Method A 4 and 54, Reference A.O.A.C. Methods, 7th edn, 23.8, 2.24 and 2.22. American Meat Institute, Analytical Methods Committee, Council on Research.

Watt, Bernice K. & Merrill, Annabel L. (1963) *Composition of Foods*. Handbook 8, U.S. Dept of Agriculture.

(*Received 1 June 1976*)

J. Fd Technol. (1977), **12**, 37–47

Differential lipid oxidation in various parts of frozen mackerel

P. J. KE,* R. G. ACKMAN, B. A. LINKE AND D. M. NASH

Summary

Kinetic variations in lipid oxidation in various parts of mackerel stored at −15, −30 and −40°C were studied by measuring the changes of POV and of TBA molar values as a function of time. Oxidation developed in the skin sample (subcutaneous fat) was found to be eight times faster in TBA change than in the white and dark muscles from mackerel held at −15°C for two months. This rapid development of rancidity in the skin was effectively inhibited by lowering the frozen storage temperature to −40°C, where relatively the degree of lipid oxidation in the dark muscle was found to be of the same order of magnitude. The *in vitro* rate of autoxidation at 60°C for the lipids extracted from skin and the fillet muscle also showed an unusual difference which indicated that unknown pro-oxidative substances in mackerel skin were fat solvent extractable. The activity of the unknown compounds in the skin was also temperature dependent even in the frozen state, but could be effectively mitigated by lowering temperature to −40°C. Lipid hydrolysis in mackerel was also retarded completely when the fish were stored at −40°C. The comparative compositions of fatty acids and volatile carbonyls were determined in head, skin, white and dark muscle, and suggested an active non-specific oxidation system in the skin fat.

Introduction

Lipids in most fatty fish, particularly in the Atlantic mackerel (*Scomber scombrus*), readily undergo oxidation on exposure to the air even in frozen storage (Ke, Ackman & Nash, 1975b). In fatty fish such as mackerel, herring, etc. depot fat occurs as extracellular globules in the muscle and in the mesentery (Flo, Hagen & Mohr, 1972; Mohr, 1972). A variable but high proportion of the total lipids are neutral lipids with substantial contents of unsaturated C_{20} and C_{22} fatty acids (Ackman & Eaton, 1971; Hardy & Keay, 1972). These acids are among the constituents of fatty fish, in free or combined forms, most suscept-

Authors' address; Fisheries and Marine Service, Department of the Environment, Halifax Laboratory, Halifax, Nova Scotia B3J 2R3.

ible to autoxidation. The oxidation products of these unsaturated fatty compounds and a number of minor lipid components contribute to the natural aromas, flavours and colour substances which make fish and fishery products attractive in minor proportions, or aesthetically objectionable if present in excess (Ackman, 1967, 1974).

In terms of controlling the onset of rancidity in lipids of frozen fish the most important problem in quality preservation would seem to be control of the initial oxidation reaction before the chain can begin to propagate. When the initial oxidation has occurred through direct interaction with oxygen or/and a free radical mechanism (Labuza, 1971), the quality of fish deteriorates, often very rapidly, and so far no practical method has appeared for preventing the development of rancidity after the lipid oxidation has proceeded beyond the induction period.

The characteristic features of lipid oxidation in fish muscle can be traced to the influence of factors other than the usual ones of concentration of reactants and temperatures. Using low storage temperatures or vacuum packaging for frozen mackerel can slow the monomolecular reactions of oxidation but do not stop problems completely (Ke, Ackman & Nash, 1976). The pro-oxidant activity of trace metals, heme pigments and other catalytically active biochemical substances in fish can lead to unexpected results, probably due to acceleration of the initiation of lipid oxidation (Schultz, Day & Shinhuber, 1962; Uri, 1961). Oxidation accelerating compounds have been found in the dorsal muscle of trout (Takama, 1974) and similar compounds may be responsible for the unusually rapid development of rancidity in mackerel. The dark and liver tissues of mackerel (*Scomber japonicus*) have both been implicated as having catalytic effects in linoleate oxidation (Nagayama, Imano & Naito, 1971). Therefore, in addition to the various contents of fats in different parts of fish, different muscles within mackerel could have a great variation in their potential oxidative susceptibilities due to the presence of unknown pro-oxidants.

This study was initially undertaken to estimate the rate of lipid oxidation in various parts of frozen mackerel and to compare their kinetic variations. Unexpectedly a rapid rate of lipid oxidation was found in skin samples during this investigation. Accordingly, our preliminary results for autoxidation lipids extracted from the skin and meats of mackerel are also presented for evaluating significance of skin as a highly sensitive site to test for the development of oxidiation.

Materials and methods

Mackerel destined for this study were obtained from the trap fisheries in the area outside Halifax Harbour. Fish caught in October (autumn) and June (spring) of 1974 were transported iced in 100-lb boxes to our laboratory within less than 6 hr after landing. The fish were washed under clean conditions and frozen in the round and unwrapped respectively at $-15°C$ for spring and

autumn mackerel, and −30 and −40 for autumn fish. After a suitable period of frozen storage, six fish were partially thawed and dissected into pooled samples designated as head, white muscle, dark muscle, belly flap, viscera, eggs and skin including the thin fat layer under the skin.

A distillation-colorimetric method (Tarladgis, Watts & Younathan, 1960) was modified and used for estimating thiobarbituric acid (TBA) molar value (μmol MA/kg fish). A fish sample (10 g) was blended with H_2O (30 ml) for 2 min at medium speed in a Waring Blendor. The homogeneous slurry sample (40 g) was diluted with H_2O (65 ml) in a 250-ml beaker. The pH was adjusted to 1.5 using 4 N HCl. The sample was transferred to a 500-ml distillation flask. A rinse of H_2O (5 ml) was combined with the sample solution in the flask. Propyl gallate (100 mg) and disodium-EDTA (100 mg) were added to the sample and the flask connected to a distillation apparatus. This consisted of a 30-cm vertical, air-cooled, reflux column and a 30-cm vertical condenser joined by a U-type connector with a double vapour bulb on the top of the reflux column. Distillate (50 ml) was collected within 15–20 min from the initiation of boiling. An aliquot (5 ml distillate) and a blank (5 ml H_2O) were reacted with 5 ml 0.02 M TBA reagent in screw cap test tubes (15 ml) placed in a boiling water bath for 40 min. The solutions were cooled to room temperature and the absorbance was measured at 538 nm. The TBA molar value was obtained from a calibration curve for the range of 0.1 to 5.0×10^{-6} M of malonaldehyde (equal to 1 to 50 μmol/kg fish), using 1,1,3,3-tetraethoxypropane as standard.

Lipids were extracted from the mackerel samples by the method of Bligh & Dyer (1959). Peroxide values (POV), the content of free fatty acids (FFA) and the iodine value (IV) were determined by the American Oil Chemists' Official Methods Cd-8-53, Ca-5a-40 and Cd-1-25 (1972). Lipids (about 50 mg) of head, skin, white and dark muscle were converted to methyl esters, respectively, by

Table 1. Average and range of lipid content and seasonal changes in various parts of mackerel

Sample	Lipid (%)*				Seasonal increase (%)†	Iodine Value‡	
	Spring		Autumn			Spring	Autumn
	Average	Range	Average	Range			
Head	13.8	11.6–16.0	17.3	15.3–18.5	25	110	112
White muscle	1.8	1.3–2.7	11.8	9.9–13.8	550	90	92
Dark muscle	13.6	10.1–19.8	19.0	15.9–20.4	40	98	104
Belly flap	15.7	12.0–19.8	38.9	34.0–44.6	150	107	112
Viscera	3.3	2.3–4.1	10.2	9.8–10.5	210	126	114
Eggs	8.4	6.7–9.1	–	–	–	116	–
Skin	26.3	20.0–32.6	47.9	39.8–54.9	82	106	105

* Average and range were obtained from six determinations.

† Seasonal increase = (fat content of autumn fish − fat content of spring fish)/(fat content of spring fish) × 100%.

‡ Iodine values were the average of two analyses.

Table 2. Changes in oxidation of lipids in various parts of frozen mackerel*

Frozen temp. (°C)	Sample	Storage time (month) 0	1	2	3	6	9	12
TBA molar value (μ mol/kg fish)								
−15	Head	2.9 (1.9)	6.0 (6.5)	13.3 (12.3)				
	Guts	4.7 (4.5)	6.6 (5.4)	11.0 (13.1)				
	White meat	2.4 (2.0)	2.6 (2.2)	6.8 (7.1)				
	Dark meat	4.2 (2.6)	5.4 (3.9)	8.2 (7.9)				
	Skin	5.8 (2.5)	14.2 (13.5)	58.9 (55.0)				
	Belly flap	2.1 (2.4)	3.5 (4.3)	9.6 (9.2)				
	Eggs	– (6.7)	– (6.1)	– (8.0)				
−30	Head	2.9	4.0	–	7.4	10.9	17.8	22.6
	Guts	4.7	5.1	–	6.6	8.9	10.1	12.2
	White meat	2.4	3.0	–	4.6	4.9	4.8	5.4
	Dark meat	4.2	5.8	–	11.0	18.7	21.4	27.5
	Skin	5.8	8.5	–	17.0	44.0	60.0	–
	Belly flap	2.1	3.8	–	8.6	9.5	10.0	10.8
−40	Head	2.9	–	3.5	–	6.7	–	10.4
	Guts	4.7	–	4.9	–	5.4	–	7.1
	White meat	2.4	–	2.9	–	3.4	–	4.5
	Dark meat	4.2	–	5.8	–	7.3	–	15.9
	Skin	5.8	–	8.6	–	12.7	–	28.9
	Belly flap	2.1	–	–	–	3.0	–	3.6
POV (m equiv./kg oil)								
−15	Head	0 (0)	0.3 (0.2)	3.1 (2.6)				
	Guts	0 (0)	0.4 (0.4)	1.4 (1.0)				
	White meat	0 (0)	0 (0)	0 (0)				
	Dark meat	0 (0)	0.1 (0)	1.2 (0.9)				
	Skin	0 (0)	0.9 (1.3)	11.4 (12.0)				
	Belly flap	0 (0)	0.1 (0)	1.0 (1.3)				
	Eggs	– (0)	– (0)	– (0)				
−30	Head	0	1.0	–	3.2	4.5	5.1	–
	Guts	0	–	–	2.9	5.2	–	8.5
	White meat	0	0	–	0	1.3	1.0	2.5
	Dark meat	0	0	–	1.1	6.4	8.8	11.6
	Skin	0	0.3	–	1.5	4.7	6.7	9.8
	Belly flap	0	–	–	1.0	2.2	2.5	–
−40	Head	0	–	0.5	–	1.9	–	3.9
	Guts	0	–	0.2	–	0.9	–	0.8
	White meat	0	–	0	–	1.1	–	1.5
	Dark meat	0	–	0.3	–	2.1	–	4.8
	Skin	0	–	0	–	1.8	–	3.7

* Data presented were from 1974 autumn mackerel except that spring mackerel of 1974 frozen at −15°C were also listed in the brackets for comparison.

refluxing for 10 min with 5 ml of 7% BF_3 in methanol (Morrison & Smith, 1964). The operations of lipid extraction and all analyses were carried out under nitrogen. The fatty acid composition was determined by GLC using a Perkin-Elmer 900 gas chromatograph with a flame ionization detector and a wall-coated open-tubular capillary column (stainless steel 46 m x 0.25 mm I.D.) coated with SILAR-5CP (Applied Science Lab.) and operated at 180°C and 2.5 kg/cm^2 He. Peaks were identified and quantitated as in a previous report (Ackman & Eaton, 1971). The monocarbonyl aldehydes from the volatiles of these lipids were also estimated by a combination GLC procedure as described elsewhere (Ke, Ackman & Linke, 1975a).

Results and discussion

The averages and ranges of lipid contents in various parts of mackerel landed in the spring and autumn of 1974 are summarized in Table 1. In general, the results agreed with the incomplete data in the previous reports (Ackman & Eaton, 1971; Hardy & Keay, 1972; Kinsella *et al.*, 1975). The skin samples always included the thin fatty layer immediately underneath the skin and had the highest lipid content of all the sites sampled, respectively 48% in autumn fish and 26% in spring fish. The lipid contents in the edible parts of mackerel (in des-

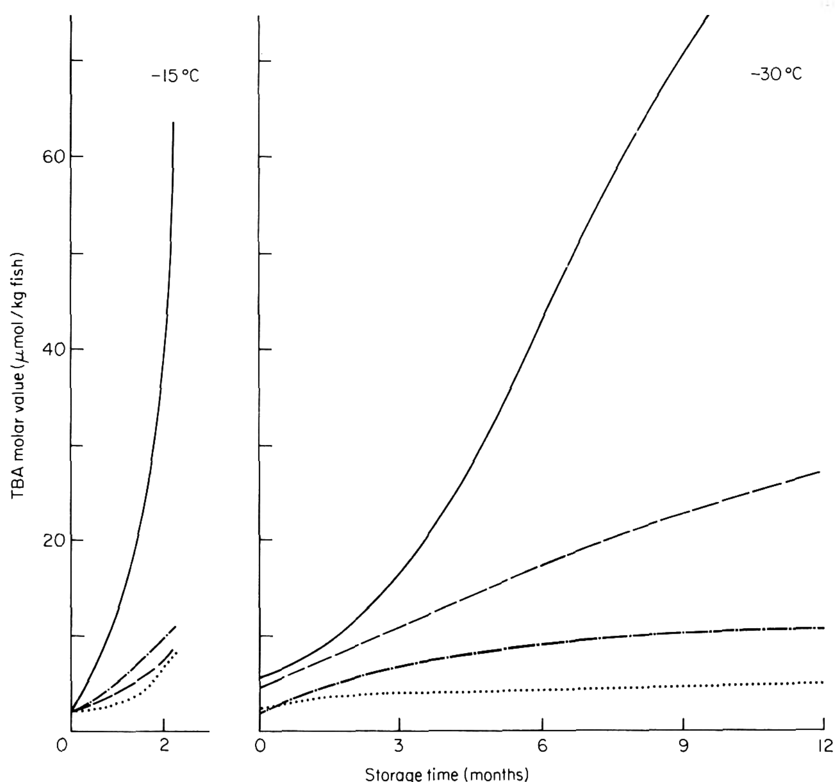


Figure 1. The reaction rates of lipid oxidation in terms of the change of TBA molar value for autumn mackerel stored at −15 and −30°C. Skin ————; white muscle ....; dark muscle — — — — —; belly flap —·—·—·—·

cending order from that of skin) were: belly-flap, dark muscle and white muscle. The latter contains the smallest amount of lipid and has the largest seasonal variation at 550% from spring to autumn mackerel. The iodine value (I.V.) of lipids from various parts of fresh mackerel were also determined for comparison of the degrees of lipid unsaturation and are listed in Table 1. The I.V. of lipids from white muscle and dark muscle were the lowest, 90 and 98 for spring mackerel, and increased slightly in the following order of skin, belly flap, head and viscera. The I.V. in most parts of the autumn mackerel were higher due to seasonal variation except a slightly lower I.V. has been found in the skin and viscera.

The development of lipid oxidation in various parts of spring and autumn mackerel stored frozen in the round at −15°C, and of autumn fish stored frozen in the round at −30 and −40°C, in terms of TBA and POV changes, has been followed for a year and the results are presented in detail in Table 2. In general, no significant seasonal variation has been observed in various parts of mackerel frozen at −15°C. The TBA value as a function of storage time has been plotted in Fig. 1 to demonstrate the rapid change in the skin sample in comparison with other tissues from autumn mackerel. As shown in Table 2, at −15°C the TBA molar value in the skin, after two months storage, has increased eight times faster than in the white and dark muscles even though the dark meat contains up to 50% of the lipid in the skin (Table 1). However, when the storage temperature was lowered to −30°C, the increase of TBA molar value become much slower in the skin, but was relatively faster in dark muscle than in white muscle. For example, the TBA molar value in the skin after nine months' storage at −30°C was thirteen times higher than in the white muscle, but only three times more than in the dark muscle. Furthermore, the TBA molar values in the skin and dark muscle were almost at the same level in the fish stored frozen at −40°C for one year (Table 2).

Comparisons of changes in POV values for the skin and dark muscle at three frozen storage temperatures are plotted in Fig. 2. From these curves of POV change, the rate of oxidation, which gives values eleven times higher in the skin lipids than in the dark meat lipids for storage at −15°C for two months, is shown to be reduced remarkedly by lowering the frozen storage temperature to −30 and −40°C. At the latter temperature POV in skin is 20% lower than in the dark muscle after one year. Based on the POV change in the white muscle lipids, the increases of POV in dark muscle were also elevated, relatively, when the frozen storage temperature was lowered (Table 2). However, it should be noted that the overall rate of lipid oxidation in the skin was significantly reduced by lowering the frozen storage temperature.

It appears that the oxidation of the reactive lipid system in the skin of mackerel which may contain some pro-oxidants, is temperature dependent. A catalytic effect on skin lipid oxidation, which can be retarded by decreasing the frozen temperature to −40°C, seems more plausible than an effect based on changes in the physical properties of this high-fat system. The reasons for a slight increase of oxidation in the lipids of dark meat, relative to skin, when the

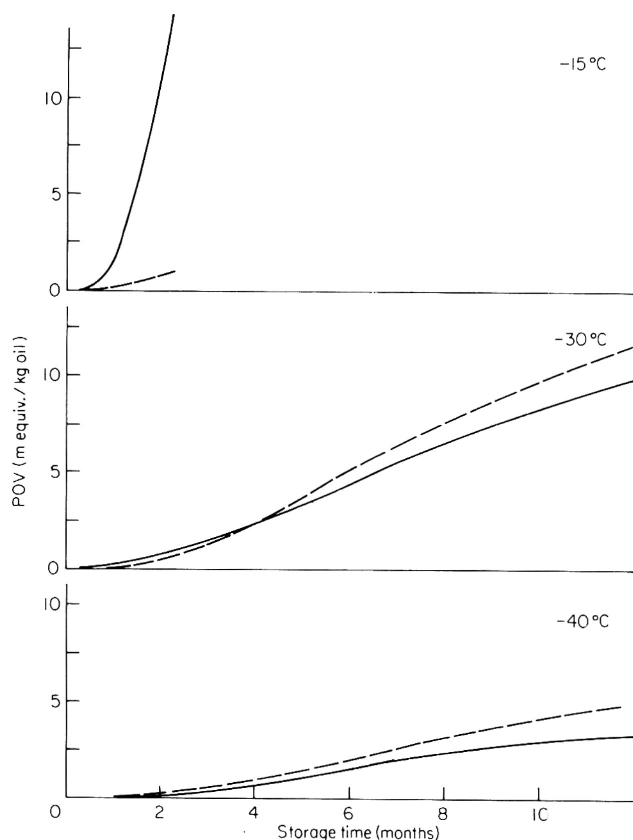


Figure 2. The rates of formation of peroxides in the skin (———) and the dark muscle (– – – – – –) of autumn mackerel in frozen storage at –15, –30 and –40°C.

temperature was lowered are not yet clear. The repression effect of lower temperatures on development of oxidation in the lipids of dark muscle seemed to be small but the physical state of systems present in the dark meat including heme compounds (Nagayama *et al.,* 1971) and liquid water (Sussman & Chin, 1966) could be responsible. The oxidation of linoleate catalysed by extracts of defatted mackerel (*Scomber japonicus*) tissue has been studied. The dark muscle and liver, which both contain high levels of theme compounds, were confirmed to be active in accelerating the oxidation reaction (Nagayama *et al.*, 1971).

The changes of FFA content in various parts of frozen mackerel are also listed in Table 2. The formation rates of FFA in different parts of frozen mackerel are approximately proportional to their fat content, except that as expected the visceral lipids were the fastest to decompose (Fig. 3). The subcutaneous (skin) lipids were very slowly hydrolysed (Fig. 3), perhaps because they are nearly pure triglyceride. Obviously the production of FFA by lipid hydrolysis in mackerel stored frozen at –15°C, takes place at a moderate rate, but for all parts of the fish has been effectively retarded in the fish stored at –30°C, and is completely inhibited at –40°C.

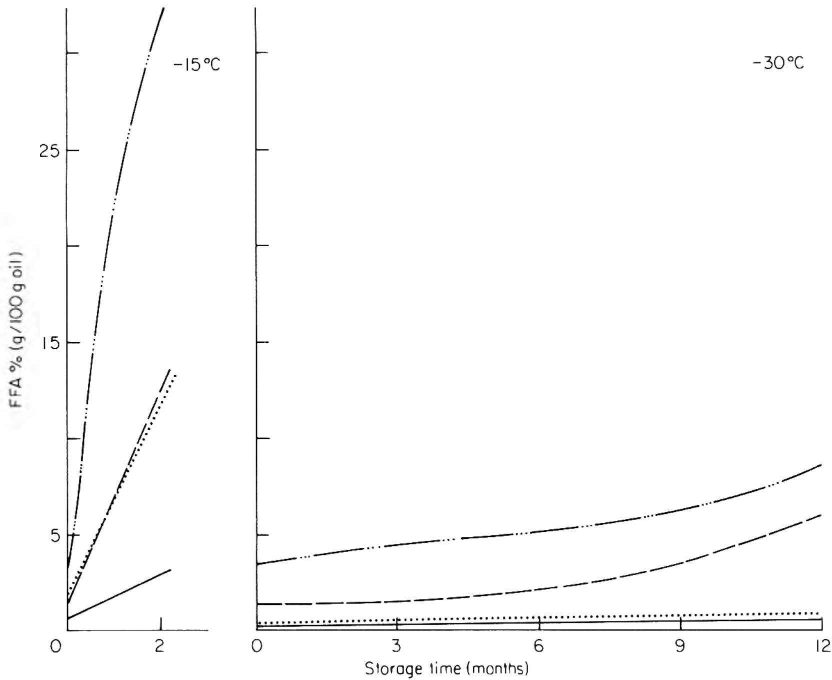


Figure 3. The change of FFA formation for autumn mackerel in frozen storage at −15 and −30°C. Skin ———; white muscle ....; dark muscle — — — — —; viscera —···—···—···—.

The primary products of lipid oxidation are hydroperoxides, but these are readily decomposed to secondary reaction products, particularly carbonyl compounds (Ke *et al.*, 1975a). Consequently, the development of oxidative rancidity should not be judged by the determination of the primary oxidation product, but rather by determining secondary oxidation products (Pokorny & Janicek, 1973). Furthermore, both the formation of certain carbonyl components (McGill, Hardy & Burt, 1974; Trofimov & Kanaev, 1973; Meijboom & Stroink, 1972) and changes of fatty acid composition (Shono & Toyomizu, 1972; Trofimchuk & Pervuninskaya, 1974) can serve as criteria for evaluation of oxidative rancidity in frozen fish. The differences of fatty acid composition and aldehydes content in total lipids of fresh white muscle, dark muscle, head and skin (sample A) are presented in Table 3. By comparing polyene index and monoene index in these four samples, it is clearly seen that proportionately the skin lipid contains the most of monounsaturated fatty acids and the least polyunsaturated acids.

The white muscle lipids contain the smallest amount of aldehydes, which are two or three times higher in the dark muscle and head lipids. Even in 'fresh' mackerel the highest quantities of all the different monocarbonyl compounds were found in the skin lipids. Since the changes of fatty acid and aldehyde are small in the first stage of frozen fish, only data from skin lipids of mackerel which had been stored frozen at −15°C for two months (sample B), are listed in Table 3 for comparison with the fresh fish analytical data. Based on these

Table 3. Composition of fatty acids and monocarbonyl aldehydes in the lipids extracted from head, skin, white and dark muscle of spring mackerel (six pooled fish)

Composition	White muscle	Dark muscle	Head	Skin*	
				A	B
Fatty acids (mol %)					
14 : 0	6.1	6.4	8.0	9.4	9.8
16 : 0	15.7	18.6	15.2	15.5	17.0
18 : 0	3.2	3.0	2.4	2.0	2.3
Total saturates	28.1	30.5	29.1	28.4	31.8
16 : 1	5.9	6.2	7.2	4.5	5.0
18 : 1	10.7	10.8	12.6	12.1	10.0
20 : 1	12.5	9.4	11.6	14.1	14.7
22 : 1	16.0	13.7	14.3	19.9	20.3
Total monoenes	46.9	42.0	47.9	51.0	51.2
18 : 4	2.7	2.8	4.2	5.8	4.7
20 : 5	7.4	6.4	5.8	5.0	4.6
22 : 6	11.6	13.2	7.6	4.6	4.4
Total polyenes	25.0	27.5	23.0	20.6	17.0
Polyene index†	0.995	0.896	0.759	0.606	0.507
Monoene index‡	1.798	1.356	1.659	1.819	1.679
Monocarbonyl aldehydes (mol/g oil x 10^{-9})					
n-propanal	4.1	8.5	9.8	16.5	39.1
n-pentanol	0.5	0.8	1.6	3.1	8.0
Total alkanals	4.8	10.7	14.1	22.9	54.9
n-hexenal	–	0.4	0.5	2.0	2.9
Total alkenals	0.2	0.9	0.8	3.7	3.5

* Skin samples A and B were obtained from the same lot of fish when fresh and after being stored frozen at −15°C for two months, respectively.
† Polyene index = (18 : 4 + 20 : 5 + 22 : 6)/(14 : 0 + 16 : 0).
‡ Monoene index = (18 : 1 + 20 : 1 + 22 : 1)/(14 : 0 + 16 : 0).

two limited sets of data, we reached the unexpected conclusion that although polyunsaturated fatty acids are definitely oxidized faster than monoenes in the skin fats of frozen mackerel, a process which has a parallel in mackerel oil autoxidized at 60° C (Ke *et al.*, 1975b), the monoenes are also oxidized fairly rapidly. Although an increase of aldehyde formation was recorded in the oxidized skin lipids and all component aldehydes have been increased, the compositional pattern is not changed. These observations suggest an active and non-selective process of autoxidation in the skin fat. The skin in fish is an important defensive mechanism and is biochemically active, secreting lipids among other materials (Lewis, 1970). The degree to which the subcutaneous fats associate physically with the dermis varies with the species (Yamada & Nakamura, 1964;

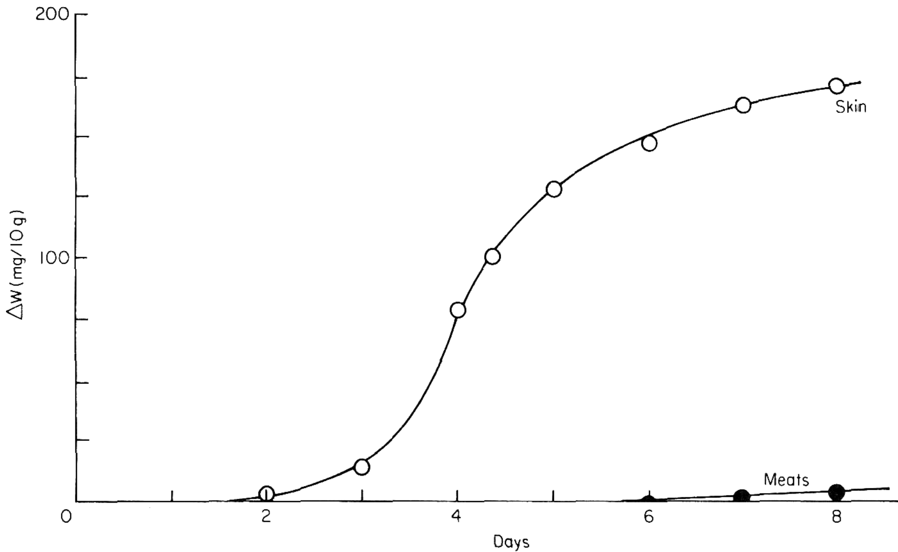


Figure 4. Comparison of the rate of oxidation reaction at 60°C for the lipids from skin and meat of mackerel.

Yamada, 1972; Flo *et al.*, 1972; Mohr, 1972). In terrestrial animals skin lipids may have different functions, for example in conserving water, but among possible recent relevant reports it has been shown that the skin is an active site of fat reactions in the human, rat, snake and chick (Ahern & Downing, 1974; Pane, Becchetti & Carinci, 1974; Wheatley *et al.*, 1971; Yeh & Leveille, 1973) and considerable amounts of various enzymes have been found in skin samples.

However, we are unaware of studies into lipid oxidation in the skin of frozen mackerel (or other fatty fish) yet, particularly as to the catalytic effect on oxidative rancidity. Lipids extracted from the skin and the meats (i.e. the skin-off fillet includes both white and dark muscles) of fresh mackerel have been oxidized *in vitro* at 60°C to compare their comparative rates of autoxidation by using a simple weight gain method (Notevarp & Chahine, 1972). The results as a function of time have been plotted in Fig. 4. The unusual difference of the reaction rate between skin and muscle lipids are indicated clearly by both the induction period and by the overall accumulation of oxidation products. The compositions of fatty acid in the skin and muscle lipids (Table 3) are not very different and cannot be regarded as a key answer to the rapid oxidation in the skin lipids. The muscle sample includes more phospholipids (cf. Ackman & Eaton, 1971). The oxidation *in vitro* shows that the faster oxidation of skin lipid is probably not due to the greater surface/mass ratio of the skin *in vitro* and that there must be some fat soluble substance in the mackerel skin lipids which catalyses their unusual lipid oxidation.

From this study we conclude that some non-selective fat solvent extractable, pro-oxidant compounds are present in the mackerel skin. Their effect is temperature dependent in the frozen condition but their catalytic activity on lipid

oxidation can be inhibited by lowering the frozen storage temperature to −40°C. The complete solution to the problem of why the skin lipids from mackerel are oxidized much faster than the meat fats is probably not simple. Interacted protein-lipids (Karel, 1973) or unknown biochemicals, not necessarily those which can still be active in lipid oxidation under frozen conditions (e.g. −15°C), may provide some leads for future research.

References

Ackman, R.G. (1967) *J. Fd Technol.* **2**, 169.

Ackman, R.G. (1974) *Fishery Products* (Ed. by R. Kreuzer). Fishing News (Books), London.

Ackman, R.G. & Eaton, C.A. (1971) *Can. Inst. Fd Technol. J.* **4**, 169.

Ahern, D.G. & Downing, D.T. (1974) *Lipids*, **9** 8.

Bligh, E.G. & Dyer, W.J. (1959) *Can. J. Biochem. Physiol.* **37**, 911.

Flo, A., Hagen, N. & Mohr, V. (1972) *Research Project* B 0103.3858. Norwegian Research Council for Technical and Natural Sciences.

Hardy, R. & Keay, J.N. (1972) *J. Fd Technol.* **7**, 125.

Karel, M. (1973) *J. Fd Sci.* **38**, 756.

Ke, P.J., Ackman, R.G. & Linke, B.A. (1975a) *J. Am. Oil Chem. Soc.* **52**, 349.

Ke, P.J., Ackman, R.G. & Nash, D.M. (1975b) *Environment Canada*, Fish. & Marine Ser., Halifax Laboratory, N.S. Circular No. 52.

Ke, P.J., Ackman, R.G. & Nash, D.M. (1976) *Can. Inst. Fd Technol. J.*

Kinsella, J.E., Posati, L., Weihrauch, J. & Anderson, B. (1975) *CRC Critical Rev. Fd Technol.* **5**, 299.

Labuza, T.P. (1971) *CRC Critical Rev. Fd Technol.* **2**, 355.

Lewis, R.W. (1970) *Lipids*, **5**, 947.

Meijboom, P.W. & Stroink, J.B.A. (1972) *J. Am. Oil Chem. Soc.* **49**, 555.

Mohr, V. (1972) *Tidsskr. Kjemi, Bergv., Mettalurgi*, **32**, 23.

Morrison, W.R. & Smith, L.M. (1964) *J. Lipid Res.* **5**, 600.

McGill, A.S., Hardy, R. & Burt, J.R. (1974) *J. Sci. Fd Agric.* **25**, 1477.

Nagayama, F., Imano, S. & Naito, Y. (1971) *Bull. Jap. Soc. Sci. Fish.* **32**, 415.

Notevarp, O. & Chahine, M.H. (1972) *J. Am. Oil Chem. Soc.* **49**, 274.

Pane, G., Becchetti, E. & Carinci, C. (1974) *Int. J. Biochem.* 5, 579.

Pokorny, J. & Janicek, G. (1973) *Die Nahrung*, **17**, 713.

Schultz, H.W., Day, E.A. & Shinhuber, R.O. (1962) *Lipids and their Oxidation.* Avi, Westport, Conn.

Shono, R. & Toyomizu, M. (1972) *Sci. Bull. Fac. Agric. (Kyushu Univ.)*, **26**, 233.

Sussman, M.V. & Chin, L. (1966) *Science, N.Y.* **151**, 3708.

Takama, K. (1974) *Bull. Fac. Fish, Hokkaido Univ.* **25**, 256.

Tarladgis, B.G., Watts, B.M. & Younathan, M.T. (1960) *J. Am. Oil Chem. Soc.* **34**, 44.

Trofimchuk, G.D. & Pervuninskaya, T.A. (1974) *Rybn. Khoz.* **9**, 50.

Trofimov, N.V. & Kanaev, N.A. (1973) *Zh. Anal. Khim.* **28**, 2033.

Uri, N. (1961) *Autoxidation and Antioxidant* (Ed. by W.O. Bundberg), Ch. 2. Wiley-Interscience, New York.

Wheatley, V.R., Hodgins, L.T., Coon, W.M., Kumarasiri, M., Berenzweig, H. & Feinstein, J.M. (1971) *J. Lipid Res.* **12**, 347.

Yamada, J. (1972) *Bull. Tokai Reg. Fish. Lab. (Japan)*, **72**, 35.

Yamada, J. & Nakamura, S. (1964) *Bull. Tokai Reg. Fish. Res. Lab. (Japan)*, **39**, 21.

Yeh, C.S. & Leveille, G.A. (1973) *Proc. Soc. Exp. Biol. Med.* **142**, 115.

(*Received 4 April 1976*)

J. Fd Technol. (1977) **12**, 49–57

The determination of freezant residues in prawns frozen by contact with dichlorodifluoromethane

J. R. CARTER AND R. S. KIRK

Summary

A method has been developed, based on headspace gas chromatography, for the determination of freezant residues in prawns frozen by contact with liquid dichlorodifluoromethane (DCDFM). Detector response was linear up to 300 μg DCDFM injected. This level is two orders of magnitude higher than the maximum permitted limit in the U.K. for residual DCDFM. Recoveries of DCDFM from 'spiked' samples were in the range 77 to 105%. A storage trial was set up to measure the fall of residual DCDFM levels with time during storage under deep freeze conditions.

Introduction

Since the first demonstration in 1969 by E.I. Du Pont de Nemours of a liquid 'Freon' freezant system (Lawler & Trauberman, 1969) there have been numerous publications (e.g. Daly, 1973; Astrom, 1975) describing the advantages of freezing foods by direct contact with liquid dichlorodifluoromethane (DCDFM) freezant. The principal advantages quoted are quality improvements for a limited range of products, e.g. individual sticky foods which do not freeze well by conventional methods, and cost savings over the other permitted contact freezants, *viz.* carbon dioxide and liquid nitrogen. Guillot (1974) has contributed a useful review and full bibliography. It was not until 15 October 1975, when an amendment (Statutory Instrument, 1975) was made to the Miscellaneous Additives in Food Regulations 1974, that DCDFM was permitted as a food additive in the United Kingdom. These amended regulations impose a limit on the amount of DCDFM in or on frozen food and specify a residue limit of 100 mg/kg determined when the food is fully thawed at, and to, 20°C.

Although there are many references to the use of DCDFM as a contact freezant, there have been only two references (Crawford, Finch & Daly, 1969;

Authors' address: Department of Industry, Laboratory of the Government Chemist, Cornwall House, Stamford Street, London SE1 9NQ.

Bucholz & Pigott, 1972) to a method for the determination of DCDFM residues in foods. Crawford *et al.* gave very brief details of the method in use at the 'Freon' Products laboratory of Du Pont de Nemours in Wilmington, Delaware, U.S.A. The method consisted of sealing samples of frozen food in aerosol cans which, after thawing at room temperature, were punctured via a suitable valve system and a vapour sample introduced on to a gas chromatographic column. Column packings recommended were 20% di-2-ethylhexyl sebacate on Chromosorb W, 60–80 mesh, and Porapak Q, 80–100 mesh, using thermal conductivity detection. Bucholz & Pigott (1972) describe a similar method including the addition of carbon tetrachloride to the sample in the can.

The object of this present work was to develop a simple method based on headspace gas chromatography for determining DCDFM residues in frozen and thawed foods. The chosen method was developed from the so called 'hot jar' technique currently being applied in this laboratory to the determination of volatile organic contaminants in foods and plastic packaging materials (Report of the Government Chemist, 1975).

From a study of the available literature, the main application of DCDFM appeared to be in the freezing of individual items of sea food and soft fruits. Prawns were selected as a suitable test material for method development and a quantity of material was frozen and kept in a commercial deep freeze cabinet for a period of more than two months. During this period it was possible to establish a 'loss with time' profile for the freezant materials. Several commercially available samples of frozen prawns were also tested for DCDFM content.

Experimental

Method development

Several gas chromatographic column packings were assessed, firstly for their ability to separate naturally occurring volatile components of the prawns from DCDFM and secondly for their ability to give reasonable retention times. The packing with the optimum properties was Porasil B (Waters Associates) mesh 80–100 packed in a 2 m x 2.2 mm internal diameter stainless steel column operated isothermally at 60°C with a nitrogen carrier gas flow of 30 ml per min. The gas chromatograph used was the Perkin-Elmer F-30 instrument equipped with flame ionization detection (FID). The detector block was maintained at 150°C and the inlet port at 100°C. At the conditions selected, the retention time of DCDFM was 1.4 min.

The method for the determination of DCDFM residues consisted of weighing a 10-g sample of frozen prawns into a shallow dish which was then transferred to a cabinet at 20°C in which the sample was allowed to thaw (time taken for prawns to reach 20°C was approximately 2 hr). Individual thawed samples were then transferred to 125 ml (nominal volume) Hypo-vials (Pierce Chemical Co.) which were immediately sealed with butyl rubber serum caps.

Frozen samples for analysis were weighed directly into the vials, large prawns being chopped with a sharp knife to facilitate transfer through the necks of the vials. Weighing and capping was carried out as quickly as possible to avoid loss of volatile material. The sealed vials were heated in an air oven at 135°C driving the volatiles into the head-space. Whereas 15 min heating was found to be sufficient for thawed samples, frozen samples required up to 30 min. On removal from the oven 0.5-ml samples of the hot headspace were transferred to the gas chromatographic column by use of a gas-tight syringe (Hamilton). The following rigidly standardized syringe transfer technique was used in order to obtain reproducible injections of hot headspace. Immediately on removal from the oven the vial was suitably lagged to avoid heat loss, the syringe was flushed with headspace a set number of times in order to pre-warm it and the actual transfer and injection performed as rapidly as possible.

Headspace gas standards were prepared by weighing amounts of DCDFM (from an inverted lecture bottle of Mathesons Gas Products 'Freon' 12) into tared vials which were quickly capped and sealed before reweighing. Standards at low concentrations were prepared by dilution of headspace gas into freshly sealed vials by use of a 5-ml gas-tight syringe (Hamilton). Headspace gas standards were chromatographed as for the prawn samples, i.e. after heat-

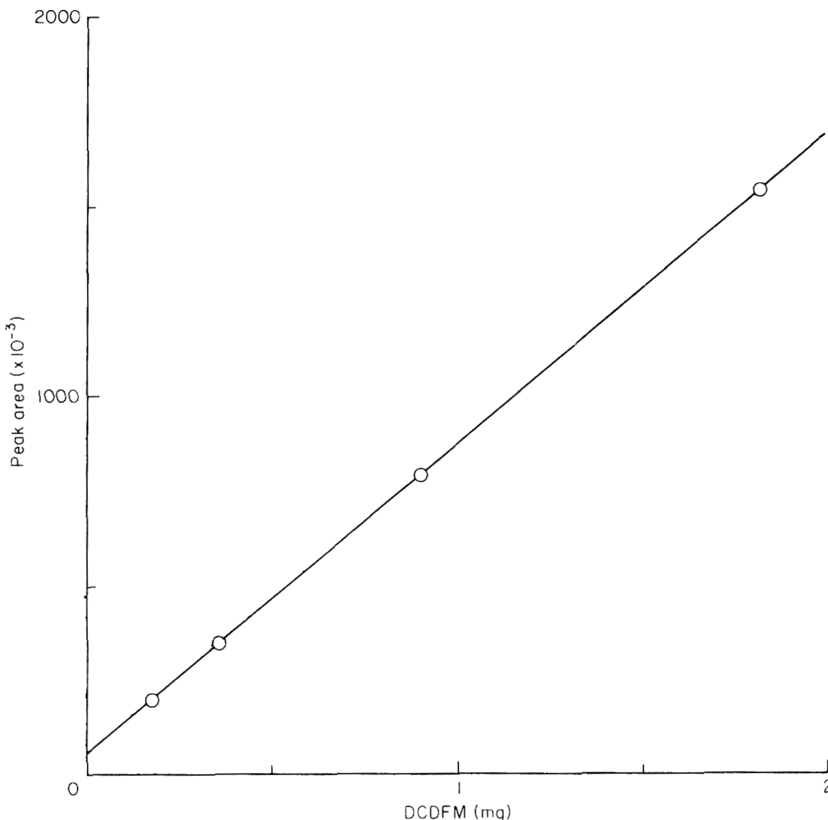


Figure 1 Calibration graph of milligrams of DCDFM per vial against integrated peak area.

ing at 135°C for 15 min and calibration curves plotted of peak areas *v.* milligrams DCDFM. An Infotronics model CRS 204 integrator was used to measure peak areas. Recovery experiments were performed by adding known amounts of DCDFM to 10-g portions of thawed prawns contained in vials and following the sampling procedure described above.

Sample preparation and examination

Fresh, whole, unpeeled prawns (*Pandalus borealis*) were purchased. Approximately 300 g were treated with liquid DCDFM in a Dewar vessel for 3 hr. Liquid DCDFM was obtained by cooling an aerosol can of laboratory 'Freon' propellant (previously demonstrated by gas chromatography to be composed solely of DCDFM) in a solid carbon dioxide-methanol freezing mixture before opening the can. After treatment, the prawns were packed in sealed polythene bags and stored in a deep freeze chest. Residual levels of DCDFM in the prawns, both frozen and after thawing, were monitored over a ten-week period.

Several commercially available samples of cooked, peeled, frozen prawns of unknown freezing history were purchased and tested for DCDFM content, both in the frozen state and after thawing. Electron capture detection (ECD) was used for some determinations to investigate any possible merits that this detector may have displayed over FID.

Results and discussion

Gas chromatography of headspace gas standards

A calibration graph over the range 0–2 mg DCDFM per vial is shown in Fig. 1. This range corresponds to 0–6.5 μg per 0.5 ml headspace gas injection. The true volume of the Hypo-vials used was 155 ml. Figure 2 is a similar calibration graph over the range 30–230 mg DCDFM per vial, corresponding to 97–742 μg per 0.5 ml injection. Loss of linearity is indicated above about 100 mg (322 μg injected). Since this corresponds to a residue level in prawns of 10 000 mg/kg it is not considered to be a serious limitation to the method. At the present U.K. statutory limit of 100 mg/kg (1 mg per vial or 3 μg injected) the flame ionization detector was well within its linear dynamic range. Flame ionization detection is normally considered to possess a wide dynamic range; however, the presence of halocarbons burning in the flame releases free halogen ions which exhibit an electron capturing effect (Lovelock, 1961). This effect, causing characteristic 'cut-off' peaks, was evident at around 1000 mg per Hypo-vial, i.e. corresponding to 3 mg injected. All headspace gas standards were injected hot following the same procedure as used for the prawn samples. However, it was found that on re-injection at room temperature the peak heights obtained were substantially constant. The headspace gas standards remained constant giving reproducible peak heights on repeated injection over a period of several weeks.

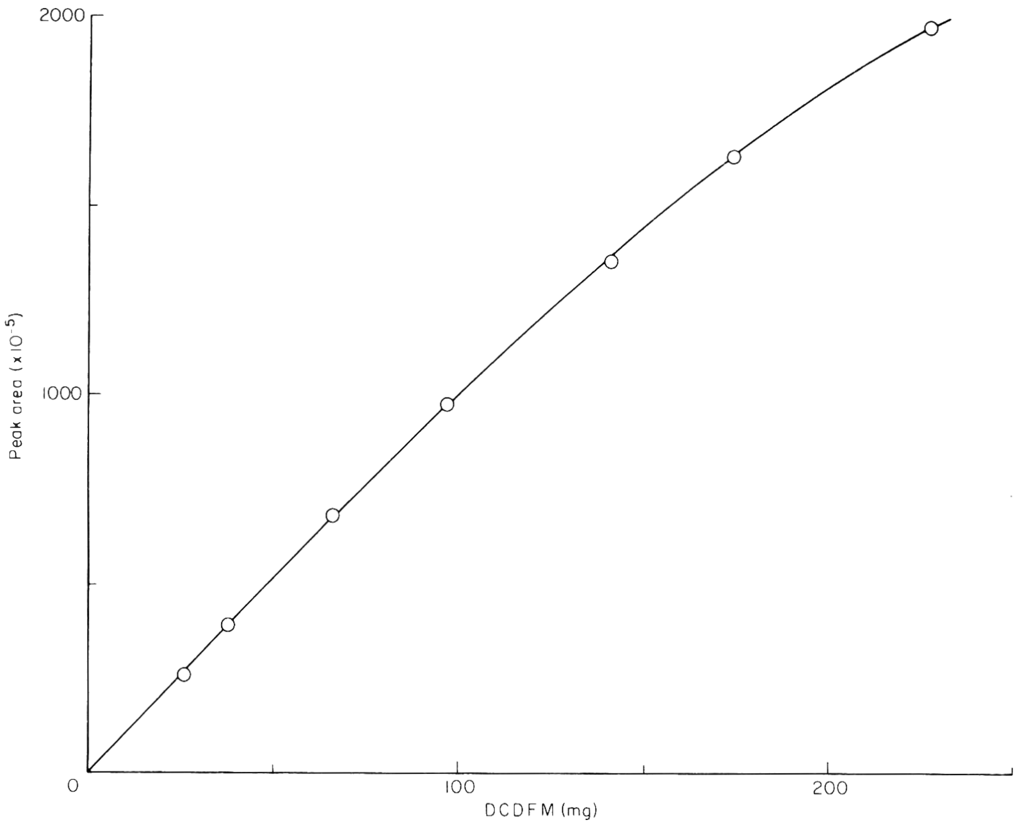


Figure 2 Calibration graph of milligrams of DCDFM per vial against integrated peak area.

Recoveries

The amounts of DCDFM recovered from duplicate analysis of 'spiked' 10-g samples of prawns are shown in Table 1.

Each determination was an average of at least three repeat injections. It can be seen that average recoveries were between 80 and 97% over the range 0.24 to 97 mg DCDFM added to 10-g prawns, equivalent to 24 to 9700 mg/kg. Such a range is adequate for examination of normal commercial material against the prescribed statutory limits.

Storage trial

Levels of residual DCDFM found in laboratory prepared frozen prawns during storage in a deep freeze cabinet are shown in Table 2.

The results given are the average of at least two determinations and each determination was based on at least three repeat injections. The DCDFM content after forty-two days can be discounted as a rogue result.

The exponential decay of residual DCDFM in frozen prawns with time is

Table 1. Recovery of DCDFM added to prawns

DCDFM added (mg)	DCDFM found (mg)		Recovery (%)	
0.24	0.21	0.25	87	104
0.40	0.32	0.36	80	90
0.79	0.71	0.80	90	101
1.59	1.42	1.50	89	94
16.8	13	14	77	83
20.5	18	19	88	93
36.6	32	32	87	87
50	49	45	98	90
97	87	100	90	103
110	116	–	105	–

Table 2. DCDFM content of frozen prawns stored in a deep freeze cabinet

Days after freezant treatment	DCDFM (mg/kg) in	
	Frozen prawns	Thawed prawns (2 hr at 20°C)
1	6500	650
5	4800	800
13	3200	130
20	1094	75
27	136	16
34	69	18
42	–	40
72	22	9

shown in Fig. 3. The very high initial levels show a rapid fall during deep freeze storage to below the permitted statutory limit after thawing at 20°C to 20°C. The effect of thawing was generally a reduction of approximately 90% of the DCDFM present in the frozen material.

These results found are somewhat at variance with figures quoted by Bucholz & Pigott (1972) who found initial levels for DCDFM residues in fish immediately after freezing of around 3000 mg/kg with a rapid decline to 100–300 mg/kg within 30 min. However, details are not given of holding conditions between freezing and sampling.

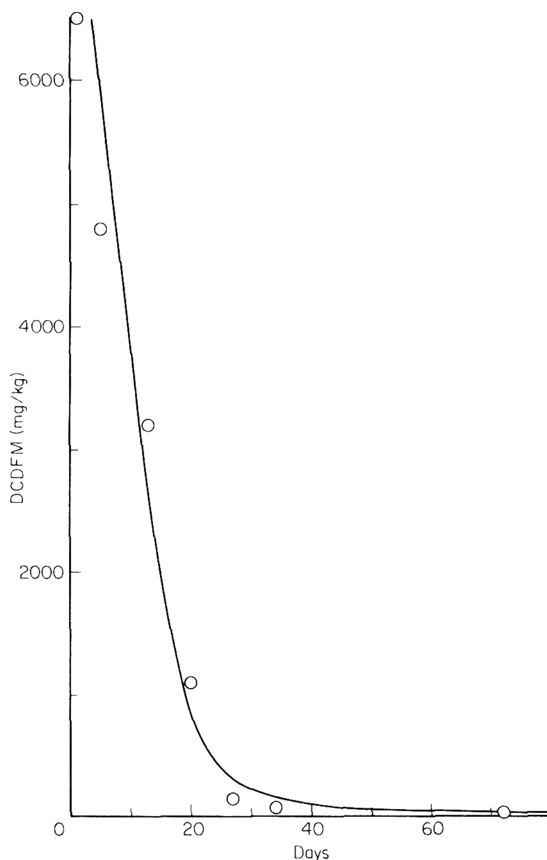


Figure 3 Residual DCDFM level (mg/kg) in frozen prawns plotted against time (days) in deep freeze storage.

All headspace gas samples above prawns were injected hot; on repeat injections after cooling to room temperature it was found that peak heights were reduced. This is contrary to the findings for the headspace gas standards and it is assumed that there was some reabsorption of DCDFM by the prawns.

Analysis of commercial samples of frozen prawns and chromatographic interference

Four retail packs of frozen prawns, from Malaysia, Canada, Singapore and the U.K., purchased in February 1976, were examined by the above method. The Canadian sample showed evidence, using FID only, of a very small peak on the chromatogram at the same retention time of DCDFM. This peak on extrapolation, expressed as DCDFM, corresponded to an insignificant level of 0.02 mg/kg after thawing. None of the samples carried a declaration on their labels that the product had been contact frozen with permitted liquid freezant. Using the column and conditions described, relatively simple chromatograms, as shown in Fig. 4, were obtained from the storage trial prawns and there was

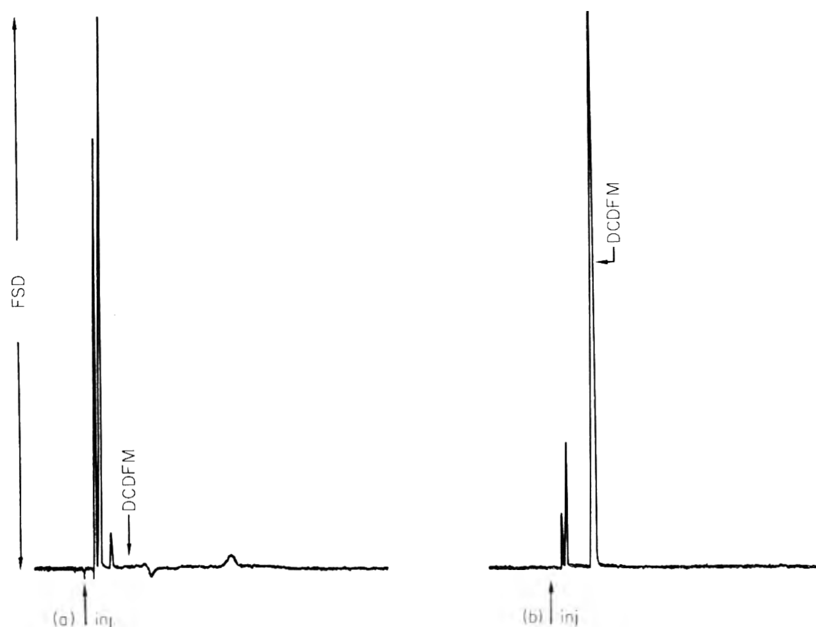


Figure 4 Chromatograms of 0.5 ml hot headspace from a vial containing 10 g thawed prawns from the storage vial. (a) Containing no DCDFM; attenuation 1 × 2. (b) Containing 0.18 mg DCDFM (corresponding to 0.58 μg injected); attenuation 1 × 16. Integrated peak area = 186 × 10^3 equivalent to 18 mg DCDFM per kilogram of prawns.

no interference on the chromatograms from other volatiles natural to the prawns. The method developed for prawns was intended to be applicable to other foods likely to be frozen using DCDFM, e.g. soft fruits. Such other foods may or may not contain interfering components. Where interference occurs, the specificity of electron capture detection (ECD) may be of value. However, the dynamic range of ECD is far more limited than that of FID. Electron capture detection was used for some prawn samples and considerably enhanced sensitivity to DCDFM was shown compared with FID. This increased sensitivity would only be of use at very low levels and may be considered of limited value when considering the relatively high permitted maximum limit set for DCDFM residues in frozen food. The advantage of the specificity of ECD was not evident in the case of prawn samples, since due to the enhanced sensitivity, there were more peaks on the chromatrograms than those obtained using FID.

Conclusions

The method described by Crawford *et al.* (1969) for the determination of DCDFM residues, although basically a headspace gas chromatographic method, requires access to equipment for filling and sealing aerosol cans and a specially

designed puncturing valve system for removing samples from the can. The 'hot-jar' method developed in this laboratory using Hypo-vials and manual injection of headspace is simple and easy to use. The initial difficulty of obtaining reproducible manual injections of hot headspace gas was overcome by use of a practised injection technique.

The electron capturing effect observed during the passage of high levels of DCDFM through the flame ionization detector was not considered to be a limitation to the method at the levels anticipated in foods. The DCDFM treatment employed for the storage trial was very severe compared with normal commercial practice. However, the results indicated that if a considerable excess of DCDFM was used in the freezing of food, resulting in massive residual levels, there was a fall to below the legal limit within about eighteen days' deep freeze storage.

It is hoped that the method developed for prawns will be applicable to other foods. The use of ECD offers no real advantages for prawns but may be of use in the examination of other foods. Alternatively, use of the Hall detector (Hall, 1974) which is known to possess high specificity towards halogenated compounds may be worthy of investigation in this context.

Acknowledgments

The authors would like to thank the Ministry of Agriculture, Fisheries and Food for sponsoring this work and the Government Chemist for permission to publish this paper.

References

Astrom, S. (1975) *Fd Manuf.* **50,** 15.
Bucholz, S.B. & Pigott, G.M. (1972) *J. Fd Sci.* **37**, 416.
Crawford, L., Finch R. & Daly, J.J. (1969) *Fd Technol., Champaign*, **23**, 549.
Daly, J.J. (1973) *Fd Process Ind.* **42**, (495), 27.
Guillot, G. (1974) *Revue fr. Diet.* **18**, 32.
Hall, R.C. (1974) *J. Chromatog. Sci.* **12**, 152.
Lawler, F.K. & Trauberman, L. (1969) *Fd Engng,* **41**, 67.
Lovelock, J.E. (1961) *Analyt. Chem.* **33**, 162.
Report of the Government Chemist (1975) H.M.S.O., London.
Statutory Instrument (1975) *The Miscellaneous Additives in Food (Amendment) Regulations* SI 1485. H.M.S.O., London.

(Received 30 July 1976)

J. Fd Technol. (1977) 12, 59–64

Colour retention in sweet red paprika (*Capsicum annuum* L.) powder as affected by moisture contents and ripening stage*

J. KANNER, STELLA HAREL, D. PALEVITCH AND I. BEN-GERA

Summary

The stability of carotenoids in paprika powders was studied at controlled humidity and temperatures during a period of eighty-five days. Reduction in colour intensity during storage at high temperature was rapid in powders with low (1%) moisture contents. Increasing the moisture contents to 14% enhanced colour stability and precluded pigment destruction. The ripening stage of the fruits from which the powder was processed also affected colour deterioration during storage. In powder stored under low moisture conditions produced from fruits allowed to dry on the plant for a considerable time, pigment deterioration was more rapid than in powder produced from fruits harvested ripe but still succulent.

Introduction

Red pepper fruit of the paprika type is used as raw material for the preparation of spices or in the production of colour concentrates for use as natural colouring in the food industries. It is obtained by extracting the lipid-oleoresins fraction. The colour components of red pepper are carotenoids, most of them specific to pepper fruits. The principal ones are Capsanthin, which constitutes about 40% of the total carotenoids present, β carotene (20%) and Capsorubin (10%) (Curl, 1962; Simpson *et al.*, 1974). The colour intensity of the raw material determines the quality of the materials derived from it. Deterioration of the pigments is likely to occur as a result of unsatisfactory storage conditions of the raw material before processing. It was found that the moisture contents of the raw material, the temperature at which it is stored and the composition of the atmosphere, are critical factors in the maintenance of pigment intensity (Lease & Lease, 1956; Chen & Gutmanis, 1968).

*Contribution from the Agricultural Research Organization, Israel, 1974 Series, No. 278-E.

Authors' addresses: Division of Food Technology and Division of Vegetable Crops, Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel.

When paprika fruits were allowed to dry in the sun on the plant itself before harvest, there was a considerable increase in the dry matter content of the subsequently harvest fruit, however, the dry matter yield did not vary during the drying period (Palevitch *et al.*, 1975). Leaving the fruit to dry in the sun resulted in a considerable saving in transport, storage volume, as well as in the energy required to dehydrate the fruits. It also opens up possibilities of once-over mechanised harvesting of high quality fruits.

The aim of the present work was to elucidate the effect of varying moisture contents of powders stored at high temperature on pigment stability in powders produced from fruits picked in a once-over harvest.

Experimental

Plant material

Fruits of cv. Mild California (Peto Seed Corporation, U.S.A.) were picked in a single once-over harvest on 1 October 1972, 146 days after sowing. The fruits were graded into three classes, in accordance with their ripening stage succulent fruits, half dry fruits and dry fruits.

Preparation of powder

The pepper powder was prepared by drying the fruits of all three classes in a drying tunnel at 55°C for 24 hr. After drying, the fruits, without stalk or seeds, were pounded in a Waring Blendor and the powder obtained was sifted through a 40 M.S. plastic mesh sieve.

Colour determinations

Colour intensity was determined by the A.S.T.A. method (Anon, 1968).

Investigation of the effect of storage period

The powders were placed in 6 cm open plastic jars. The depth of the powder layer was 0.5–1.0 cm. The jars were then put in desiccators in the base of which a saturated salt solution was placed in order to obtain different degrees of water activity.

Different degrees of moisture were obtained as a result of the use of different salts, as detailed below (Rockland, 1960).

Salt solution	Water activity	% Moisture of ground powders
$CaCl_2 + CaSO_4$ anhydrous	0.01	1.0
$NgCl_2$	0.32	4.0
$Mg(NO_3)_2$	0.52	7.5
$CH_3COOLi\ 2H_2O$	0.64	14.0
NaCl	0.75	28.0

The desiccators with the salts and with the paprika powders were placed in an incubator at 37°C. They were opened daily for several minutes to allow fresh air to penetrate. According to Rockland (1960), relative moisture values obtained by saturated solutions of these salts are not affected by temperatures in the range of 5–40°C.

Results and discussion

In Fig. 1 are presented the data recorded when paprika powder was stored for eighty-four days at 37°C. The powder was produced from fruit picked in a single harvest at various stages of ripening.

The results show that the moisture level and ripening stage considerably affected the deterioration rate of the colour intensity of the stored powder. Increases in the a_W values increased the colour stability during storage, with greatest stability when the a_W was 0.64 (relative moisture, 14%). Increasing the moisture level above this value caused a reduction in colour intensity. The results indicate that when the powder is kept at the appropriate moisture there is only a minimal loss in colour intensity even at a high storage temperature, for a period of about eighty days.

Initial colour intensity was highest in powder produced from dry fruits left to dry on the plant. Whereas in succulent fruits (85–80% moisture content) immediately after red-ripening, initial colour intensity was only 160 A.S.T.A. units (Fig. 1(a)), in half dry and dry (73–70% and 40–25% moisture content) fruits, the corresponding value was over 265 and 270 units respectively. This is similar to results obtained with cayenne pepper (Lease & Lease, 1956): initial colour intensity of fruits allowed to dry on the plant was higher than in fruits harvested while still succulent.

In powders produced from dry fruits, colour stability was very low when the moisture content of the powders was 0.01 and 0.32 a_W. During storage there was a rapid decline in the colour intensity of these powders. However, in-

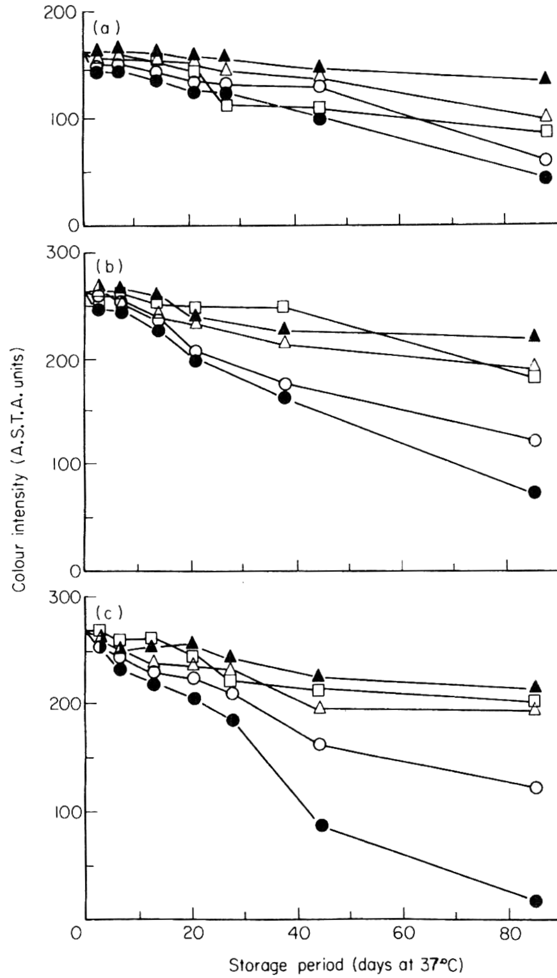


Figure 1. Colour stability of paprika powder influenced by moisture and stage of ripeness. (a) Succulent fruits; (b) half-dry fruits; (c) dry fruits ● 0.01 a_w, 1% moisture; ○ 0.32 a_w, 4% moisture; △ 0.52 a_w, 7.5% moisture; ▲ 0.64 a_w, 14% moisture; □ 0.75 a_w, 28% moisture.

creasing the moisture level stabilized the colour intensity, especially at a_W = 0.64 (Fig. 2). After about eighty days' storage colour intensity was higher in these fruits than the initial colour in fruits harvested while still succulent. This relative stabilization of carotenoids at high moisture levels in the powders is now under investigation in our department. The findings obtained in the present work on sweet paprika are in agreement with those obtained by Chen & Gutmanis (1968), who investigated the effect of moisture contents on the colour stability in chili pepper powder. It is possible that the differences in the stability of the carotenoids in powders prepared from fruit at various stages of maturity can be attributed to differences in the composition and contents of the lipids. It was found that the lipid level rises with increasing maturity (Kanner, 1974). The lipids in pepper fruit are rich in linoleic acid, which undergoes

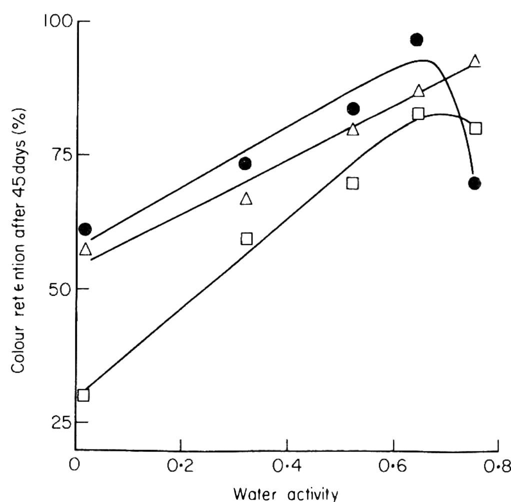


Figure 2. Influence of the water activity on the colour retention after storage for forty-five days at 37°C. ● Succulent; △ half dry; □ dry.

rapid autoxidation (Philip, Nawar & Francis, 1971). 'Coupled' oxidization of carotenoids with unsaturated fatty acids has been found by several workers (Budowski & Bondi, 1960; Blain, Patterson & Pearce 1968; Ben Aziz *et al.*, 1971). We found that the colour loss in pepper, due to deterioration of carotenoids, is accomplished by a coupling process with unsaturated fatty acids (Kanner, 1974). The effect of moisture contents on colour stability in paprika powder can be compared with the effect of this factor on the stability of linoleic acid in solid models (Labuza, Tannenbaum & Karel, 1970; Heidelbaugh & Karel, 1970).

It should be noted that while rate of colour deterioration decrease with high moisture contents, the rate of browning and caking increase under these conditions. In powders intended for pigment extraction this is not detrimental since the browning and subsequent caking have no adverse effect on the final product.

In conclusion, it can be inferred that by storing the powder at the appropriate moisture level it is possible to prevent the deterioration of carotenoids and the loss of colour during storage. This is of special importance when the powder is produced from fruits picked in a single harvest after a certain period of drying on the plant.

References

Anon. (1968) *Official Analytical Methods of the American Spice Trade Association*, A.S.T.A., New York.

Ben Aziz, A., Grossman, S., Ascarelli, L. & Budowski, P. (1971) *Phytochem.* **10**, 1445.

Blain, J.A., Patterson, J.D.E. & Pearce, M. (1968) *J.Sci.Fd Agric.* **19**, 715-7.

Budowski, P. & Bondi, A. (1960) *Arch.Bioch.Biophys.* **89**, 66-73.

Chen, S.L. & Gutmanis, F. (1968) *J.Fd Sci.* **33**, 274.
Curl, A.C. (1962) *J.Agric.Fd Chem.* **10**, 504.
Heidelbaugh, N.D. & Karel, M. (1970) *J.Am.Oil Chem. Soc.* **47**, 539-44.
Kanner, J. (1974) Ph.D. thesis, Hebrew University, Jerusalem. (In Hebrew, English summary.)
Labuza, T.P., Tannenbaum, S.R. & Karel, K. (1970) *Fd Technol., Champaign,* **24**, 546.
Lease, J.G. & Lease, E. (1956) *J. Fd Technol.* **10**, 368.
Palevitch, D., Harel, S., Kanner, J. & Ben-Gera, I. (1975) *Scientia Hort.,* **3**, 143.
Philip, T., Nawar, W.W. & Francis, F.J. (1971) *J. Fd Sci.* **36**, 98.
Rockland, L.B. (1960) *Analyt. Chem.* **32**, 1375.
Simpson, D.J., Rahman, F.M.M., Buckle, R.A. & Lee, T.H. (1974) *Aust. J.Pl. Physiol.* **1**, 135.

(Received 26 April 1976)

J. Fd Technol. (1977) **12**, 65–72

Freeze drying encapsulation of water soluble citrus aroma

I. J. KOPELMAN, S. MEYDAV AND P. WILMERSDORF

Summary

A freeze drying method is proposed for production of citrus aroma powders to be used as natural flavour ingredient in soft drink dry mixes. Approximately 75% of the initial aroma volatiles was retained in the optimal maltodextrin sucrose carrier, as against 50% by the best other method reported (Sugisawa, Kitson & Moyls, 1970).

The aroma powders restored the original flavour in reconstituted orange juice. The low moisture content essential for prevention of losses during storage is obtainable without difficulty.

Introduction

Because of the growing demand for convenience foods, there has been considerable interest in dehydrated citrus based instant soft drinks, with the natural aroma components included in the powder.

Close similarity to the natural flavour can be achieved, when both the oil soluble flavour ingredient (cold-pressed essential oil) and the water soluble aroma essence are incorporated in the product (Mannheim *et al.*, 1967). The degree of concentration of aroma essences in the various industrial recovery systems does not normally exceed a factor of 200; consequently, not less than 5% of the preparation (mostly water) should be added to the dry powder for full cutback of the aroma components. Addition of so much water to a dry citrus base, consisting mainly of sugars and citric acid, is obviously impractical; hence the need to incorporate the aroma in pre-prepared dry form.

While encapsulation or 'locking-in' of citrus essential oils on a dry carrier is well known (Schultz, Dimick & Markower, 1956; Schultz & Talburt, 1961), only scant information is available regarding similar processes for water soluble essences (Sugisawa, Kitson & Moyls, 1970). The present study was undertaken

Authors' address: Department of Food Engineering and Biotechnology, Technion–Israel Institute of Technology, Haifa, Israel.

with a view to a freeze drying method for encapsulation of water soluble citrus aroma in carbohydrate carriers. Extensive work by Thijssen and coworkers (Thijssen & Rulkens, 1969; Menting, Hoogstad & Thijssen, 1970; Rulkens & Thijssen, 1972), and by Flink & Karel (1970a, b) indicated that retention of volatile compounds during freeze drying is highly dependent on composition: in various model systems retention increased with decreasing molecular weight of the carbohydrates and increasing total soluble solids (up to about 20%). On the other hand, substantial losses are known to be incurred through collapse or melting frequently encountered in freeze drying of sugar solutions (Rey & Bastien, 1962; MacKenzie, 1965), thus reduction of the molecular weight may act both ways.

The specific aims of the study were, accordingly: (a) evaluation of the effect of various combinations of carbohydrates (mono- and disaccharides and maltodextrins) on retention of orange aroma volatiles under constant drying conditions and (b) determination of certain properties of the aroma powders.

Materials and methods

Materials

Aroma. Commercial 150-fold water-soluble orange aroma essence, freshly obtained from a Gulf evaporator.

Corn syrup solids. Fifteen, thirty-two and forty-two D.E., by American Maize-Products Co., Hammond, Indiana, U.S.A.

Orange concentrate. Sixty degrees Bx frozen concentrate, obtained from local industry.

Essential oil. Cold-pressed Valencia peel oil.

Chemical. Glucose, sucrose, lactose – all analytical grade.

Experimental procedure

Solutions for freeze drying were prepared by dissolving various blends of corn syrup solids and sugars (mono- and disaccharides) in the aroma solution at the level of 25% w/w. The solutions were frozen overnight at –20°C in a deep freezing cabinet, and subcooled thereafter with liquid nitrogen, granulated and screened (2–5 mm) and evenly spread upon the freeze drier trays. Freeze drying was carried out in 2.5 m^2 Grenco RI-25 pilot plant unit. The product was charged at 6 kg/m^2 and dried for 20 hr at 0.2–0.01 Torr with the heating plate maintained at 40°C. The heat was supplied by radiation from both sides of the product tray. Condenser temperature was kept at –45 to –50°C.

C.O.D. determination. According to Dougherty (1968) with a slight modification, colorimetric readings taken (Beckman D.U. Spectrophotometer) at 605 nm – the wavelength of a major absorbance peak observed in advance for

C.O.D. reaction products of standard sucrose solutions and of the aroma distillate.

D-limonene. By bromide-bromate titration (Scott & Velhuis, 1966).

Moisture content. By drying in a vacuum oven for 24 hr at 55°C, a procedure found to yield results similar (within the experimental error) to a series of determinations by the Karl Fischer method (McComb & Wright, 1954).

Gas chromatography. Aroma powder was dissolved in distilled water at 1 : 3. Ten per cent (w/w) of NaCl was added, the solution extracted with 100 ml methylene chloride in a laboratory homogenizer (Polytron, by Kinematical GmbH), and the organic phase drawn off in a 250 ml separation funnel. Extraction was repeated three times, and the combined extracts were dried over anhydrous Na_2SO_4. The extract was concentrated to a final volume of 1 ml in a two-step procedure on a rotary film evaporator (Rotovapor EL, Büchi-Switzerland) with the water bath kept at 30°C. Five microlitre samples of the concentrated extract were injected into the gas chromatograph (Packard model 7600) equipped with a dual flame ionization detector for programmed GLC analysis, using two identical glass columns (8 ft × 1/8 in.), charged with 20% carbowax 20 M on 60/80 mesh Chromosorb P DMCS. Carrier gas was N_2 at 30 ml/min. Oven temperature was programmed as follows: (1) 85–180°C at 4°C/min; (2) isothermally at 180°C for 15 min; (3) increase to 220°C at 4°C/min and kept there until end of run. The injection-port and detector temperatures were 150 and 235°C, respectively.

Major peaks were identified by comparing retention times of pure substances typical of orange essence (Wolford, Alberding & Attaway, 1962), injected after addition to the aroma extract at known concentration.

Sorption isotherms. Plotted at 25°C by exposure to water vapour under vacuum over constant-humidity salt (Rockland, 1960) and sulphuric acid (Anon, 1951) solutions.

Organoleptic evaluation. Ranking tests by a panel of three highly trained experts with at least two years' experience in citrus juice.

Results and discussion

Throughout the tests, the orange aroma was successfully incorporated into combinations of carbohydrate carriers to yield free flowing concentrated aroma powders, which restored the original flavour in addition to reconstituted juice prepared from frozen aroma stripped concentrate. The degree of improvement was correspondent with that of aroma retention.

Aroma retention

Table 1 shows that aroma retention improved with increase of the dextrose equivalent of the corn syrup solids. These findings are in agreement with those

Table 1. Aroma retention in freeze dried maltodextrin carriers

Dextrose equivalent (D.E.)	C.O.D. retention		D-limonene retention		Moisture content (%)
	ppm	%	ppm	%	
15	38 200	42	640	97.8	0.2
32	55 500	62	610	93.3	0.5
42	59 500	67	630	96.3	0.7

Initial strength 90 000 ppm (dry powder basis).

reported for freeze drying of model systems (Flink & Karel, 1970a, b) and with the theory that retention of volatiles in drying processes is directly related to the content of low MW carbohydrates.

None of the freeze drying difficulties or failures, frequently occurring in low MW carbohydrates solutions (Rey & Bastien, 1962; MacKenzie, 1965), were encountered with the corn syrup solids systems. Further evidence of their ease of drying is the low moisture content (<1% of all samples).

The results of the subsequent attempt at further improvement by admixture of mono- and disaccharides are shown in Table 2. Such improvement was achieved with 20% glucose added to 15 D.E. maltodextrin (calculated D.E. 32); in fact, the degree of retention thus obtained was the same as for 32 D.E. corn syrup solids (Table 1), even though the two systems are totally dissimilar in carbohydrate composition. Further increase of the glucose content resulted in a poorer product, apparently, due, *inter alia,* to impaired release of moisture as evidenced visually by spot-melting and puffing.

Admixture of disaccharides improved the strength of the aroma powder, this time without any observed difficulties in dehydration (Table 3). It is seen that a 2 : 3 sucrose–maltodextrin 15 D.E. mixture yielded a powder retaining close to 75% of the initial volatiles, i.e. well above the 50% level reported for other water soluble aroma encapsulation method (Sugisawa *et al.,* 1970).

Table 2. Aroma retention in freeze dried 15 D.E. dextrin–glucose system

Composition		D.E. of system	C.O.D. retention		Moisture content (%)
Glucose (%)	Dextrin 15 D.E. (%)		ppm	%	
0	100	15	38 500	43	0.3
20	80	32	56 000	62	0.9
40	60	49	46 000	51	1.8
60	40	66	45 000	50	2.4

Table 3. Effect of added sugars on aroma retention during freeze drying

Composition		C.O.D. retention		
Sugars	Dextrin 15 D.E. (%)	ppm	%	Moisture content (%)
Glucose 40%	60	46 000	51	1.8
Sucrose 40%	60	65 800	73	1.2
Glucose 20% + lactose 20%	60	64 000	71	1.4
Sucrose 20% + lactose 20%	60	65 600	73	0.9

Aroma composition

The C.O.D. method, which yields the overall organic volatiles content, is clearly of limited value here, as citrus aroma essence contains non aromatics (methanol, ethanol, acetone) (Walford *et al.*, 1962), which have little effect upon flavour and taste. By contrast, more specific evaluation is possible by means of gas chromatography.

Results for the aroma solution, and for the corresponding aroma powder, are given in Figs 1 and 2, and some of the identified compounds known to contribute to citrus flavour (Wolford *et al.*, 1962), are listed in Table 4. It is seen that most of the compounds are retained at a fairly high level; the much lower loss found by this method (compared with the 30% figure by the C.O.D.

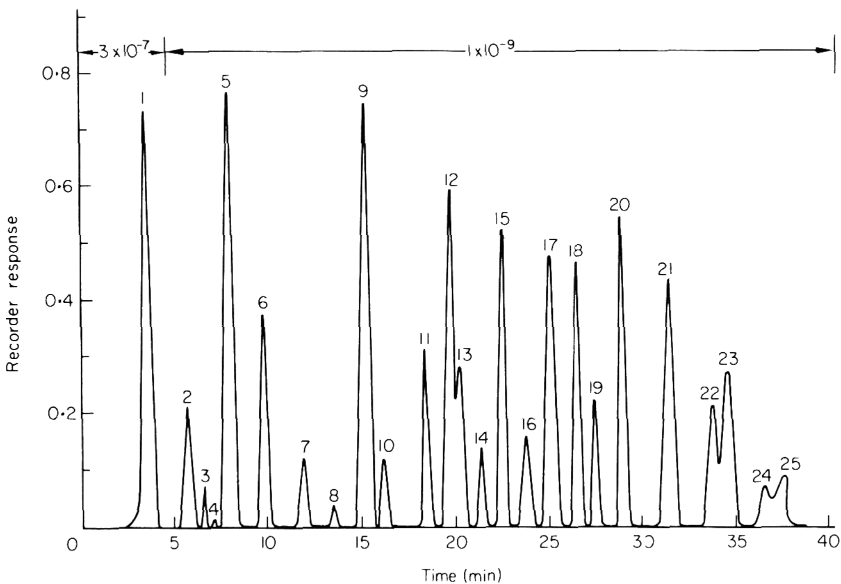


Figure 1. Gas-Chromatogram of volatiles extract from aroma essences.

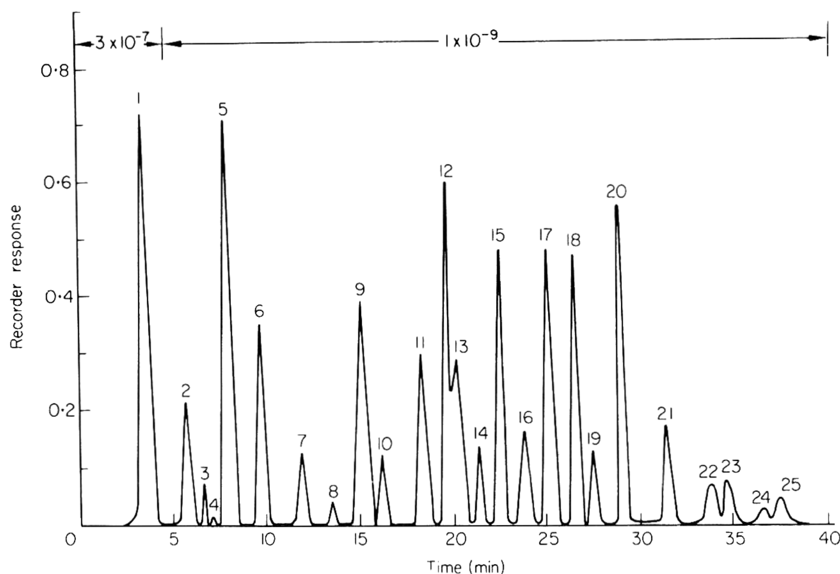


Figure 2. Gas-chromatogram of volatiles extract from freeze dried aroma powder, based on 2 : 3 sucrose dextrin 15 D.E. carrier.

method) is attributable to selective retention of compounds with higher molecular weight, as reported by Thijssen & Rulkens (1969), thus, it can be assumed that the actual aroma strength of the freeze-dried powder is higher than would seem from the C.O.D. test.

Storage stability

The percentage retention of volatiles in aroma powders stored for ninety days at different relative humidities and 35°C is given in Table 5. Up to R.H. 11% the relative losses are small for all types of carriers; for R.H. 22% and above, there is considerably heavier depletion which makes storage under such conditions commercially impractical. These increased losses may be correlated

Table 4. Aroma compounds identified in volatiles extracts from aroma essence

Peak no.	Compound	Peak no.	Compound
1	Methylene chloride (solvent)	17	Furfural
	Methanol, Ethanol	19	m-Decanal, Citronellal
8	β-Myrcene	20	Linalool
9	d-Limonene, 2-Hexanal	21	Terpinene-4-ol
12	n-Octanal	22,23	α-Terpineol, Neral
14	n-Hexanal	24,25	Citronellol, Geranial
16	n-Nonanal		

Table 5. Effect of R.H. on aroma losses during three months' storage at 35°C

Carrier (D.E.)	Percentage of initial aroma volatiles (C.O.D.) retained			
	R.H. 6%	R.H. 11%	R.H. 22%	R.H. 33%
15	95	86	55	30
32	97	94	68	42
42	96	92	50	28

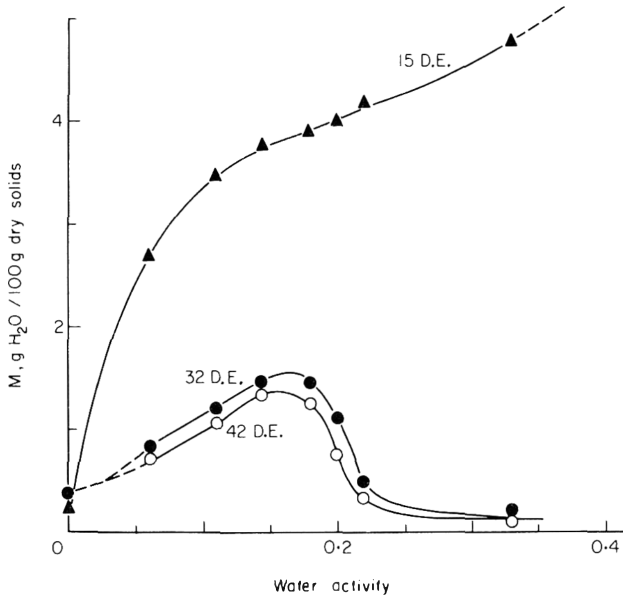


Figure 3. Water sorption isotherms of aroma powders at 35°C.

with the water sorption properties of the powders, as shown in the sorption isotherms in Fig. 3. The powder based on maltodextrin D.E. 15 shows a monotonic increase in moisture content with increasing water activity. This curve is typical of high MW products, such as starch, pectin and proteins (Gane, 1950; Salwin 1963). By contrast, the powders based on the 32 and 42 D.E. carriers absorb water to a much lesser extent, with a peak of 1.5 g H_2O/100 g solids at a_w = 0.15–0.20, beyond which the equilibrium moisture content drops to a negligible level at a_w = 0.33; such a pattern is typical of amorphous materials subject to recrystallization due to increase in water activity, as reported for glucose and sucrose (Makower & Dye, 1956). In the case of the 15 D.E. carrier, the volatiles losses are probably due directly to the water sorption process, which induces structural changes in the micro regions where the aroma components are encapsulated. In the case of the 32 and 42 D.E. carriers, the losses are probably caused by matrix rearrangement during the recrystallization process. Similar disruption of the matrix, accompanied by loss of volatile organic

alcohols, as a result of water sorption above the monolayer and of recrystallization of amorphous lactose, was observed by Flink & Karel (1972) in freeze dried model systems.

References

Anon. (1951). A.S.T.M. designation E104/51.
Dougherty, M.H. (1968) *Fd Technol., Champaign,* **22**, 1456.
Flink, J. & Karel, K. (1970a) *J. Agric. Fd Chem.* **18**, 295.
Flink, J. & Karel, M. (1970b) *J. Fd Sci.* **35**, 444.
Flink, J. & Karel, M. (1972) *J. Fd Technol.* **7**, 199.
Gane, R. (1950) *J. Sci. Fd Agric.* **1**, 42.
MacKenzie, A.P. (1965) *Annex of New-York of Science* **125**, 522.
Makower, R. & Dye, W. (1956) *J. Agric. Fd Chem.* **4**, 72.
Mannheim, H.C., Bomben, J.L., Guadagni, D.G., & Morgan, A.T., Jr (1967) *Fd Technol, Champaign,* **21**, 469.
McComb, E.A. & Wright, H.W. (1954) *Fd Technol., Champaign,* 8, 73.
Menting, L.C., Hoogstad, B. & Thijssen, H.A.C. (1970) *J. Fd Technol.* **5**, 127.
Rey, L.R. & Bastien, M.C. (1962) *Freeze-Drying of Foods* (Ed. by F.R. Fisher) pp. 25-42. National Academy of Science, National Research Council, Washington, D.C.
Rulkens, W.H. & Thijssen, H.A.C. (1972) *J. Fd Technol.* **7**, 79.
Rockland, L.B. (1960) *Analyt. Chem.* **32**, 1375.
Salwin, J. (1963) *Fd Technol, Champaign,* **17**, 1114.
Schultz, T.H., Dimick, K.P. & Markower, B. (1956) *Fd Technol, Champaign,* **10**, 57.
Schultz, T.H. & Talburt, W.F. (1961) *Fd Technol, Champaign,* **15**, 188.
Scott, W.C. & Velhuis, M.K. (1966) *J. ass. off. analyt. Chem,* **49**, 628.
Sugisawa, H., Kitson, J.A. & Moyls, W. (1970) *Confructa,* **15**, 280.
Thijssen, H.A.C. & Rulkens, W.H. (1969) *Recent Developments in Freeze-Drying, Vol. I,* pp. 99–113. IIR, Commission X, Lausanne, Switzerland.
Wolford, R.W., Alberding, G.E. & Attaway, J.A. (1962) *J. Agric. Fd Chem.* **10**, 297.

(Received 4 June 1976)

J. Fd Technol. (1977), 12, 73–84

The expansion of wafer and its relation to the cracking of chocolate and 'bakers' chocolate' coatings*

L. F. BARRON

Summary

The linear expansion of a wafer was found to be directly proportional to any increase in moisture content over most of the range from 0.5% to the moisture content in equilibrium with the atmosphere to which the wafer was exposed. The average gain in linear dimension of the wafer per 1% increase in moisture content was 0.4%, which was sufficient to cause cracking of the chocolate coating. The time taken for the coating to crack, as a result of adsorption of water vapour from the atmosphere through holes in the coating, was measured as a function of type and thickness of coating, initial moisture content of the wafer and the relative humidity of the atmosphere. The results were examined in relation to commercial attempts to minimize cracking of the coating by prior conditioning of the wafer sheet. It was concluded that cracking will only be inhibited if (i) there is uniform distribution of moisture within the wafer and (ii) there are no holes or flaws in the coating.

Introduction

Chocolate-coated wafers usually consist of two to four layers of wafer sheet sandwiched with one to three layers of cream filling, the composite sandwich being subsequently coated with chocolate by either an enrobing or a moulding

* In this paper 'chocolate' refers to those coatings containing cocoa butter and, where appropriate, milk fat as the only fats, while 'bakers' chocolate' refers to those coatings in which the cocoa butter has been largely replaced by fractionated vegetable fats. The generic term 'coatings' has been used to describe both groups. It should be noted that the nomenclature of chocolate products is controlled by the Cocoa and Chocolate Regulations 1976, which fully come into operation in May 1977. These regulations prescribe compositional standards for 'chocolate' and forbid the use of this name to describe products which do not conform to these standards. Coatings such as 'bakers' chocolate' must be described in such a way that they cannot be confused with 'chocolate'. A designation such as 'chocolate-flavoured coating' appears to be acceptable for 'bakers' chocolate' and similar products.

Author's address: Flour Milling and Baking Research Association, Chorleywood, Rickmansworth, Herts. WD3 5SH.

process. It is widely believed that cracking of the chocolate coating on such products is produced by changes in dimensions of the wafer during storage caused by (i) redistribution of moisture within the wafer, resulting in simultaneous expansion and contraction of different parts and (ii) expansion of the wafer due to adsorption of water vapour through holes in the chocolate coating.

It is common practice in most factories manufacturing chocolate-coated wafers for the wafers to be conditioned, either before or after creaming, in an atmosphere of controlled temperature and humidity for periods varying from a few hours to several days. The objects of this conditioning process are (i) to permit an even distribution of moisture within the wafer sheet and (ii) to allow the wafer to adsorb a limited amount of moisture from the atmosphere which would reduce the subsequent rate of adsorption of water vapour through any holes present in the chocolate coating. In some instances wafers are conditioned to a final moisture content of about 5%, but they become tough and their eating quality deteriorates if the moisture content rises to more than about 6%.

A survey in industrial plants of the distribution of moisture in wafer sheets throughout the process of baking, creaming, conditioning and chocolate enrobing gave evidence supporting the mechanisms of cracking of the coating described above and confirmed the usefulness of the objects of conditioning.

The present paper describes an investigation in the laboratory of (i) the relation between the adsorption of water vapour by a wafer sheet and its linear expansion and (ii) the relation between the time taken for various types of chocolate coating to crack and the conditions determining the expansion of the wafer, the object being to provide quantitative evidence which might be of value in the design of conditioning procedures.

Linear expansion of a wafer sheet

Apparatus

The apparatus used for the measurement of water vapour adsorption and linear expansion of a wafer sheet is shown in Figs 1 and 2. Air was circulated by a diaphragm pump A in a closed system consisting of two Drechsel bottles B, containing a saturated solution of a suitable salt to control the relative humidity at the desired level, a cotton wool trap C and the Perspex container D holding the wafer sample. Suspended from the top of the container was a calibrated spring balance E to which the wafer sheet was attached by means of two hooks. The spring consisted of twenty-two turns, 2.45 cm in diameter, of 30 SWG copper/beryllium wire which had been tempered for 1 hr at 315°C. A fine pointer was attached horizontally to the lower end of the spring, and a scale graduated in millimetres was located adjacently. The whole apparatus was enclosed in a thermostatically controlled incubator at 24°C, having an inner glass door through which the pointer and scale were observed by means of a travelling microscope.

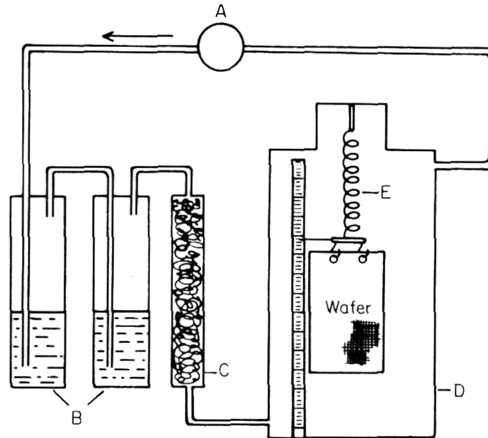


Figure 1. Diagram of apparatus. A, Diaphragm circulating pump; B, Drechsel bottles; C, Cotton wool trap; D, Perspex container; E, spring balance.

Experimental

The spring balance was calibrated by hanging a series of known weights on the spring. The scale readings (spring extension) were graphed against the weights applied and all the points lay on a straight line passing through the origin (calibration constant 5.3 cm/g).

The wafers used in this investigation were produced on the pilot plant at the Flour Milling and Baking Research Association and details of their preparation are as follows (Pritchard & Stevens, 1973):

Formula

English flour	2520 g
Water	3700 g
Powdered lecithin	24 g
Groundnut oil	60 g
Salt	6 g
Sodium bicarbonate	8 g

Processing

Gap between wafer plates	1.78 mm
Baking time	90 sec at 195°C

Immediate closure of plates after deposition of batter.

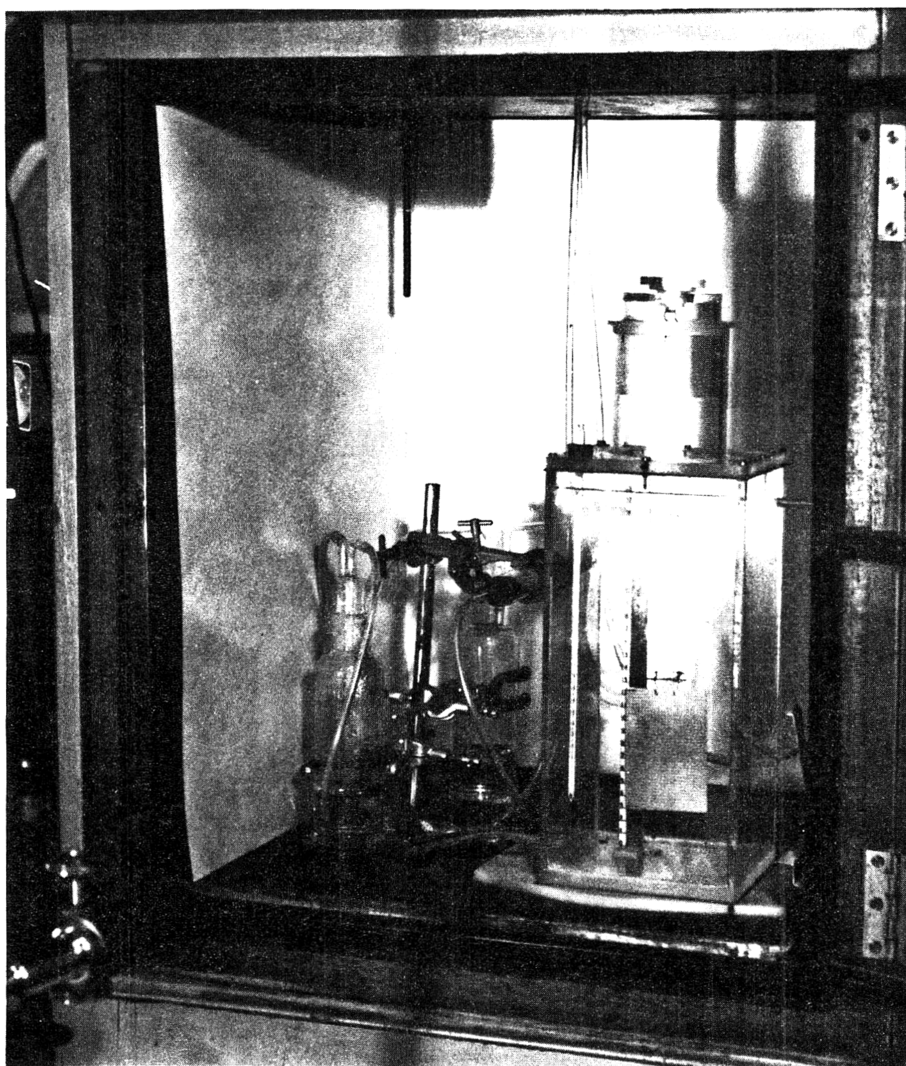


Figure 2. Apparatus for measuring expansion of wafer.

The wafer sheets were cut into nine equal pieces (157 x 98 mm) five of which were placed in a pile and packed in three layers of polyethylene. This procedure prevented adsorption of water vapour during storage for 48 hr, required to ensure an even distribution of moisture. The centre wafer from the pile was then removed and a piece measuring 102 x 64 mm was cut from it. Thin black ink lines were drawn at the top and bottom of the wafer to provide reference points for length measurements, and it was then rapidly attached to the hooks of the spring balance. When the wafer came to rest (20–30 sec) the spring balance scale reading was noted and the distance between the two ink marks on the wafer was measured, all readings being taken by means of the travelling microscope. Subsequent readings were made at hourly intervals for the first 8 hr, and thereafter at daily intervals until equilibrium was reached. As

Table 1. Equilibrium relative humidities of saturated salt solutions at 24°C.

Saturated salt solution	Equilibrium humidity (%)
Lithium chloride	11.7
Magnesium chloride	32.9
Potassium carbonate	43.7
Magnesium nitrate	53.0
Sodium nitrite	64.6
Sodium chloride	75.5

soon as the first readings had been taken, moisture determinations (oven method, 90 min at 130°C) were carried out on the two pieces of wafer which had been in contact with the centre test piece during the 48 hr storage period. The mean figure obtained was taken as the moisture content of the test piece at zero time, as it had been shown experimentally that the moisture contents of the three pieces of wafer stored under these conditions were identical.

The gain in weight (adsorption of water vapour) and increase in linear dimension of the wafer were determined at six levels of relative humidity using suitable saturated solutions as shown in Table 1 (Wink & Sears, 1950; Wexler & Hasegawa, 1954; Young, 1967), the air temperature being maintained throughout at 24°C. Three determinations were carried out at each level of relative humidity and the results were averaged.

Results

The true moisture content of the wafer at the time of each reading was calculated from the moisture content at zero time and the gain in weight as measured by the spring balance. The rate at which water vapour was adsorbed by a wafer sheet in all cases followed a curve of the type shown in Fig. 3, which gives results obtained at 53.0% relative humidity for the change in moisture content as a function of time. The rate of linear expansion of the wafer sheet under the same conditions is shown in Fig. 4 and, as would be expected, the shape of this curve is similar to that in Fig. 3. The shape of the equilibrium adsorption isotherm was sigmoidal (Fig. 5).

When the gain in moisture content was graphed against the gain in length of the wafer, expressed as a percentage of the length at zero time, the relation was found to be linear over most of the range at each level of relative humidity. The graph illustrating this relation at 53.0% relative humidity is shown in Fig. 6. The departure from linearity near the origin probably indicates that in this re-

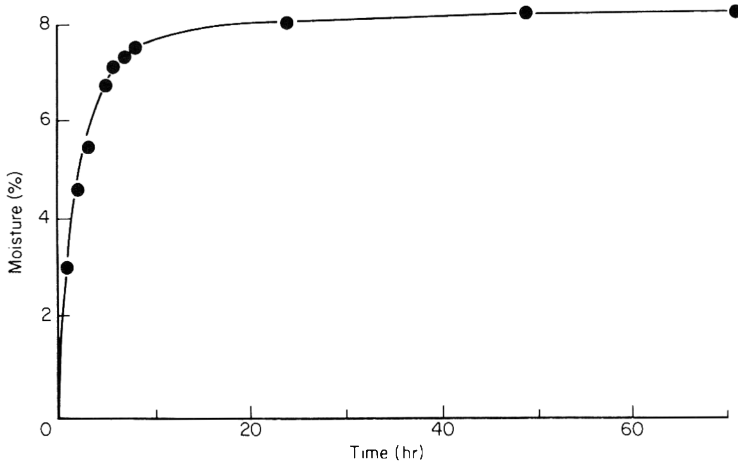


Figure 3. Rate of adsorption of water vapour by a wafer sheet in atmosphere of 53.0% relative humidity.

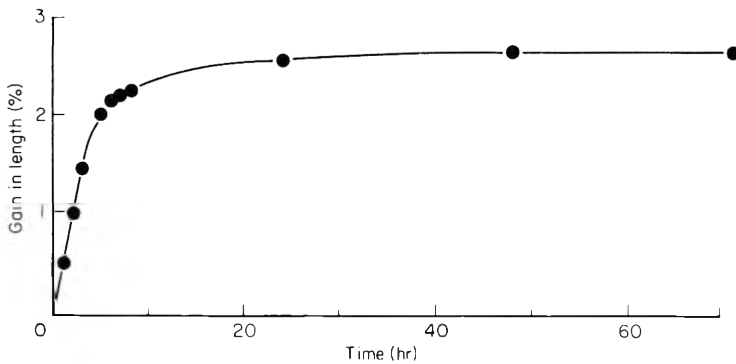


Figure 4. Rate of gain in length of a wafer sheet in an atmosphere of 53.0% relative humidity.

gion the rate of expansion of the wafer is relatively lower than the initially very rapid adsorption of moisture. Throughout the linear region the average gain in length of the wafer per 1% gain in moisture was 0.42% over the normal ambient range of relative humidities (43.7–75.5%). At lower levels of relative humidity (11.7–32.9%) the average gain in wafer length was 0.33%.

Determination of the relation between wafer expansion and the time taken for a coating to crack

Experimental

The procedure employed for the preparation of the wafer samples was the same as that described in the previous section. After the 48 hr storage period the five pieces of wafer were each cut into nine smaller pieces measuring

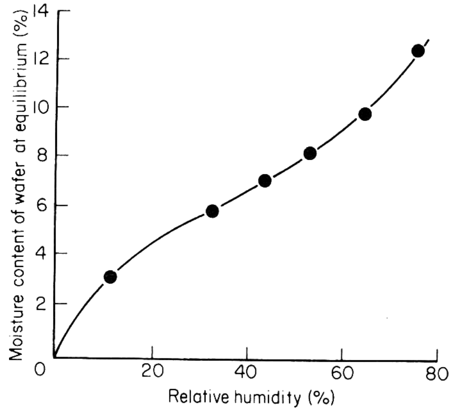


Figure 5. Isotherm showing the moisture content of a wafer sheet in equilibrium with various relative humidities.

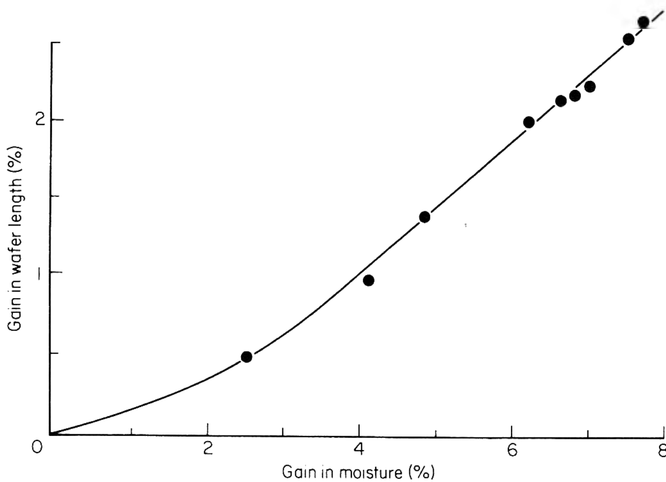


Figure 6. Relation between the gain in length of a wafer sheet and the gain in moisture content in an atmosphere of 53.0% relative humidity.

52 x 33 mm which were immediately sealed in a polyethylene bag so that no change in moisture content would take place. Approximately half of these wafer pieces were used for determination of moisture content, while the remainder were individually weighed and hand-dipped in coating. The plain chocolate was melted to 46–49°C and tempered by cooling to 28°C and then heating to 31–32°C. Milk chocolate was similarly tempered but the final temperature was 29–30°C. The 'bakers' chocolate' was melted to 49°C and then cooled to 37–43°C; no tempering was required. Particular care was taken to ensure that the coating was continuous and that there were no holes through which water vapour could pass. After cooling, each coated wafer was stored in an individual polyethylene bag in a tin. Any faulty samples containing holes in the coating developed cracks after 48 hr storage and were discarded. In order to hang the coated wafer on the hooks of the spring balance two suitable holes

1.65 mm in diameter were drilled with a sharp pointed steel nail through the sample at one end. An additional two holes were drilled, one at the centre and one at the lower end of the sample so that there was a total of four points where the wafer was exposed to the atmosphere. The sample was placed on the spring balance, the time was noted and when the sample had come to rest the scale reading was noted by means of the travelling microscope. The sample was kept under continual observation and, as soon as a crack in the coating appeared in the vicinity of one of the holes, the time and scale reading were noted. The sample was then removed from the balance and broken along the line of the crack in the coating. The thickness of the coating at the line of fracture was measured at a number of points by means of the travelling microscope and the average figure recorded.

This procedure was carried out using plain and milk chocolate and dark and light 'bakers' chocolate'. In each case various thicknesses of coating and three levels of relative humidity, *viz.* 32.9%, 53.0% and 75.5%, were employed. With plain chocolate and dark 'bakers' chocolate' additional observations were made at 100% relative humidity.

A separate series of experiments used wafers of different moisture contents which were coated with milk chocolate, with the object of determining the relation between the moisture content of the wafer and the time taken for the chocolate to crack.

Results

Relation between the time taken for the coating to crack and the thickness of the coating. When the time taken for cracks to appear in the coating was graphed against the average thickness of the coating along the line of fracture, a straight line passing through the origin was obtained at each level of relative humidity. As anticipated, the lower the relative humidity the lower was the rate of moisture adsorption and the longer the time taken for the coating to crack. The results obtained for plain chocolate are shown in Fig. 7, and in this case additional measurements were made at 100% relative humidity.

Similar sets of results were found for milk chocolate, dark and light 'bakers' chocolate', and also for milk chocolate coated wafers having different moisture contents (2%, 3%, 4.5% and 5.5%).

Relation between the time taken for the coating to crack and the relative humidity of the atmosphere. The times taken for cracks to appear in coatings of constant thickness (arbitrarily chosen at 0.65 mm) were read off from the graphs of time *v.* coating thickness, and these figures were then graphed against the relative humidity of the atmosphere for all four types of coating (Fig. 8). In each series of observations the initial moisture content of the wafer was 2%. The curves are rectangular hyperbolae which are asymptotic to the equilibrium relative humidity of wafer at 2% moisture (7% r.h.) and to the time taken for the coating to crack in an atmosphere of 100% relative humidity. Significant

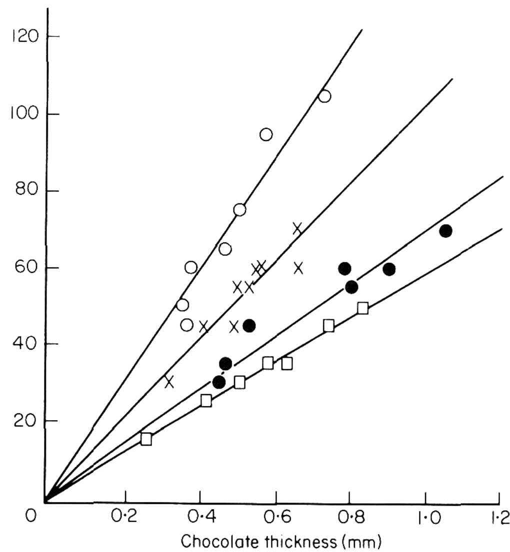


Figure 7. Relation between the time taken for a plain chocolate coating to crack and the thickness of the chocolate at various humidities (○ 32.9%; x 53.0%; ● 75.5%; □ 100.0%). Initial moisture content of wafer = 2%

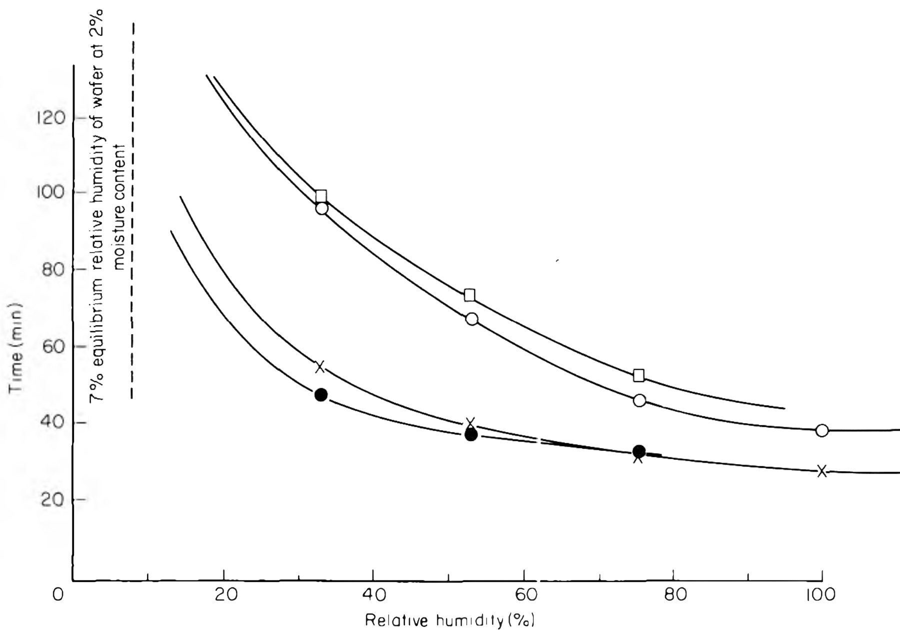


Figure 8. Relation between the time taken for coatings to crack and the relative humidity of the atmosphere. Initial moisture content of wafer = 2%; thickness of coating = 0.65 mm. ○ Plain chocolate; x milk chocolate; ● dark 'bakers' chocolate'; □ light 'bakers' chocolate'.

differences between the rates of cracking of the four types of coating are apparent. The fact that plain chocolate takes longer to crack than milk chocolate under the same conditions may be explained in terms of the compositions of the fats in the two chocolates. The fat in the plain chocolate was 100% cocoa

butter whereas that in the milk chocolate was a mixture of cocoa butter and butter fat contributed by full-cream milk crumb. It is suggested that since the butter fat/cocoa butter mixture was softer than pure cocoa butter it would offer less resistance to the forces set up by the expansion of the wafer and the milk chocolate would therefore fracture earlier than the plain chocolate.

The two 'bakers' chocolates' gave reversed results, however, since the dark variety fractured earlier than the light variety. The light variety contained fractionated fats and also a small proportion of cocoa butter derived from the cocoa powder used in its manufacture. The cocoa solids content was greater in the dark variety than in the light variety and the proportion of cocoa butter in the total fat was therefore greater. The fractionated fat and cocoa butter would form an eutectic mixture with a lower melting point which would be softer than the fat in the light variety which was affected to a lesser extent. In addition the total fat content of the light variety was about 6% less than that of the dark variety and this contributed to the difference in hardness of the two coatings.

Relation between the time taken for milk chocolate to crack and the moisture content of the wafer. Wafer pieces having moisture contents of 2%, 3%, 4.5% and 5.5% were coated with milk chocolate and the times taken for the chocolate to crack at various chocolate thicknesses were measured at two levels of relative humidity (53.0% and 75.5%). From the graphs of time *v.* chocolate thickness the times taken for cracks to appear in chocolate of 0.65 mm thickness were read off and these figures were plotted against the moisture content of the wafer (Fig. 9). The curves were asymptotic to the equilibrium moisture contents of the wafer at each level of relative humidity, and increasing the moisture content of the wafer from 2% to 5.5% doubled the time taken for the chocolate to crack.

Discussion

The results confirm that, with a standard area of wafer exposed to the atmosphere, the time taken for a coating to crack is dependent on the thickness of the coating, the moisture content of the wafer, and the relative humidity of the atmosphere. With a wafer of 2% moisture content coated with chocolate of average thickness (0.65 mm) and exposed to an atmosphere of low relative humidity (32.9%) the time taken for the chocolate to crack was short – 45 min for milk chocolate and 90 min for plain chocolate. It must be remembered that there were four holes of 1.65 mm diameter in each test piece, and the area of wafer exposed to the atmosphere was greater than would be found in an average commercial sample. An accelerated effect was thus assured, but even so, if holes are present in the coating on a commercial wafer sandwich, cracks must be expected to appear within a relatively short time of the enrobed pieces leaving the cooler unless they are wrapped immediately. Wrapping in a material of low water vapour permeability will retard the adsorption of water vapour pro-

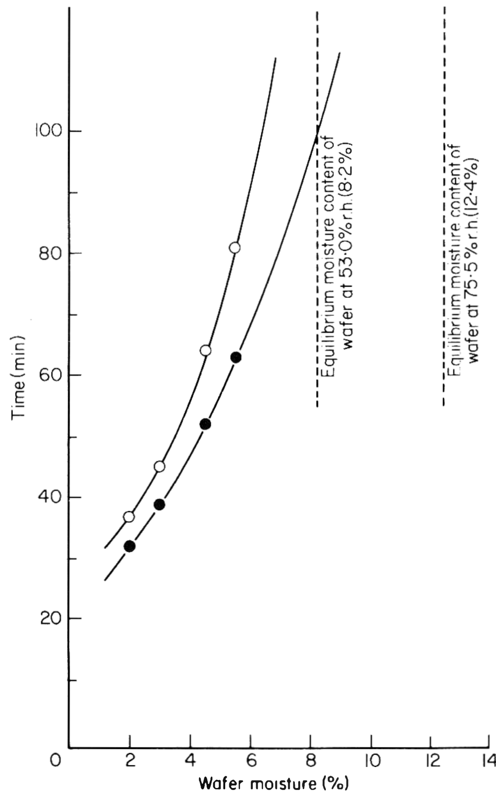


Figure 9. Relation between time taken for milk chocolate to crack and moisture content of wafer. Thickness of chocolate coating = 0.65 mm. ○ 53.0% r.h.; ● 75.5% r.h.

viding the pack is perfectly sealed. The moisture content of the wafer must increase by about 1% before cracks will appear in chocolate 0.65 mm thick and, since the weight of wafer in an average commercial product is about 2–3 g, the weight of water vapour which must be adsorbed amounts at most to 0.03 g. A wrapper (130 cm^2) with a low water vapour permeability (1 $g/m^2/24$ hr) and suitable for an average size commercial product, will transmit this amount of water vapour in about three days under temperate conditions (25°C and 75% r.h.). Under average commercial storage conditions (19°C and 50–60% r.h.) cracks in the coating would be expected to appear within one or two weeks after manufacture. If the wafer is conditioned before enrobing to an evenly distributed moisture content of about 5% and there are holes in the coating, the time taken for cracks to appear will be doubled. Even so this conditioning would only increase the time to about two to four weeks with a perfectly sealed wrapper. Cracking of chocolate coating containing holes would only be eliminated if a perfectly sealed wrapper were used which was completely impermeable to water vapour. Such a wrapper, e.g. a multifoil laminate, would in most cases be uneconomical.

It is evident that to prevent cracking the most important requirement is that the coating should be continuous and that there should be no holes in it. If this

requirement is achieved the moisture content of the wafer is of secondary importance, except that the lower the moisture, the crisper will be the wafer. However, it is essential that the moisture should be evenly distributed within the wafer and that there should be no moisture gradients. If these two criteria are satisfied the coating will not crack.

Conclusions

(1) Determinations of the expansion of a wafer sheet under controlled conditions showed that at each level of relative humidity there was a linear relation between the percentage gain in length of the wafer sheet and the gain in moisture content over most of the range.

(2) The average gain in wafer length per 1% increase in moisture content was 0.42% over the normal ambient range of relative humidity (43.7–75.5%). At lower levels of relative humidity (11.7% and 32.9%), the average gain in wafer length was 0.33% per 1% increase in moisture content.

(3) The time taken for a coating on a wafer to crack is dependent on the thickness of the coating, the moisture content of the wafer and the relative humidity of the atmosphere. The size and number of holes in the coating also affect the time.

(4) When the moisture content of the wafer was increased from 2% to 5.5% by suitable conditioning prior to enrobing the time taken for the coating to crack was doubled.

(5) In order to ensure that the coating will not crack it must be continuous and entirely free from holes and the moisture in the wafer must be evenly distributed.

Acknowledgments

The author's thanks are due to the directors of Lesme Ltd who provided the chocolate and 'bakers' chocolate' used in this investigation and to the staff of the Confectionary and Biscuit Sections at Chorleywood for assistance with the experimental work.

References

Pritchard, P.E. & Stevens, D.J. (1973) *Food Trade Review*, **43**, 11.
Wexler, A. & Hasegawa, S. (1954) *J. Res.Natn.Bur.Stand*, **53**, 19.
Wink, W.R. & Sears, G.R. (1950) *TAPPI*, **33**, (Sept.), 96A.
Young, J.F. (1967) *J.appl.Chem.* **17**, 241.

(Received 30 June 1976)

J. Fd Technol. (1977) 12, 85–91

Technical note: Acid sensitivity of freeze injured salmonellae in relation to their isolation from frozen vegetables by pre-enrichment procedure

A. Y. SADOVSKI

Introduction

The potential public health hazards existing in frozen foods requires adequate control measures in order to ensure the absence of pathogenic bacteria. The recommended methods for the isolation of salmonellae from foods often suggest a pre-enrichment step and most commonly in lactose broth (Angelotti, 1963; Thatcher & Clark, 1968; A.O.A.C., 1970; F.D.A., 1972).

In using lactose broth and other media for pre-enrichment of salmonellae from frozen vegetables, we have observed a fast decrease in pH. Considering the sensitivity of salmonellae to low pH (Chung & Goepfert, 1970; Huhtanen, 1975; Prost & Reiman, 1967) we found it necessary to study the influence of this fact on the recovery of freeze injured salmonellae from frozen vegetables.

Materials and methods

Salmonellae serotypes were isolated in this laboratory and identified by the Government Central Laboratories, Salmonellae Center of Israel, Jerusalem. *S. typhimurium, S. senftenberg* and *S. concord* were isolated from sewage effluent and *S. anatum* from samples of corn kernels taken from a production line of a commercial vegetable freezing plant just before blanching. The cultures were routinely maintained on Trypticase Soy Agar (TSA, Difco) slants. Cultures used in the experiments were 17–18-hr old and were prepared by three consecutive subculturings in nutrient broth (NB, Difco). All dilutions were made in saline (0.85% NaCl). Viable cell count was performed by triplicate plating on nutrient agar (NA, Difco). Pre-enrichments were made in 0.5% lactose broth and in buffered peptone water (BPW), which was made of 10.0 g Bactopeptone; 5.0 g NaCl; 9.0 g $Na_2HPO_4 . 12H_2O$; 1.5 g KH_2PO_4 and 1 litre distilled water (Edel & Kampelmacher, 1973). The samples were added to the broth in blender jars (Omnimixer, Sorvall) kept in an ice bath and blended at high speed for 2.5 min.

Author's address: Department of Agricultural Biochemistry, Unit of Food Science, Faculty of Agriculture, Hebrew University, Rehovot, P.O. Box 12, Israel.

Frozen vegetables and hamburger steaks were purchased in retail stores and prepared for the experiments by thawing overnight at 6°C. Commercially prepared egg and milk powders were received from Dr D. Hartal of the Vita Co. and Dr S. Gordin of the Volcani Institute, respectively.

pH was measured with a Radiometer 25 pH meter. Freezing was performed in dry ice in ethanol and temperature determined and recorded with copper-ircn thermocouples attached to a recorder. Before freezing the cultures were washed by three consecutive centrifugations followed by resuspensions in saline which was also the freezing medium.

The resultant recovery and injury was estimated by plating on NA and on Salmonella Shigella Agar (SS, Difco) and injury calculated as following:

$$\text{Injury} = \left[1 - \frac{\text{Recovery on selective medium}}{\text{Recovery on nonselective medium}}\right] \times 100$$

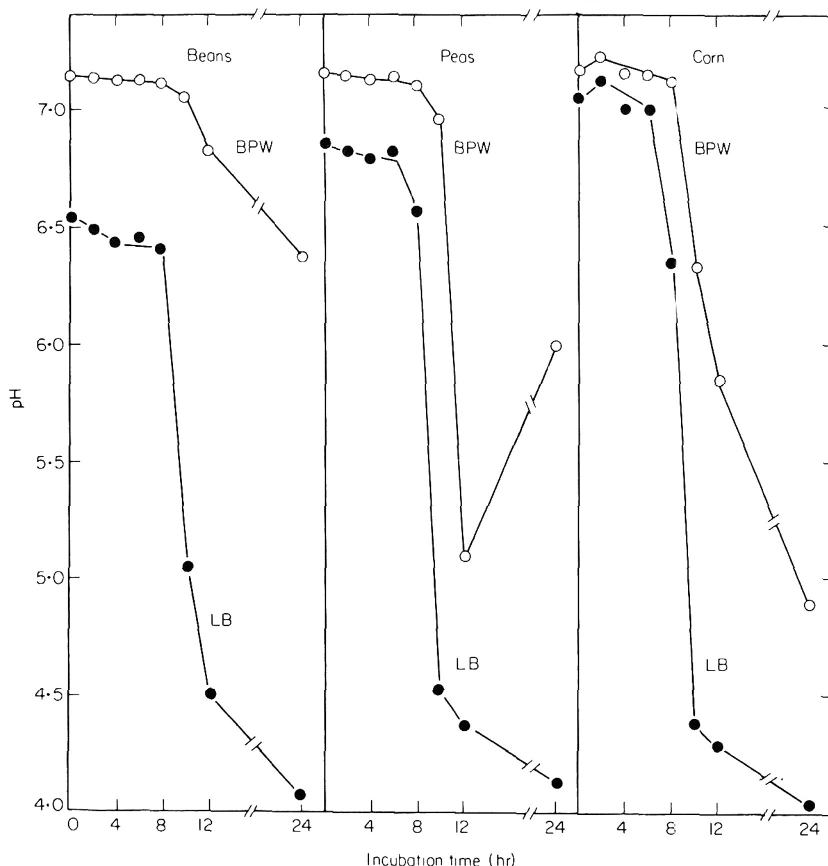


Figure 1. Typical decrease in pH during pre-enrichment incubation of frozen vegetable samples in lactose broth (LB) and in BPW. The pre-enrichments in lactose broth were prepared by blending 50 g sample in 150 ml of broth (Israeli Standard, 1974) and in BPW by blending 25 g sample in 225 ml broth (Edel & Kampelmacher, 1973).

All incubations were performed at 37°C. All reported observations were confirmed in at least three trials with vegetables from different commercial sources.

Results and discussion

Monitoring the change in pH of frozen vegetable pre-enrichments in lactose broth and in BPW during 24 hr of incubation indicated a sharp decrease after a lag period which lasted for 6-8 hr (Fig. 1). Table 1 presents typical results of pH determinations of eleven samples incubated both in lactose broth and in BPW. The pH of all the frozen vegetable pre-enrichments in lactose broth decreased to 4.5 in less than 24 hr. In four out of eight vegetables pre-enrichment samples of pH of 4.5 was recorded after less than 12 hr and approached the value of 4 after 24 hr of incubation.

The increase in acidity in frozen vegetables was predominant and occurred to a much lesser extent in the non-vegetable pre-enrichments of the egg and milk powders and of the frozen hamburger steak. Similarly, the pre-enrichments of all samples in BPW maintained a higher pH. Different ratios of sample size to pre-enrichment broth volume are used by different workers. Harvey & Price (1974) recommend a 1/4 ratio, A.P.H.A. (1966) recommends a 1/5 and the F.D.A. (1972) and the A.O.A.C. (1970) recommend a 1/10 ratio.

Table 1. Typical pH changes during pre-enrichment incubation of frozen vegetables and other food samples in LB and BPW*

Food sample	pH values Incubation time (hr)						Time of incubation to pH 4.5
	0		10		24		
	LB	BPW	LB	BPW	LB	BPW	
Carrot	6.60	7.14	5.92	6.07	4.21	4.89	18
Brussel sprouts	6.51	7.13	5.51	6.83	4.37	6.55	12
Sweet corn	7.04	7.18	4.37	6.34	4.01	4.88	10
Snap green beans	6.54	7.13	5.04	7.06	4.06	6.37	12
Peas	6.87	7.18	5.54	6.89	4.07	6.01	10
Summer squash	6.74	7.11	3.96	5.50	3.80	6.21	8
Broccoli	6.66	7.15	4.22	6.30	4.14	6.59	9
Okra	6.52	7.12	5.03	6.64	4.42	6.02	>12<24
Hamburger steak	7.25	7.25	5.15	6.46	4.77	6.51	–
Egg powder	6.35	6.95	6.14	6.70	5.38	6.35	–
Milk powder	6.37	6.80	6.22	6.64	5.45	5.02	–

*The pre-enrichments were prepared as described in the legend to Fig. 1.

Table 2. Increase in acidity and microbial growth in pre-enrichments of different sample sizes to broth volume ratio*

Pre-enrichment medium	Sample weight (g)	Broth volume (ml)	Ratio	pH values			Growth (count/ml)		
				Incubation time (hr)					
				0	8	22	0	8	22
Lactose	50	150	1 : 4	7.01	6.93	4.14	4.7×10^3	2.4×10^8	3.3×10^8
Broth	20	80	1 : 5	6.95	6.88	4.11			
	10	90	1 : 10	6.93	6.93	4.15			
BPW	50	150	1 : 4	7.10	7.06	4.54			
	20	80	1 : 5	7.11	7.05	4.81			
	10	90	1 : 0	7.12	7.05	4.98	2.8×10^3	4.2×10^8	2.9×10^9

*The pre-enrichments at the shown sample sizes to both volume ratios were prepared as described in the Methods.

Table 3. Sensitivity of salmonellae to incubation at pH 4.0*

Salmonella serotype	D-value (hr) at pH 4.0
S. typhimurium	5–6
S. senftenberg	5–6
S. concord	2–3
S. anatum	2–3

* Lactose broth acidified with lactic acid was inoculated with 10^3 organisms/ml. Viable counts were determined at intervals of 1 hr for 7 hr of incubation at 37°C. The 90% destruction time was estimated from the logarithmic destruction curves.

To determine the effect of this ratio on the rate of increase in acidity, pre-enrichment of frozen sweet corn at different ratios of lactose broth and BPW were studied. As shown in Table 2, the ratio of sample weight to broth volume had little or no effect on pH. In addition, it is shown that the differences between lactose broth and BPW in promoting acid conditions should not be attributed to differences in bacterial growth, as the increase in bacterial colony counts per millilitre was faster in the BPW pre-enrichment. Thus, it is suggested that due to the availability of easily fermentable carbohydrates the buffer capacity of the medium used for salmonellae pre-enrichments from frozen vegetables should be carefully controlled.

Differences in growth of different salmonella serotypes under inhibiting acidic conditions were shown by Huhtanen (1975) using pH gradient plates. *S. senftenberg* could grow down to pH 4.75 while *S. anatum* and *S. typhimurium* grew to pH 5.05. Since acidity accumulation in the pre-enrichment media may result in lower pH values, the relative resistance of bactericidal conditions on four serotypes was determined in lactose broth acidified to pH 4.0. The results (Table 3) show that the 90% destruction time may differ considerably and for the different serotypes it ranged between 2 and 6 hr of incubation. Considering that the commonly practiced pre-enrichment incubation time is 24 and 48 hr and that acidification to pH 4.0 may happen within 24 hr or less (Table 1), these expressions of acid sensitivity become highly significant.

In view of the fact that salmonella contamination at the post blanching stage of frozen vegetable production may have been subjected to the freezing process, the effect of freeze injury on acid sensitivity was studied. A suspension of *S. typhimurium* was frozen and recovery, injury and acid sensitivity were determined. The results (Table 4) show that prior to freezing *S. typhimurium* could grow at pH as low as 4.4. However, the frozen culture that has suffered a 48% injury could not grow at that pH nor at pH 4.71, but could at pH 5.52. Increasing the extent of injury by freeze storage at −18°C to 72% did not alter that result.

Table 4. Acid sensitivity of freeze injured *S. typhimurium**

	Colony count/ml		Recovery (%)		Freeze injury (%)	Growth in lactose broth at pH			
	NA	SS	NA	SS		6.65	5.52	4.71	4.40
Before freezing	1.4×10^5	1.4×10^5†	100	100	–	+	+	+	+
Freezing for 18 hr	2.6×10^4	6.1×10^3†	18.4	9.5	48	+	+	–	–
Freezing for 30 days	4.5×10^2	5.7×10^1†	0.32	0.09	72	+	+	–	–

* A log phase culture was frozen to −27°C at an average rate of 15.2°C/min and stored frozen at −18°C. Growth of the freeze-injured cells was determined in lactose broth acidified with lactic acid. A + mark was denoted to multiplication of at least 10-fold after 22 hr of incubation at 37°C.

† The colony counts of unfrozen cultures on SS were consistently lower than the counts on NA and the mean recovery valuc in three different experiments was 43.9 ± 2.2%. Since our interest was to study the effect of freeze-injury only, a correction factor of 2.27 was applied to the counts on SS to eliminate the effect of the selective medium on uninjured cells.

The need to control pH when using lactose broth as a pre-enrichment medium for salmonellae was suggested before (F.D.A., 1972). Our data draw attention to the frozen vegetables which is shown to be a particular sensitive system due to two main reasons. The first is the apparent lower buffering capacity of the vegetable tissue compared with protein-rich foods and the second is the higher sensitivity to low pH of freeze injured salmonellae which may contaminate frozen vegetables. The high acid conditions may interfere with epidemiological investigations by selecting more acid tolerant serotypes, and may result in low recoveries and ultimately 'false negative' results.

In conclusion it is suggested that lactose broth, a commonly used medium, is not suitable for the pre-enrichment of salmonellae from frozen vegetables and a better buffered medium such as BPW is preferred.

Acknowledgment

The technical assistance of Varda Sandowsky is gratefully acknowledged.

References

Angelotti, R. (1963) In: *Microbiological Quality of Foods* (Ed. by I.W. Slanetz, C.O. Chichester, A.R. Gaufin & Z.J. Ordal), p. 149. Academic Press, New York.

A.O.A.C. (1970) *Official Methods of Analysis of the A.O.A.C.*, 11th edn (Ed. by W. Horowitz, P. Chichilo & H. Reynolds), p. 845. Association of Official Analytical Chemists, Washington, D.C.

A.P.H.A. (1966) *Recommended Methods for the Microbiological Examination of Foods*, 2nd. edn (Ed. by J.M. Sharf), p. 154. American Public Health Association, New York.

Edel, W. & Kampelmacher, E.H. (1973) *Bull. Wld Hlth Org.* **48**, 167.

Chung, K.C. & Goepfert, J.M. (1970) *J. Fd Sci.* **35**, 326.

F.D.A. (1972) *Bacteriological Analytical Manual.* Part 4, Salmonella. Food and Drug Administration, Washington, D.C.

Harvey, R.W.S. & Price, T.H. (1974) *Isolation of Salmonellas.* Public Health Laboratory Service. Monograph Series No. 8, H.M.S.O., London.

Huhtanen, C.N. (1975) *Appl. Microbiol.* **29**, 309.

Israeli Standard (1974) *Frozen Fruits and Vegetables: General.* No. 877, Israeli Standards Institute, University St, 42, Tel-Aviv.

Prost, E. & Reiman, H. (1967) *Ann. Rev. Microbiol.* **21**, 495.

Thatcher, F.S. & Clark, D.S. (1968) *Microorganisms in Foods, their Significance and Methods of Enumeration*, pp. 92-95. University of Toronto Press, Canada.

(*Received 11 June 1976*)

J. Fd Technol. (1977) **12**, 93–97

Book Reviews

Protein Metabolism and Nutrition. Ed. by D. J. A. Cole, K. N. Boorman, P. J. Buttery, D. Lewis, R. J. Neale and H. Swan.
London: Butterworths, 1976. Pp. 515. £16.

The book is the outcome of a symposium held by the European Association of Animal Production at the University of Nottingham in July 1974. This statement could be very misleading to the potential reader who may then anticipate a publication devoted to the relative merits of protein-rich feedstuffs for the production of more eggs, lamb triplets, and larger litters of piglets.

Far from it, the first sixteen of the twenty-seven contributions are almost pure biochemistry and are quartered under the headings 'Protein Synthesis and Turnover' which includes a masterly introductory chapter by Professor H. N. Munro on the intricacies of protein synthesis at the cellular level, 'Digestion and Availability', 'Nitrogen–Energy Relationships' and 'Measurement of Protein Adequacy'. Nutritionists tend to think of protein mainly in terms of amino acids but this symposium rightly investigated its intrinsic energy value and also the energy requirements involved in the metabolism of protein.

The remaining eleven papers are of a more applied nature, but still with a biological content, and are divided between the protein nutrition of ruminants and non-ruminants; however, as to be expected from a gathering of agriculturalists, *homo sapiens* has no place in the latter section!

Therefore, animal nutritionists will find this publication unrivalled as an up-to-date source of information about protein; human nutritionists and biochemists would be well advised to study the first half of the book; however, food scientists and food technologists will find comparatively little information relevant to their professional interests unless they are directly concerned with the animal feedstuff industry. The editors and publishers are to be congratulated on the very clean-cut and uniform presentation which makes for reading without strain.

K. Mary Clegg

Commercial Processing of Fruits: Food Technology Review No. 30. By L. P. Hanson.
New Jersey: Noyes Data Corporation, 1976. Pp. xi + 299. US$36.

This review covers the U.S. patent literature from 1965–75 with references to one or two earlier patents. The ten sections dealing with 174 patents include handling fresh fruit, general dehydration processes, other general processes,

e.g. peeling, canning, freezing and stabilization, and specific products. citrus fruit, pomes, drupes, grapes, bananas, avocados, dates, melons, coconuts, and pineapples.

The sections on the post harvest handling of fruits are of particular interest. Several methods for extending shelf life and maintaining quality prior to processing are dealt with together with methods of peeling. Dehydration processes include freeze drying, drum drying, air drying and other conventional methods as well as reverse osmosis and drying with hot oils under vacuum. Many of the products are of no direct or immediate interest to U.K. food processors; however, there is a good deal of information on apples and other tree fruit as well as berry fruits.

In general the book is well written and attractively produced. Those who are concerned with fruit handling and processing will find a wealth of information in this book.

S. D. Holdsworth

Lipids. (2 Vols.) Ed. by R. Paoletti, G. Porcellatti and G. Jacini.
New York: Raven Press, 1976. Pp. xvi + 282 + 271. US$59.50 (both volumes).

Lipids consists of the invited lectures and symposia presented at the 12th International Congress on Fat Research, held in Milan, Italy, in September 1974. The book is divided into Volume 1 on 'Lipid Biochemistry' (twenty-nine papers), and Volume 2 on 'Lipid Technology' (thirty-one papers). The contributions, which are all in English, range from small reviews to minor research papers, and most have between fifteen and twenty-five references.

The contents do not give a comprehensive survey of lipid research, as the title might suggest, but provide an up-to-date (1974) discussion of progress in selected topics. About one-third of the papers are likely to be of interest to food scientists and technologists.

In Volume 1, mechanisms of lipid oxidation are the subject of papers by Boldingh (soya lipoxygenase-1), McCay *et al.* (enzyme-generated free radicals and singlet oxygen), and Mead and Wu (autoxidation of fatty acids in monolayers). Some nutritional aspects of dietary lipids are described by Holman (essential fatty acids), Berra *et al.* (brain gangliosides), Galli *et al.* (essential fatty acids in brain lipids), and Fidanza (coronary heart disease).

In Volume 2, problems in olive oil research are described in the plenary lecture by Jacini, and in related papers by Bozzini (improvement in oil production), Pallotta (proof of genuineness), and Fedeli *et al.* (insect infestation of olives).

Analytical methodology is contained in papers in several sections of both volumes. The topics covered are sterol analysis (Seher, plenary lecture), liquid chromatography of non-ionic surfactants (Aitzetmüller), determination of double bond positions by NMR (Bus and Frost) and Raman spectroscopy in aqueous dispersions (Larsson), quantitative thin-layer chromatography

(Mukherjee), metals in oils by flameless atomic absorption (Prevot and Kundu) and preparative gas chromatography of flavour volatiles (Prevot and Mordret).

There is very little on the industrial processing of oils and fats. Letan and Koslowsky describe a method for fractionating oils, and Seher reviews new developments in hydrogenation and transesterification. Readers with a special interest in oils and fats processing would find the contemporary June 1976 issue of the Journal of the American Oil Chemists Society far more comprehensive.

Papers on lipids in food technology are scattered throughout several sections in Volume 2. They deal with the control of fat polymorphism and physical properties (Berger), medium-chain triglycerides (Bracco), the physical state of fat in dispersions (Walstra), fats in baked goods (Menger), off-flavours (Pokorny), and alteration of fats used in frying (Chang and Blumenthal; Fedeli *et al.*).

Lipids provides a broad perspective on progress in what the Congress organizers consider to be particularly active and important areas of research, and as such it is good background reading. However, for the reader who has developed a specialized interest the book could prove rather disappointing.

W. R. Morrison

Staphylococci and their Significance in Foods. By T. E. Minor and E. H. Marth. Amsterdam, Oxford, New York: Elsevier Scientific Publishing Company, 1976. Pp. xiv + 297. US$33.50.

This book is a comprehensive review of *Staphylococcus aureus* and staphylococcal enterotoxins in food and is an extension of earlier reviews which were written by the same authors and which appeared in the *Journal of Milk and Food Technology* (1971–72) and the *Indian Journal of Nutrition and Dietetics* (1972). The book is directed at research workers in academic, government and industrial food microbiology laboratories, quality control personnel and management in the food industry, public health authorities, personnel engaged in food related regulatory activities and students of food microbiology. Some background information is included for the benefit of readers who are unfamiliar with the more difficult concepts.

Subjects covered include some historical aspects of staphylococcal infections; taxonomy and a general description of staphylococci; resistance of *Staphylococcus aureus* to biochemical and chemical agents in artificial environments; physiological characteristics suggest as indicators of staphylococcal enterotoxigenicity; isolation and identification; properties, synthesis, purification, detection and mode of action of staphylococcal enterotoxins; source and transmission of enterotoxigenic strains; behaviour in dairy foods, in meat, fish and poultry products and in cream filled pastries and delicatessen foods; and surveillance and control of staphylococcal foodborne intoxications in the

U.S.A. Methods for the isolation and enumeration of *S. aureus* from foods and the detection of enterotoxins are thoroughly discussed and a detailed description of some of these is given.

Most chapters are followed by an extensive bibliography. However, with the exception of the bibliographies of *Staphylococcus aureus* in foods and the surveillance and control of staphylococcal foodborne intoxications there are few references after 1973. It is disappointing that some of the European literature is not mentioned or discussed, for example the work carried out by Untermann and Sinell in Germany on the occurrence of enterotoxigenic strains. A slight irritation is the use of the word 'staphylococci' as a synonym for *S. aureus*.

This book brings together for the first time, into one volume, information about the characteristics of *Staphylococcus aureus* and of staphylococcal enterotoxins, the behaviour of *S. aureus* in foods and the role of this organism in foodborne illness. Altogether an excellent source of reference that gives a clear picture of the development of our knowledge of *S. aureus* and its enterotoxins in relation to foods.

Antonnette A. Wieneke

The Chemical Analysis of Foods, 7th Edn. By David Pearson.
Edinburgh: Churchill Livingstone, 1976. Pp. xii + 575. £13.50.

This seventh edition appears exactly fifty years after the first and reflects the advances made during that time in analytical chemistry and their applications to food, and also the developments in legislation that have resulted in the setting of rigorous standards for the quality and safety of present-day foodstuffs.

In the first edition the author stated that the methods given were those which had been well tried and found reliable, and it is a tribute to the pioneers of food analysis that several of those methods have stood the test of time and are included in the current edition, e.g. the Gerber and Werner-Schmidt methods for fat estimation, determination of crude fibre, King's method for pectin and the microscopical identification of starches. The more recent methods given in the new edition are also of proved reliability and it is the inclusion of the working details of so many accepted methods that makes this book such a valuable bench-side companion for the food analyst.

The sixth edition was approximately twice the size of the first and had probably reached the maximum size desirable for a book of this nature. Thus the author faced the problem of revising the text to include new material without increasing the size of the book, and it is the reviewer's opinion that Dr Pearson has succeeded admirably in this task; in fact the new edition contains fewer pages than its predecessor.

Legal requirements and standards are now derived not only from U.K. Regulations but also from EEC Directives and the Codex Alimentarius and the

author's declared aim was to effect a compromise between the present and the probable future. In the sixth edition the chapter on Food Legislation occupied eighteen pages, some of which repeated details already given in other chapters on specific commodities. This chapter has been omitted and Food Legislation is summarized in three pages of the Introduction. In other chapters details of some of the more elaborate procedures, especially those likely to be used only rarely, if at all, have been deleted. For example, the determination of calcium as oxalate is not described in detail but its determination by atomic absorption spectrophotometry is. Estimation of sodium and potassium by flame photometry are among other new methods described in detail. Descriptions of arsenic, lead, tin and zinc determinations have been shortened, often by eliminating alternative methods, but that of mercury is described in greater detail. The long description of the Filth Test is omitted. However, appropriate literature references have been retained and new ones added; the references have been taken out of the text and collected at the end of each chapter.

There has been a minor rearrangement of material in certain chapters, e.g. starch products are grouped with baking powders, etc. instead of with cereals. There has also been a partial changeover to the SI system but in most instances the older equivalent is also stated.

This book can be very highly recommended to all laboratories concerned with food analysis; it should be kept close to the bench.

T. L. Parkinson

Books Received

The Man/Food Equation. Ed. by F. Steele and A. Bourne.
London: Academic Press, 1975. Pp. xv + 289. £7.00.

A collection of papers presented at a symposium in which the prospects of food production for a growing world population were considered.

Food Flavouring Processes. By N. D. Pintauro.
New Jersey: Noyes Data Corporation, 1976. Pp. xii + 208. US$39.00.

A survey of the American patent literature on food flavours and processes for their production.

Sausages and Small Goods Production. Sixth edition. By F. Gerrard.
London: Northwood Publications Ltd, 1976. Pp. ix + 254. £4.75.

A guide for students and practising butchers.

Food for Life. By F. E. Deatherage.
New York: Plenum Press, 1975. Pp. xii + 422. £23.40.

An integrated approach to problems of food, food production and nutrition intended for students.

Erratum

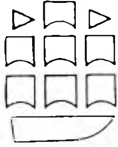
A. E. Bender and M. Zia. Meat quality and protein quality. *Journal of Food Technology* 1976, **11**, 495.

The table on p. 497 should be replaced by:

Table 1. Quality of meat protein and collagen content

Sample	NPU duplicates	Mean	Hydroxyproline (g/100 g protein)	Collagen (g/100 g Protein)
Shin	68, 70	69	3.31	23.6
Shin + methionine	88, 90	89		
Fillet	81, 83	82	0.36	2.5
Fillet + methionine	99, 97	98		
[Shoulder (cooked)	76, 80	78	1.9	13.5] *

* From Bender & Husaini, 1976.



Churchill Livingstone

CHEESE

J. G. Davis

Volume 2 Annotated Bibliography with Subject Index
288 pages £3.75

Volume 3 Practical-Manufacturing Methods
580 pages, illustrated £17.00

Volume 4 Annotated Bibliography with Subject Index
264 pages £10.00

It is expected that this series will become the standard work in the English language for all those concerned with the scientific, technological and practical aspects of cheese.

Primarily written for cheesemakers and all concerned with cheese in the dairy industry and also for managers, technologists, teachers, research workers, graders and quality controllers, these books will also be of interest and value to a much wider readership.

Churchill Livingstone 23 Ravelston Terrace Edinburgh EH4 3TL

The Journal of General Microbiology

Partial contents of Vol. 98, Part 1, January 1977

DEVELOPMENT AND STRUCTURE

D. C. KAUSHAL and O. P. SHUKLA Excystment of Axenically Prepared Cysts of *Hartmannella culbertsoni*.

C. G. ELLIOTT and E. SANSOME The Influence of Sterols on Meiosis in *Phytophthora cactorum*.

M. T. DAVISON and P. B. GARLAND Structure of Mitochondria and Vacuoles of *Candida utilis* and *Schizosaccharomyces pombe* Studied by Electron Microscopy of Serial Thin Sections and Model Building.

PHYSIOLOGY AND GROWTH

D. WESTMACOTT and S. B. PRIMROSE The Effect of Nalidixic Acid on the Cell Cycle of Synchronous *Rhodopseudomonas palustris* Cultures.

R. K. POOLE Fluctuations in Buoyant Density during the Cell Cycle of *Escherichia coli* K12: Significance for the Preparation of Synchronous Cultures by Age Selection.

BIOCHEMISTRY

G. S. COLEMAN and J. I. LAURIE The Metabolism of Starch, Glucose, Amino Acids, Purines, Pyrimidines and Bacteria by the Rumen Ciliate *Polyplastron multivesiculatum*.

D. F. NIVEN, P. A. COLLINS and C. J. KNOWLES Adenylate Energy Charge during Batch Culture of *Beneckea natriegens*.

L. HANKIN and S. L. ANAGNOSTAKIS Solid Media Containing Carboxymethylcellulose to Detect C_x Cellulase Activity of Micro-organisms.

GENETICS AND MOLECULAR BIOLOGY

J. SYKES, E. METCALF and J. D. PICKERING The Nature of the Proteins in 'Chloramphenicol Particles' from *Escherichia coli* A19 (Hfr *rel met rns*).

J. SYKES, E. METCALF and J. D. PICKERING The Nature of the Proteins Present in the 'Relaxed Particles' from Methionine-starved *Escherichia coli* A19 (Hfr *rel met rns*).

MEDICAL MICROBIOLOGY

W. C. NOBLE Variation in the Prevalence of Antibiotic Resistance of *Staphylococcus aureus* from Human Skin and Nares.

TAXONOMY

P. A. D. GRIMONT, F. GRIMONT, H. L. C. DULONG DE ROSNAY and P. H. A. SNEATH Taxonomy of the Genus *Serratia*.

L. ALSHAMAONY, M. GOODFELLOW, D. E. MINNIKIN, G. H. BOWDEN and J. M. HARDIE Fatty and Mycolic Acid Composition of *Bacterionema matruchotii* and Related Organisms.

Volumes 98–103 (1977) £15.50 ($42.00 U.S.A. and Canada) each. Single parts £10.00 ($26.00 U.S.A. and Canada) each. Enquiries about advertising in the journal should be sent to the Press.

CAMBRIDGE UNIVERSITY PRESS

Bentley House, 200 Euston Road, London NW1 2DB
32 East 57th Street, New York, N.Y. 10022

// Journal of the Science of Food and Agriculture

Volume 28, Number 4, April 1977

CONTENTS

329 *I. F. Penny* The effect of temperature on the drip, denaturation and extracellular space of pork *Longissimus dorsi* muscle

339 *H. W.-S. Chan, G. Levett and N. M. Griffiths* Light-induced flavour deterioration. The effect of exposure to light of pork luncheon meat containing erythrosine

345 *T. A. Gough, M. F. McPhail, K. S. Webb, B. J. Wood and R. F. Coleman* An examination of some foodstuffs for the presence of volatile nitrosamines

352 *D. S. Mottram and R. L. S. Patterson* The effect of ascorbate reductants on N-nitrosamine formation in a model system resembling bacon fat

355 *T. S. Banerjee, B. R. Roy and B. Majee* Interaction of copper ion with some food dyes

365 *K. Herrmann and M. Woldecke* The flavanol content of peas as influenced by variety and light, and a note on the flavanol content of broad beans

369 *Y. Ohyama, S. Hara and S. Masaki* The use of caproic acid to prevent aerobic deterioration of silages after opening, with special reference to the amounts and time of application

375 *M. Fafunso and M. Byers* Effect of pre-press treatments of vegetation on the quality of the extracted leaf protein

381 *S. Sudarmadji and P. Markakis* The phytate and phytase of soybean tempeh

384 *D. Adomako* Fatty acid composition and characteristics of *Pentadesma butyracea* fat extracted from Ghana seeds

387 *M. J. Fishwick and P. A. T. Swoboda* Measurement of oxidation of polyunsaturated fatty acids by spectrophotometric assay of conjugated derivatives

394 *M. J. Fishwick, A. J. Wright and T. Galliard* Quantitative composition of the lipids of cucumber fruit (*Cucumis sativus*)

Published for the Society of Chemical Industry, 14 Belgrave Square, London SW1X 8PS by Blackwell Scientific Publications.

Annual subscription price £45.00 (U.S.A. and Canada $150.00) post free.

JOURNAL OF FOOD TECHNOLOGY: NOTICE TO CONTRIBUTORS

The Journal of Food Technology publishes original contributions to knowledge of food science and technology and also review articles in the same field. Papers are accepted on the understanding that they have not been and will not be, published elsewhere in whole, or in part, without the Editor's permission. Papers accepted become the copyright of the Journal. This journal is covered by *Current Contents*.

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

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

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

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

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

SI UNITS

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

NON SI UNITS

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

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

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

Offprints. Fifty offprints will be issued free with each paper but additional copies may be purchased if ordered on the printed card which will be sent to the senior author with the proofs.

Journal of Food Technology

Volume 12 Number 1 February 1977

Contents

1 Volatile constituents of white bread crust
D. F. Folkes ond J. W. Gramshaw

9 Methylated amino acids as indices in meat products. II. Further examination of protein sources and the practical application of methylamino acid titres in predicting meat content
W. R. D. Rangeley and R. A. Lawrie

27 A study of the composition of three popular varieties of fish in Israel, with a view towards further processing
S. Angel and R. C. Baker

37 Differential lipid oxidation in various parts of frozen mackerel
P. J. Ke, R. G. Ackman, B. A. Linke and D. M. Nash

49 The determination of freezant residues in prawns frozen by contact with dichlorodifluoromethane
J. R. Carter and R. S. Kirk

59 Colour retention in sweet red paprika (*Capsicum annum* L.) powder as affected by moisture contents and ripening stage
J. Kanner, Stela Harel, D. Palevitch and I. Ben-Gera

65 Freeze drying encapsulation of water soluble citrus aroma
I. F. Kopelman, S. Meydav and P. Wilmersdorf

73 The expansion of wafer and its relation to the cracking of chocolate and 'bakers' chocolate' coatings
L. F. Barron

Technical Note

85 Acid sensitivity of freeze injured salmonellae in relation to their isolation from frozen vegetables by pre-enrichment procedure
A. Y. Sadovski

93 Book Reviews

97 Books Received

Printed by Adlard and Son Ltd, Bartholomew Press, Dorking