Food Chemistry 8 (1982) 1–9

SPORE FATTY ACID COMPOSITION IN *BACILLUS NATTO*, A FOOD MICROORGANISM

EIJI ICHISHIMA, MOTOYA KATO, YOSHIAKI WADA, HISAYA KAKIUCHI, MICHIO TAKEUCHI & TAKESHI TAKAHASHI

Department of Agricultural and Biological Chemistry, Tokyo Noko University, Fuchu, Tokyo 183, Japan

&

KOICHI TAKIMAMI & YOSHIO HIROSE

Central Research Laboratories, Ajinomoto Co., Inc., Suzukicho, Kawasaki 220, Japan

(Received: 7 July 1981)

ABSTRACT

The levels of fatty acids and their distribution were determined in spores from twenty-five strains of Bacillus natto *isolated from a fermented soybean food, natto, or subcultured from the culture collection. The major fatty acid components of spores, consisting of about 80 to 90% of the total, were* anteiso-C_{15}, anteiso-C_{17}, iso-C_{15} *and* iso-C_{17}*; the other fatty acids, at a level of 10 to 20% of the total, were* iso-C_{14}, iso-C_{16}, n-C_{14}, n-C_{16} *and* n-C_{18}. *The amount of fatty acid in spores was highest with* anteiso-C_{15}, *followed in order by* iso-C_{15}. *In addition to the nine fatty acids, some of the strains produced six extra fatty acids, three branched* (anteiso-C_{13}, iso-C_{12} *and* iso-C_{13}) *and three normal* (n-C_{12}, n-C_{15} *and* n-C_{17}).

INTRODUCTION

For about a thousand years Japanese people have been familiar with handling microorganisms through the production of such traditional food as Itohikinatto (natto) (Kiuchi *et al.*, 1976; Ohta, 1975). Natto is a typical, popular and economical fermented soybean food in Japanese diets. In 1976, 124,000 tonnes of natto were produced in Japan. *Bacillus natto* (Sawamura, 1906, 1913; Hanzawa & Tamura, 1934) is the important starter for processing of the fermented soybean food, natto. To make natto, soybean is cooked and on its surface *Bacillus natto* is grown, characteristic tastes and flavours, i.e. tetramethylpyrazine (Kosuge *et al.*, 1971), being produced. When fermented by *Bacillus natto*, the surface of soybean is

Food Chemistry 0308-8146/82/0008-0001/$02·75

covered with a characteristic mucin consisting of 58 % γ-polyglutamic acid and 40 % polysaccharide (Saito *et al.*, 1974).

A nutritional requirement of biotin is essential for vegetative growth of *Bacillus natto* (Kita *et al.*, 1956). *Bacillus subtilus* has no nutritional requirement of biotin for growth. Furthermore, bacteriophage typing has also been reported as an effective differentiation method for the *Bacillus natto* strain within the *Bacillus subtilus* strain (Fujii *et al.*, 1975). In spite of these experimental results, original cultures of *Bacillus natto* Sawamura 1906 and 109 were listed as identical to *Bacillus subtilis* in *Bergey's Manual of Determinative Bacteriology* (eighth edition) (Buchanan & Gibbons, 1974).

Bacterial membranes are associated with almost all of the fatty acids and lipid components found in the cell. The first attempt to correlate lipid composition with taxanomic classification was made by Abel *et al.* (1963), who showed that quantitative fatty acid analyses could be used to differentiate between various organisms.

In this paper an investigation of the fatty acid composition *Bacillus natto* spores grown in the sporulation medium is described.

MATERIALS AND METHODS

Microorganisms

Twenty-two strains of *Bacillus natto* were isolated from the commercial fermented soybean food, natto, as shown in Table 1. Three cultures of *Bacillus natto* and one of *Bacillus subtilus* were subcultured from the culture collection. All the organisms were maintained as stock cultures on the medium containing 1 % beef extract, 1 % polypeptone and 0·5 % NaCl at pH 6·5.

Culture for sporulation

The medium contained 0·3 % beef extract, 0·5 % polypeptone, 0·1 % glucose, 0·1 % KCl, 0·025 % $MgSO_4$, 10^{-3} M $CaCl_2$, 10^{-6} M $FeSO_4$ and 2×10^{-3} M $MnCl_2$ in distilled water at pH 6·8, according to Hanson *et al.* (1964). Each strain was transferred from a stock subculture into 100 ml of the culture medium and first incubated for 72 h at 30 °C. Ten millilitres of active growing culture thus obtained were transferred to 3000 ml Sakaguchi flasks containing 1000 ml of the culture medium and cultures were incubated for 72 h at 30 °C. The flasks were incubated on a reciprocal shaker at 120 strokes/min.

Spore preparation

Spores collected from the flask cultures by refrigerated centrifugation at 22 000 × *g* for 10 min and 0 °C were washed twice with 0·85 % NaCl and rinsed twice with distilled water. A washed crude spore suspension (60 ml) was added to 120 ml of

TABLE 1
LIST OF STRAINS OF *Bacillus natto* ISOLATED FROM COMMERCIAL SOYBEAN FERMENTED FOOD, NATTO

Strains	*Sources*
NB 1	Ugo Food Co., Senhokugun, Akita Prefecture
NC 2-1	Naruse Ferment. Chem. Inst., Nerima-ku, Tokyo
ND 1	Amanoya, Kanda, Chiyoda-ku, Tokyo
NE 1	National Food Research Institute
NH 1	Azuma chlorella-natto, Utsunomiya, Tochigi Prefecture
NI 1	Azuma Food Co., Utsunomiya, Tochigi Prefecture
NJ 1	Torisen Co., Tatebayashi, Gunma Prefecture
NK 1	Azuma Food Co., Utsunomiya, Tochigi Prefecture
NL 1	Echigoya, Itabashi-ku, Tokyo
NN 1	Isolated from rice straw in Tokushima Prefecture
NO 1	Isolated from rice straw in Saitama Prefecture
NP 1	Asahi Food, Namekata-gun, Ibaraki Prefecture
NQ 1	Tamanatto, Fuchu, Tokyo
NR 1	Adachi Food, Hachioji, Tokyo
NS 1	Isolated from Hikiwari-natto, Takahashi Food, Sendai, Miyagi Prefecture
NS 2	Takahashi Food, Sendai, Miyagi Prefecture
NT 1	Dai-ichi Food, Akita, Akita Prefecture
NT 2	Isolated from Hikiwari-natto, Dai-ichi Food, Akita, Akita Prefecture
NU 1	Sugaya Food, Kodaira, Tokyo
NV 1	Isolated from rice straw in Yamagata Prefecture
NW 1	Kofuji Food, Oomuta, Fukuoka Prefecture
NZ 1	Isolated from Hikiwari-natto, Ikeda Co., Suginami-ku, Tokyo

50 % (w/w) Carbowax-4000, 38 ml of 3 M phosphate buffer (pH 7·1) and 22 ml of water. The mixture was homogenised for 2 min in a Nihonseiki HC type Waring blender.

Culture for vegetative cells

The medium contained 1 % beef extract, 1 % polypeptone and 0·5 % NaCl at pH 7·2. Each strain was transferred from a stock subculture according to the previously described method. Cultures were incubated for 18 h at 30 °C. The vegetative cells were washed twice with 0·85 % NaCl and rinsed twice with distilled water, and then lyophilised.

Extraction and methylation of the fatty acids

The procedure was performed as described by Uchida & Mogi (1972). The dried spores or dried cells (200 ml each) were directly saponified at 105 °C overnight with 7 ml of 3 % KOH in 50 ml of methanol in nitrogen in a sealed tube. Non-saponifiable material was extracted from the saponified solution with three 10-ml portions of petroleum ether discarded. The methanol layer was acidified by the addition of 2 ml of 6N H_2SO_4 and the fatty acids were extracted with three 10-ml portions of petroleum ether. After the solvent was evaporated to dryness, the fatty acids were

redissolved in 1 ml of ether and methylated by the addition of 2 ml of freshly prepared diazomethane in ether in the cold. The solvent and the excess reagent were then evaporated under reduced pressure and the methyl esters of fatty acids thus obtained were dissolved in 0·5 ml of petroleum ether. These samples were analysed immediately or stored at −20 °C.

Gas–liquid chromatography

The fatty acid methyl esters were analysed by the use of a Shimazu GC–6 AM gas chromatograph equipped with a hydrogen flame ionisation detector (FID). A glass column (3 mm × 2 m) coated with 20 % diethylene glycol succinate/Johns-ManVille Chromosorb W was used. Nitrogen was used as the carrier gas with a flow rate of 30 ml/min. Operation parameters of this instrument were as follows: injection temperature, 240 °C; column (oven) temperature, 150 °C. A sensitivity of 10^3 and a range of 128 were used. Fatty acids were primarily identified by comparison of the relative retention time of their methyl esters with that of standard fatty acids (14:0, 15:0, 16:0) and of a standard strain of *Bacillus subtilis* IAM 1523 (Uchida & Mogi, 1973).

RESULTS AND DISCUSSION

The formation of straight chain fatty acids and branched chain fatty acids bears an important relationship to the metabolism of amino acids. Fatty acid distribution patterns may possibly be of some use in the identification and taxonomy of bacterial species (Abel *et al.*, 1963; Uchida & Mogi, 1972, 1973; Shaw, 1974), since fatty acids are known to be primary metabolites.

Figure 1 shows a typical gas chromatogram of the methyl esters of fatty acids extracted from a representative strain of *Bacillus natto*. To ascertain the reproducibility of the fatty acid determination of bacterial cells and spores, the strains were cultured and analysed repeatedly under identical conditions.

The fatty acid spectra of vegetative cells and spores of *Bacillus natto* NC 2–1 and *Bacillus subtilis* IAM 1523 are presented in Table 2. No distinguishable difference in fatty acid patterns of vegetative cells could be detected between *Bacillus natto* and *Bacillus subtilis*. The major fatty acid components of vegetative cells of *Bacillus natto* NC 2–1, consisting of about 80 % of the total, were branched chain fatty acids; the other fatty acids, at a level of about 20 % of the total, were normal fatty acids. *Iso*-fatty acids (*i*-C_{17}, *i*-C_{15} and *i*-C_{13}) of the odd-numbered branched chain type were the main components in vegetative cells. The amount of fatty acid in vegetative cells was highest with *i*-C_{17} (subtiloheptadecanoic acid (Saito, 1960)) followed in order by *i*-C_{15} (subtilopentadecanoic acid (Saito, 1960)).

Branched chain fatty acids were major components in the spores of *Bacillus natto* (Table 2) and among these, *anteiso*-fatty acids (*a*-C_{15} and *a*-C_{17}) of the odd-numbered branched chain type were main components. The amount of fatty acid of

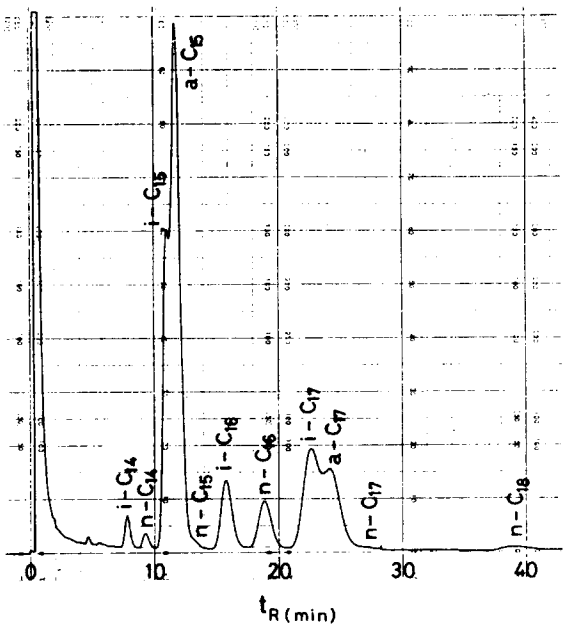


Fig. 1. Gas chromatogram of spore fatty acids from *Bacillus natto* NW 1.

TABLE 2

FATTY ACID SPECTRA IN VEGETATIVE CELLS AND SPORES OF *Bacillus natto* AND *Bacillus subtilis* (PERCENTAGE COMPOSITIONS OF FATTY ACIDS ARE SHOWN)

Fatty acids	Bacillus natto *NC 2-1*		Bacillus subtilis *IAM 1523*	
	Vegetative cells	*Spores*	*Vegetative cells*	*Spores*
a-C_{13}	1·8	—	1·7	—
a-C_{15}	7·2	40·4	8·2	34·6
a-C_{17}	4·8	11·6	8·5	11·6
i-C_{13}	6·4	0·1	5·4	0·1
i-C_{15}	19·9	16·8	20·0	23·3
i-C_{17}	24·2	13·3	27·7	15·7
i-C_{12}	0·6	—	0·5	—
i-C_{14}	6·0	1·8	4·8	2·8
i-C_{16}	9·4	7·1	8·1	10·1
i-C_{18}	0·4	—	0·3	—
n-C_{13}	tr	—	tr	—
n-C_{15}	0·6	0·3	0·4	—
n-C_{17}	2·9	0·1	3·7	—
n-C_{12}	0·3	—	0·3	—
n-C_{14}	3·2	0·5	3·4	0·1
n-C_{16}	12·2	6·2	6·8	1·7
n-C_{18}	0·1	1·8	0·2	—

tr, trace.

spores was highest with *a*-C_{15} followed in order by *i*-C_{15}, and *i*-C_{17} and/or *a*-C_{17}. The ratio of *a*-C_{15}: *i*-C_{17} in vegetative cells and spores was remarkably different. Odd-numbered straight chain fatty acid (*n*-C_{15} and *n*-C_{17}) and even-numbered straight chain fatty acid (*n*-C_{12}, *n*-C_{14}, *n*-C_{16} and *n*-C_{18}) fractions in the spores showed a sharply lowered ratio of those to the total fatty acids as compared with vegetative cells. The foregoing data indicate a shift in fatty acid proportions during the course of spore formation. A similar shift in the fatty acid compositions was obtained in the analyses of vegetative cells and spores from *Bacillus subtilis*.

The chemical composition of many bacterial components can be affected by a variety of external factors—for example, temperature of growth, substrate composition, environmental pH, time of harvesting, etc. (Scandella & Kornberg, 1969). In the present paper, we describe the relative abundance of fatty acids during the resting state of spores in *Bacillus natto* because fatty acid compositions showed more evident spectra in the synchronous state of the spore phase than in the non-synchronous state of vegetative cells.

Relative proportions of fatty acids in twenty-two strains of *Bacillus natto* isolated from fermented soybean food, natto, and three strains of *Bacillus natto*, subcultured from the culture collection, are shown in Table 3. The major fatty acid components of spores in *Bacillus natto*, consisting of about 90% of the total, were six branched chain fatty acids (*a*-C_{15}, *a*-C_{17}, *i*-C_{15}, *i*-C_{17}, *i*-C_{14} and *i*-C_{16}).

The amounts of straight chain fatty acids (*n*-C_{14}, *n*-C_{16} and *n*-C_{18}) were 4 to 15% of the total fatty acids. Although the total amount of straight chain fatty acids was low, it is of interest that all strains of *Bacillus natto* produced even-numbered straight chain fatty acids, mostly *n*-C_{16}, at a much higher level than that of odd-numbered straight chain fatty acids (Table 3). The relationship between the amount of *i*-C_{16} and the amount of *n*-C_{16} in the *Bacillus natto* spores is shown in Fig. 2. There were slight differences in the percentage composition of *n*-C_{16} in the spores produced between the *Bacillus natto* strains and the standard *Bacillus subtilis* IAM 1523 strain (Fig. 2).

Although only twenty-five strains of *Bacillus natto* have been examined in this work, it seems probable that the occurrence of a particular spectrum of fatty acids in *Bacillus natto* may be a reliable identification of its species.

Natto is a fermented-protein food, like cheese. Natto contains a significant number of native enzymes (Ohta, 1975), especially alkaline proteinase (Yoshimoto *et al.*, 1971) and α-amylase, which are important hydrolysing enzymes in fermented food processing. Alkaline proteinase from *Bacillus natto* bears a similarity to subtilisin Carlsberg from *Bacillus subtilis* in enzymatic activity and physico-chemical properties (Yoshimoto *et al.*, 1971). Although natto is produced from a vegetarian source, it contains significant amounts of vitamin B_{12}. The B_{12} is produced by *Bacillus natto*, as it is in the rumen of livestock from which most people obtain their requirement in the form of meat or dairy products (Shurtleff & Aoyagi, 1976). *Bacillus natto* seems to be the best presently available organism for the

TABLE 3

PERCENTAGE COMPOSITION OF THE SPORE FATTY ACIDS IN *Bacillus natto*

Fatty acids	Bacillus natto																							
	NB 1	*NC 2-1*	*ND 1*	*NE 1*	*NH 1*	*NI 1*	*NJ 1*	*NK 1*	*NL 1*	*NN 1*	*NO 1*	*NP 1*	*NQ 1*	*NR 1*	*NS 1*	*NS 2*	*NT 1*	*NT 2*	*NU 1*	*NV 1*	*NW 1*	*NZ 1*	*IAM 1207*	*IAM 1225*	*IAM 1230*
a-C_{13}	—	—	0·5	—	—	—	—	—	—	—	0·1	—	0·2	—	—	—	—	—	—	—	—	—	—	1·2	0·6
a-C_{15}	33·4	40·4	41·2	42·0	42·6	43·5	40·4	40·1	39·4	39·4	34·7	44·3	39·1	45·4	39·1	41·5	42·4	43·7	46·2	42·5	42·5	38·8	31·4	34·6	30·6
a-C_{17}	10·1	11·6	12·1	11·2	9·4	12·2	11·8	8·0	8·3	13·2	10·3	10·5	17·1	12·0	11·4	15·0	14·5	14·1	14·8	12·4	11·9	11·3	10·7	15·0	12·7
i-C_{13}	0·8	0·1	0·1	0·9	—	tr	0·6	0·1	0·2	0·1	0·1	tr	0·2	0·1	tr	0·3	0·3	0·3	tr	tr	—	tr	0·2	—	0·1
i-C_{15}	25·2	16·8	21·2	20·8	18·9	14·6	21·3	19·2	23·2	18·0	19·6	18·7	13·7	17·3	16·4	17·9	14·3	14·7	14·0	18·7	20·0	19·3	31·2	22·3	27·0
i-C_{17}	9·7	13·3	11·4	11·0	12·1	12·1	12·3	12·8	11·7	13·3	11·4	11·1	12·2	8·5	9·3	6·7	10·9	11·0	9·7	12·6	12·5	13·4	14·8	15·2	17·0
i-C_{12}	0·7	—	0·3	1·1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	tr	tr	0·2	—	—	—	—
i-C_{14}	9·4	1·8	2·3	4·7	1·5	2·6	2·1	4·0	2·4	2·1	1·7	2·2	2·0	3·0	1·6	2·5	2·1	2·3	1·6	2·1	1·3	1·7	1·6	1·7	1·6
i-C_{16}	5·7	7·1	6·9	5·5	4·8	9·5	6·3	7·2	7·3	6·2	5·3	7·4	9·8	9·9	6·3	9·2	9·9	8·2	8·4	7·9	5·4	5·6	3·7	4·7	5·3
i-C_{18}	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
n-C_{13}	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
n-C_{15}	—	0·3	0·1	—	1·0	0·4	—	0·2	—	0·6	0·8	—	—	—	—	—	—	—	—	—	0·3	—	0·2	—	—
n-C_{17}	—	0·1	tr	—	—	—	—	—	—	—	0·4	—	—	—	—	—	—	—	—	—	—	—	—	—	—
n-C_{12}	—	—	—	—	0·3	—	—	—	—	—	1·1	0·2	0·2	0·2	0·5	0·5	0·6	0·5	—	tr	—	0·2	—	—	—
n-C_{14}	—	0·5	0·2	—	1·4	0·7	0·3	0·9	0·9	0·5	1·7	0·6	0·5	0·5	1·9	0·6	0·5	0·6	0·5	0·4	0·7	0·9	0·7	0·5	0·3
n-C_{16}	3·7	6·2	3·6	2·4	7·4	3·9	4·8	6·8	5·8	5·2	8·9	4·8	3·4	2·5	9·3	4·8	3·9	3·9	4·1	3·4	4·5	6·5	5·0	4·8	4·7
n-C_{18}	1·3	1·8	0·1	0·4	0·8	0·6	—	0·6	0·7	1·4	3·7	tr	0·5	0·5	4·1	0·8	0·6	0·8	0·7	tr	0·5	2·3	0·5	—	tr

tr, trace.

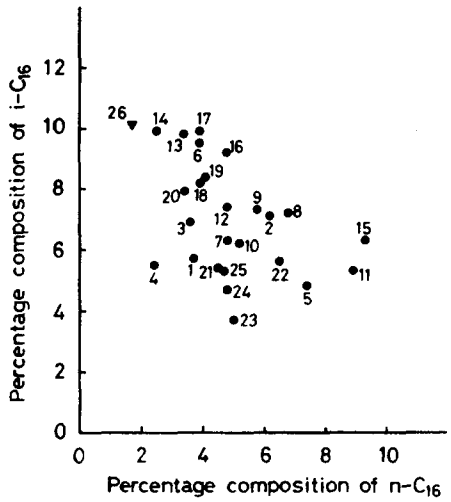


Fig. 2. Relationship between the amount of i-C_{16} and that of n-C_{16} in the spores from *Bacillus natto* and *Bacillus subtilis*.

1, *Bacillus natto* NB 1. 2, NC 2-1. 3, ND 1. 4, NE 1. 5, NH 1. 6, NI 1. 7, NJ 1. 8, NK 1. 9, NL 1. 10, NN 1. 11, NO 1. 12, NP 1. 13, NQ 1. 14, NR 1. 15, NS 1. 16, NS 2. 17, NT 1. 18, NT 2. 19, NU 1. 20, NV 1. 21, NW 1. 22, NZ 1. 23, IAM 1207. 24, IAM 1225. 25, IAM 1230. 26, *Bacillus subtilis* IAM 1253.

production of a fermented food which contains animal vitamin B_{12}. Kosuge *et al.* (1971) obtained a characteristic flavour, tetramethylpyrazine, from natto. Tetramethylpyrazine has also been isolated from the total volatiles of shallow fried beef (Watanabe & Sato, 1971). When fermented by *Bacillus natto*, the surface of soybean is covered with a characteristic mucin consisting of 58 % of γ-polyglutamic acid and 40 % of polysaccharide (Saito *et al.*, 1974). The ratio of L- and D-glutamic acid was determined to be 58:42 using L-glutamic acid dehydrogenase. The antagonistic action of *Bacillus natto* was demonstrated by Udo (1936). He reported that *Bacillus natto* elaborated an antibacterial metabolite, dipicolinic acid, which is active against susceptible microorganisms.

Whilst *Bacillus subtilis* has none of the above-mentioned properties, *Bacillus subtilis* is, without doubt, the organism of fermented food poisoning.

DNA–DNA hybridisation was studied by the DNA–agar method using ^{32}P-labelled DNA of *Bacillus subtilis* Marburg *sfr* and unlabelled DNA of the two strains of *Bacillus natto* 8-1259 and 7-119a (Takahashi *et al.*, 1966). Although high homology indices of 70–90 % were obtained in the two cases, complete homology (100 %) was not obtained. The term 'homology index' implies the relative recovery percentage of hot DNA in heterologous combination compared with the recovery percentage of hot DNA in isologous combination. It may be suggested that the slight difference of the fatty acids spectra in spores between *Bacillus natto* and *Bacillus subtilis* reflects the slight difference of the DNA homology index of the two

groups. Further investigations are required to elucidate the relationship between the two groups of strains, *Bacillus natto* and *Bacillus subtilis*.

REFERENCES

ABEL, K., DESCHMERTZING, H. & PETERSON, J. L. (1963). Classification of microorganisms by analysis of chemical composition. I. Feasibility of utilizing gas chromatography. *J. Bacteriol.*, **85**, 1039–44.

BUCHANAN, R. E. & GIBBONS, N. E. (1974). *Bergey's Manual of Determinative Bacteriology*, (Eighth edition), Baltimore, The Williams & Wilkins Co.

FUJII, H., SHIRAISHI, A., KABE, H., SHIBAGAKI, M., TAKAHASHI, S. & HANADA, A. (1975). Abnormal fermentation in natto production and *Bacillus natto* pharges. *J. Ferment. Technol. Osaka*, **53**, 424–8.

HANSON, R. S., BLICHARSKA, J. & SZULMAJESTER, J. (1964). Relationship between the tricarboxylic acid cycle enzymes and sporulation of *B. subtilis*. *Biochim. Biophys. Res. Commun.*, **17**, 1–7.

HANZAWA, J. & TAMURA, Y. (1934). Studies on natto producing strain. *J. Agric. Chem. Soc. Japan*, **10**, 520–1.

KANEDA, T. (1967). Fatty acids in the genus *Bacillus*. I. *Iso*- and *anteiso*-fatty acids as characteristic constituents of lipids in ten species. *J. Bacteriol.*, **93**, 894–903.

KITA, S., HASHIDA, T. & TERAMOTO, S. (1956). Nutritional studies on natto and *Bacillus natto*. *J. Ferment. Technol. Osaka*, **34**, 542–6.

KIUCHI, K., OHTA, T., HOH, H., TAKAHASHI, T. & EBINE, H. (1976). Studies on lipid of natto. *J. Agric. Fd. Chem.*, **24**, 404–7.

KOSUGE, T., ZENDA, H., TSUJI, H., YAMAMOTO, T. & NARITA, H. (1971). Studies on flavour components of foodstuffs. Part I. Distribution of tetramethylpyrazine in fermented foodstuffs. *Agric. Biol. Chem. Tokyo*, **35**, 693–6.

OHTA, T. (1975). Science and technology of natto production. In: *The history of natto* (Hoshina, K. (Ed)), Tokyo, Sankei Press, 325–63.

SAITO, K. (1960). Studies on bacterial fatty acids. Structure of subtilipentadecanoic and subtiloheptadecanoic acids. *J. Biochem. Tokyo*, **47**, 710–19.

SAITO, T., ISO, N., MIZUNO, H., KANEDA, H., SUYAMA, H., KAWAMURA, S. & OSAWA, S. (1974). Conformational change of natto mucin in solution. *Agric. Biol. Chem. Tokyo*, **38**, 1941–46.

SAWAMURA, S. (1906). On the microorganisms of natto. *Bull. Agric. Coll. Tokyo Univ.*, 7, 107–10.

SAWAMURA, S. (1913). On *Bacillus natto*. *J. Coll. Agric. Univ. Tokyo*, **5**, 189–91.

SCANDELLA, C. J. & KORNBERG, A. (1969). Biochemical studies of bacterial sporulation and germination. XV. Fatty acids in growth, sporulation, and germination of *Bacillus megaterium*. *J. Bacteriol.*, **98**, 82–6.

SHAW, N. (1974). Lipid composition as a guide to the classification on bacteria. *Adv. Appl. Microbiol.*, **17**, 63–108.

SHURTLEFF, W. & AOYAGI, A. (1976). Miso as a food. In: *The book of miso*. (Shurtleff, W. & AOYAGI, A. (Eds)), Kanagawa-ken, Autumn Press, 22–28.

TAKAHASHI, H., SAITO, H. & IKEDA, Y. (1966). Genetic relativeness of spore bearing bacilli studied by the DNA agar method. *J. Gen. Appl. Microbiol.*, **12**, 217–22.

UCHIDA, K. & MOGI, K. (1972). Cellular fatty acid spectra of *Pediococcus* species in relation to their taxonomy. *J. Gen. Appl. Microbiol.*, **18**, 109–29.

UCHIDA, K. & MOGI, K. (1973). Cellular fatty acid spectra of *Sporolactobacillus* and some other *Bacillus-Lactobacillus* intermediates as a guide to their taxonomy. *J. Gen. Appl. Microbiol.*, **19**, 129–40.

UDO, S. (1936). Studies on the constituents of 'natto', the fermented soybeans. Part I. On the effect of dipicolinic acid produced in 'natto'. *J. Agric. Chem. Soc. Japan*, **12**, 386–94.

WATANABE, K. & SATO, Y. (1971). Some alkyl-substituted pyrazines and pyridines in the flavour components of shallow fried beef. *J. Agric. Food Chem.*, **19**, 1017–19.

YOSHIMOTA, T., FUKUMOTO, J. & TSURU, D. (1971). Studies on bacterial protease. Some enzymatic and physical properties of the alkaline protease from *Bacillus natto*. *Int. J. Protein Res.* **III**, 285–95.

Food Chemistry 8 (1982) 11-19

EFFECT OF PROCESSING ON PHOSPHORUS AND PHYTIC ACID CONTENTS OF SOME EGYPTIAN VARIETIES OF LEGUMES

S. S. GAD, M. S. MOHAMED, M. ESMAT EL-ZALAKI & S. ZEINAB MOHASSEB

Food Science and Technology Division,
College of Agriculture, Alexandria University, Egypt

(Received: 17 June, 1980)

ABSTRACT

Phytic acid and total phosphorus contents were determined in different varieties of dry legume seeds—broad beans, lentils, lupin, fenugreek, chickpea and peas. These seeds are widely used in Egypt. The dry seeds were subjected to different processing methods according to the mean of their consumption in Egypt. These processing methods include decortication, steeping, sprouting, boiling and cooking. Phytic acid and total phosphorus contents of seeds differed according to the kind, the variety and the processing method. Steeping and sprouting decreased the phytic acid content of the seeds. The phytic acid content of lupin was greatly reduced by decortication and by steeping of seeds.

INTRODUCTION

Cuthbertson (1968) reported that legumes contain phytic acid capable of interfering with the utilisation of mineral elements. Asada *et al.* (1968) concluded that phytate could be considered a final product of phosphorus metabolism in the ripening process. The phytic acid content of seeds was found to differ according to the kind of seed, the variety and the degree of maturity, as investigated by Makower (1969) and Lolas & Markakis (1975). The large amount of phytin present in seeds may be regarded as a storage of inorganic phosphate which can be liberated on germination, as detected by Kumar *et al.* (1978) and Mayer & Poljakoff-Maybor (1978). The present work was carried out with the aim of studying the total phosphorus and phytic acid contents of six different kinds and twelve different varieties of legume seeds consumed widely in Egypt. The effects of decortication, steeping, sprouting, boiling and cooking on the phytic acid and total phosphorus contents of the seeds were also assessed.

Food Chemistry 0308-8146/82/0008-0011/$02·75

MATERIALS AND METHODS

Legumes

Different kinds of legume were obtained from the Plant Breeding Department, Ministry of Agriculture, Giza, Egypt. The dry seeds under investigation are described below.

1. Broad beans: *Vicia faba* L. Varieties Giza 1, Giza 2 and Rebaya 40.
2. Chickpea: *Cicer arietinum* L. Varieties Giza 1 and Ala 2.
3. Fenugreek: *Trigonella foenum-graecum* L. Varieties Giza 2 and Giza 30.
4. Lentils: *Lens esculenta*, Varieties Giza 9 and Pakistany.
5. Lupin: *Lupinus termis*. Varieties Giza 1 and Giza 2.
6. Peas: *Pisum sativum*. Variety Little Marvel.

Decortication

The dry seeds were decorticated mechanically. The steeped and sprouted seeds were decorticated manually.

Baked beans (medamis)

The baked broad bean is a popular food in Egypt, known locally as medamis. In the present study canned baked broad beans were prepared. The dry seeds were cleaned, washed and steeped in water for 6 h. No. 1 cans (211 × 409) were filled with beans (150 g) and boiled water (200 ml), exhausted, seamed and heat processed at 240 °F for $4\frac{1}{2}$-h, a period long enough to soften and cook the beans.

Stewed broad bean paste (bisara)

Stewed broad bean paste is used in the preparation of a delicious and popular food known in Egypt as bisara. Dry, decorticated broad beans were cooked in sufficient water in a normal proportion (1:4). The cooking period differed according to the variety, the beans being cooked until they disintegrated into a paste upon mixing. The beans were cooked for $1\frac{1}{2}$-h for the Giza 1 variety and for $2\frac{1}{4}$-h for both the Giza 2 and Rebaya 40 varieties. The cooked paste was strained, then allowed to cool and set.

Sprouted broad beans

Sprouting of bean seeds was carried out by first steeping them in cold tap water for 24 h. The bean seeds were then spread between moistened cloth sheets for 3 days.

Cooked sprouted broad beans

The sprouted beans were cooked with water in a normal proportion (1:2) for 45 min in the case of the Giza 2 variety and for 30 min in the case of the Rebaya 40 and Giza 1 varieties.

Whole cooked lentils

The seeds were cooked in water (1:3) for 2 h in the case of the Giza 9 variety and for $1\frac{1}{2}$-h in the case of the Pakistany variety, where the seeds disintegrated upon mixing.

Decorticated cooked lentils

Dry, decorticated lentils were cooked in water (1:4) for $2\frac{1}{2}$-h in the case of the Giza 9 variety and for 2 h in the case of the Pakistany variety. The paste was strained and allowed to cool and set.

Steeped lupin seeds

The dry seeds were steeped overnight in water, boiled in water for 10 min and soaked for 4 days in sufficient water to cover them. The water was changed twice a day throughout the soaking period.

Sprouted fenugreek

The dry seeds were steeped in cold tap water for 24 h, then spread between moistened cloth sheets for 3 days.

Boiled fenugreek

The dry seeds were boiled in water (1:3) for 20 min, drained and cooled.

Sample preparation

Dry seeds were cleaned, ground and passed through a 30 mesh sieve. The processed products were dried in an air oven at 55 °C to a constant weight, ground and passed through a 30 mesh sieve.

Moisture

The moisture content was determined, using an air-oven at 135 °C, according to the methods of the AOAC (1970).

Total phosphorus

The colorimetric method, using ammonium molybdate and hydroquinone as reported by Kent-Jones & Amos (1967), was followed.

Phytic acid

Phytic acid content was determined in terms of its phosphorus content, according to Kent-Jones & Amos (1967). The method is based on the separation of phytic acid as ferric phytate which is transformed into sodium phytate. The organic matter was destroyed by heating with concentrated sulphuric acid and perchloric acid. The neutralised solution was used for the colorimetric determination of phytic acid phosphorus.

RESULTS AND DISCUSSION

Dry legume seeds

The phytic acid and total phosphorus contents differed according to the legume species and variety, as shown in Table 1. The results shown in the Table indicate that the dry seeds most rich in phytic acid (mg/100 g) were broad beans (274·9) followed in a descending order by peas (222·7), fenugreek (190·2), chickpea (184·5), lentils (149·7) and lupin (91·9). The total phosphorus content differed from the above-mentioned sequence. The seeds containing the highest amount of total phosphorus (mg/100 g) were broad beans (518·2), followed in a descending order by fenugreek (369·7), lentils (357·5), peas (345·2), lupin (340·1) and chickpea (203·7). Accordingly, the ratio of phytic acid phosphorus as a percentage of total phosphorus was different.

TABLE 1
PHYTIC ACID AND TOTAL PHOSPHORUS CONTENTS OF SOME WHOLE DRY LEGUME SEEDS

Legume seed varieties	*Moisture content* (%)	*Phytic acid phosphorus* (*mg/100 g on a dry weight basis*)	*Total phosphorus*
Broad beans			
Giza 1	9·4	241·5	441·5
Giza 2	10·3	319·2	574·1
Rebaya 40	10·0	263·9	538·9
Chickpea			
Giza 1	9·2	151·4	170·6
Ala 2	9·2	217·5	236·7
Fenugreek			
Giza 2	9·6	207·4	320·8
Giza 30	10·4	173·0	418·5
Lentil			
Giza 9	9·9	123·5	305·2
Pakistany	9·7	175·9	409·7
Lupin			
Giza 1	8·8	98·7	378·3
Giza 2	8·9	85·1	301·9
Peas			
Little Marvel	10·2	222·7	345·2

Broad beans

The results shown in Table 2 indicate that the phytic acid and total phosphorus contents of broad beans were reduced by baking, stewing, sprouting and cooking of the seeds. The extent of this reduction differed according to the processing treatment and the variety. The results shown in Table 2 indicate that the most pronounced loss of phytic acid was demonstrated in baked seeds, followed in a descending order by cooked sprouted, sprouted and stewed seeds. The obvious decrease in phytic acid

content of the baked and cooked sprouted broad beans was attributed mainly to the phytase activity during the steeping of seeds prior to baking and cooking.

The results presented in Table 2 indicate that the loss of phytic acid phosphorus was more pronounced than that of total phosphorus following the various processing treatments. This was expected as a result of the phosphorus release upon the degradation or hydrolysis of phytic acid by the effect of heat treatments and phytase activity. Belavady & Banerjee (1953) found that phytic acid phosphorus diminished and water-soluble phosphorus increased during seed germination. According to Chang *et al.* (1977) incubation of beans in water followed by cooking in boiling water increased inorganic phosphorus concentration and 50% hydrolysis of bean phytate was observed.

TABLE 2
THE EFFECT OF PROCESSING AND COOKING ON PHYTIC ACID AND TOTAL PHOSPHORUS CONTENTS OF BROAD BEANS

Broad bean varieties	*Moisture content* (%)	*Phytic acid phosphorus mg/100 g on a dry weight basis* (*Loss* %)	*Total phosphorus* (*Loss* %)
Giza 1			
Whole beans	9·4	241·5	441·5
Decorticated beans	9·5	279·0 —	436·4 (1·2)
Baked beans (medamis)	75·0	119·1 (50·7)	335·5 (24·0)
Stewed beans paste (bisara)	65·7	253·0 (9·3)	412·2 (5·5)
Sprouted beans (nabet)	55·0	202·5 (16·1)	415·8 (5·8)
Cooked sprouted beans	70·0	191·0 (20·9)	394·3 (10·7)
Rebaya 40			
Whole beans	10·0	263·9	538·9
Decorticated beans	10·4	309·8 —	535·6 (0·6)
Baked beans (medamis)	80·0	121·6 (53·9)	508·1 (5·7)
Stewed beans paste (bisara)	67·4	256·9 (17·1)	513·8 (4·1)
Sprouted beans (nabet)	61·4	202·8 (23·3)	518·3 (3·8)
Cooked sprouted beans	70·0	155·4 (41·1)	493·5 (8·4)

In the present study broad bean variety Rebaya 40 showed a high reduction in phytic acid—and a low reduction in total phosphorus—upon sprouting and cooking, as shown in Table 2. Accordingly, this variety can be recommended for the preparation of cooked sprouted beans, a delicious popular food known in Egypt as bisara.

Lentils

Decortication of lentils raised the phytic acid and total phosphorus contents of the seeds, as shown in Table 3. This indicated that the hulls of lentil seeds were almost free from those components. Cooking of the decorticated lentil seeds reduced the phytic acid content of both Giza 9 and Pakistany lentils by 38·4% and 21·7%, respectively, as shown in Table 3. The decrease was more pronounced upon cooking the decorticated lentils than the decrease in whole cooked seeds.

TABLE 3
THE EFFECT OF DECORTICATION AND COOKING ON PHYTIC ACID AND TOTAL PHOSPHORUS CONTENTS OF LENTIL SEEDS

Lentil varieties	*Moisture content* (%)	*Phytic acid phosphorus mg/100 g on a dry weight basis* (*Loss %*)	*Total phosphorus* (*Loss %*)	*Phytic acid-P as % of total phosphorus*
Giza 9				
Whole seeds	9·9	123·5	305·2	40·5
Decorticated seeds	10·6	137·1 —	313·6 —	43·7
Whole cooked seeds	68·1	108·2 (12·4)	297·6 (2·5)	36·4
Decorticated cooked seeds	70·0	83·4 (38·4)	231·2 (26·3)	36·1
Pakistany				
Whole seeds	9·7	175·9	409·7	42·9
Decorticated seeds	9·9	183·1 —	416·2 —	44·0
Whole cooked seeds	68·0	167·7 (4·7)	400·4 (2·1)	44·9
Decorticated cooked seeds	70·4	143·6 (21·7)	343·7 (17·4)	41·8

Lentils of the Giza 9 variety had a lower phytic acid content and a higher total phosphorus content than the Pakistany variety, which makes the Giza 9 variety preferable nutritionally.

Lupin seeds

The results presented in Table 4 show that decortication of lupin seeds greatly lowered the phytic acid and total phosphorus contents of the seeds. The decreases in phytic acid content amounted to 40·2% and 32·5% in Giza 2 and Giza 1, respectively. This is an indication that lupin hulls contained considerable amounts of phytic acid.

Steeping of whole lupin seeds decreased the phytic acid and total phosphorus contents of the seeds by 50·8–56·7% and 7·1–12·8%, respectively. Decortication of

TABLE 4
THE EFFECT OF DECORTICATION AND WATER STEEPING ON PHYTIC ACID AND TOTAL PHOSPHORUS CONTENTS OF LUPIN SEEDS

Lupin varieties	*Moisture content* (%)	*Phytic acid phosphorus mg/100 g on a dry weight basis* (*Loss %*)	*Total phosphorus* (*Loss %*)	*Phytic acid-P as % of total phosphorus*
Giza 1				
Whole seeds	8·8	98·7	378·3	26·1
Decorticated seeds	8·0	66·6 (32·5)	367·4 (2·9)	18·1
Whole steeped seeds	69·8	48·6 (50·9)	329·7 (12·8)	14·7
Decorticated steeped seeds	67·2	24·1 (75·6)	316·5 (16·3)	7·6
Giza 2				
Whole seeds	8·9	85·1	301·9	28·2
Decorticated seeds	8·8	49·3 (40·2)	291·6 (3·4)	16·9
Whole steeped seeds	71·9	36·8 (56·7)	280·5 (7·1)	13·1
Decorticated steeped seeds	68·7	21·4 (74·2)	266·4 (11·4)	8·0

the steeped seeds further lowered the phytic acid and total phosphorus contents. The diminution of phytic acid upon steeping and decortication varied according to the lupin variety.

It is of interest to notice from Fig. 1 that decortication of steeped whole seeds reduced 74–75 % of the phytic acid content of the seeds whilst steeping diminished 50–56 %. Accordingly, decortication of steeped lupin seeds before consumption must be recommended, a practice not normally followed, particularly by children, who consume large amounts of whole steeped and boiled lupin seeds as a popular snack in Egypt. According to Rackis (1974), the utilisation of Ca, Fe, Mg and other metals is affected by the phytic acid content of the food.

Fenugreek

The phytic acid content of fenugreek seeds was greatly diminished (45·9–49·8 %) upon steeping but decreased only slightly (4·15–5·9 %) upon boiling, as illustrated in Fig. 2.

There was an equal loss of phytic acid and total phosphorus after sprouting the

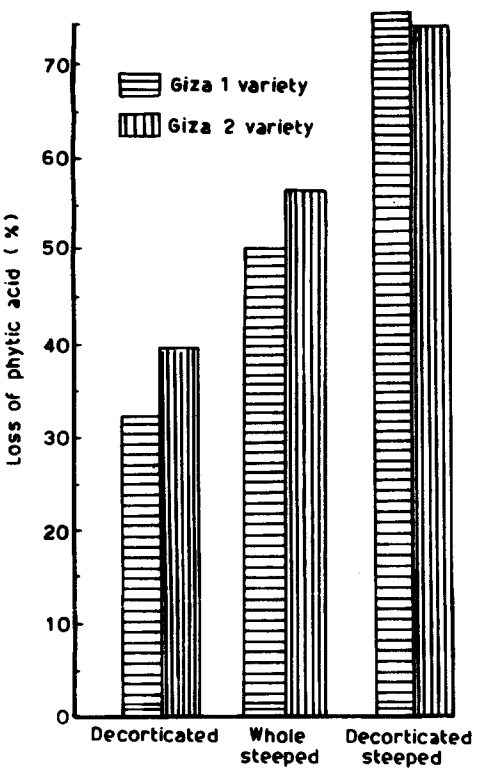


Fig. 1. The effect of decortication and water steeping on phytic acid content of lupin seeds.

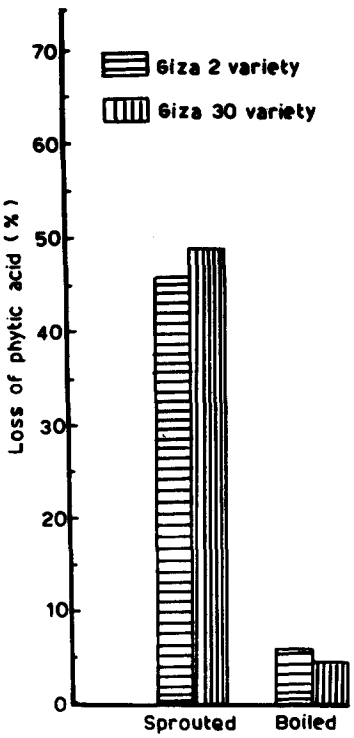


Fig. 2. The effect of sprouting and boiling on phytic acid content of fenugreek seeds.

TABLE 5

THE EFFECT OF SPROUTING AND BOILING ON PHYTIC ACID AND TOTAL PHOSPHORUS CONTENTS OF FENUGREEK SEEDS

Fenugreek varieties	*Moisture content* (%)	*Phytic acid phosphorus mg/100 g on a dry weight basis* (*Loss %*)	*Total phosphorus* (*Loss %*)	*Phytic acid-P as % of total phosphorus*
Giza 2				
Dry seeds	9·6	207·4	320·8	64·7
Sprouted seeds	76·4	112·1 (45·9)	173·8 (45·8)	64·4
Boiled seeds	74·5	195·1 (5·9)	280·2 (12·7)	69·6
Giza 30				
Dry seeds	10·4	173·0	418·5	41·3
Sprouted seeds	76·6	86·8 (49·8)	279·8 (33·1)	31·0
Boiled seeds	74·6	165·2 (4·5)	366·7 (12·1)	45·1

seeds of the Giza 2 variety, which indicates a constant ratio of phytic acid to total phosphorus of 64:100, as shown in Table 5. In the case of the Giza 30 variety, the loss in the two components varied and therefore the ratio decreased from 41:100 before sprouting to 31:100 after sprouting. The results also indicate that boiling the seeds had the same effect of lowering the phytic acid and total phosphorus levels in the two varieties but the reduction of total phosphorus was greater than that of phytic acid. The pronounced reduction of phytic acid content due to the sprouting of the seeds was mainly due to the action of phytase, which hydrolysed the phytic acid to inositol and phosphoric acid but, upon boiling, the destruction was due to the effect of heat, Belavady & Banerjee (1953) demonstrated that, during germination, the phytic phosphorus diminished and the water-soluble phosphorus increased. They also observed that phytase and the phosphatase activities were increased by seed germination.

REFERENCES

ASADA, K., TANAKA, K. & KASAI, Z. (1968). Phosphorylation of inositol in ripening rice and wheat grains: incorporation of phosphate- 32P and inositol- 3H into inositol polyphosphates. *Plant Cell Physiol.*, **9**, 195.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS (1970). *Official methods of analysis AOAC*, Washington, DC, USA.

BELAVADY, B. & BANERJEE, S. (1953). The effect of germination on the phosphorus values of some common Indian pulses. *Food Res.*, **18**, 223–6.

CHANG RUTH, SCHWIMMER, S. & BURN, K. HORACE (1977). Phytate removal from whole dry beans by enzymatic hydrolysis and diffusion. *J. of Fd. Sci.*, **42**, 1098–101.

CUTHBERTSON, D. (1968). *Natural antinutritive substances in foodstuffs and forages*. S. Karger, Basel, New York.

KENT-JONES, D. W. & AMOS, A. J. (1967). *Modern cereal chemistry*. Food Trade Press Ltd, Great Britain.

KUMAR, GANESH K., VENKATARAMON, L. V., JAYA, T. V. & KUSHNAMURTHY, K. (1978). Cooking characteristics of some germinated legumes. Changes in phytin, Ca, Mg and Pectins. *J. of Fd. Sci.*, **43**, 85–8.

LOLAS, G. M. & MARKAKIS, P. (1975). Phytic acid and other phosphorus compounds of beans (*Phaseolus vulgaris* L.). *J. Agr. Food Chem.*, **23**, 13.

MAKOWER, R. U. (1969). Changes in phytic acid and acid soluble phosphorus in maturing pinto bean. *J. Sci. Food Agric.*, **20**, 82–4.

MAYER, A. M. & POLJAKOFF-MAYBOR, A. (1978). *The germination of seeds*, Pergamon Press, New York.

RACKIS, J. J. (1974). Biological and physiological factors in soybeans. *J. Amer. Oil Chem. Soc.*, **51**, 161A.

Food Chemistry 8 (1982) 21–25

A STATISTICAL EVALUATION OF THREE METHODS FOR MEASURING LIPID OXIDATION IN A MODEL SYSTEM*

BRUCE MACDONALD,† J. IAN GRAY, MARK A. UEBERSAX & IAN D. MORTON

Department of Food Science and Human Nutrition, Michigan State University, East Lansing, MI 48824, USA

(Received: 8 September, 1980)

ABSTRACT

The extent of oxidation in purified linoleic acid as a simple lipid model system was measured by total carbonyls (TC), peroxide values (PV) and thiobarbituric acid (TBA) values. Regression equations established relationships between individual methods and between each method and time, temperature and air flow rate independently. Correlation coefficients were statistically significant ($P < 0{\cdot}05$) *and practically the same for each method over time. Significant correlations* ($P < 0{\cdot}05$) *were also found between the TBA and PV methods, as well as between the TC and TBA procedures. A smaller but still significant correlation was found between TC and PV determinations.*

INTRODUCTION

The oxidation of lipids in foods is an important area of study. Lipid deterioration can give rise to rancidity, unpleasant aromas and loss of essential fatty acids and, under exceptional conditions, oxidised material which can be harmful to animals (Artman, 1969; Alexander, 1978). There are many reports in the literature of studies involving the oxidation of oils and other fats (Chang *et al.*, 1978; Graziano, 1979). Some reports have shown that linoleic acid is preferentially oxidised before oleic acid (Rosas-Romero & Morton, 1975, 1977). Many studies have used the peroxide value (PV) or total carbonyl value (TC) without appreciating possible drawbacks and limitations. The thiobarbituric acid (TBA) value, while in theory estimating the malonaldehyde content, gives a clear indication of the state of oxidation of oils

* Published as Michigan Agriculture Experiment Station journal article No. 9249.
† Present address: Canada Packers Ltd., Toronto, Canada.

Food Chemistry 0308-8146/82/0008-0021/$02·75

(Gray, 1978). We initiated the study reported here to establish in a pure lipid system, linoleic acid, the significance of the peroxide values, total carbonyls and thiobarbituric acid numbers for measuring lipid oxidation and, in addition, the relationships, if any, between them.

EXPERIMENTAL

Lipid oxidising system

Linoleic acid (Fisher Scientific Co.) was checked for purity by gas chromatography. No traces of linoleic or other polyunsaturated fatty acids were found. The purified linoleic acid was oxidised by passing air through samples held in glass vessels at various temperatures. Compressed air was purified by passing through solutions of 2,4-dinitrophenylhydrazine (0·2% in 2 N HCl), potassium dichromate (2% in 1% H_2SO_4) and concentrated H_2SO_4, respectively. Air flow rate was controlled by Roger Gilmont flowmeters (Canlab, Ontario) equipped with micrometer valves. Factorial combinations of storage temperature (15 °C, 40 °C and 65 °C), air flow rate (10, 100 and 240 ml/min) and holding time (0, 5, 10, 15, 20, 25, 30, 35, 40 and 45 h) were performed in duplicate. Lipid oxidation was monitored by each method for all experimental conditions. When necessary, the collected samples were flushed with nitrogen and stored at −20 °C before analysis.

Measurement of lipid oxidation

Peroxide values, expressed in terms of milliequivalents of peroxide per kilogramme of sample, were measured by the AOCS official method (AOCS, 1971).

Thiobarbituric acid (TBA) values were determined using the distillation method of Tarladgis *et al.* (1960). A K value of 5·41 was obtained as previously described and results were expressed as milligrammes of malonaldehyde per kilogramme of sample.

Total carbonyl compounds were determined following the calorimetric method of Lappin & Clark (1951) using an average ε_{max} of $2{\cdot}72 \times 10^4$.

Statistical design and analysis

Results from each method were analysed as to split–split plot design with temperature as main plots, air flow rate as sub-plots and holding time as sub-sub plots according to Cochran & Cox (1957) by means of analysis of variance. The three methods were evaluated as indices of lipid oxidation by means of simple linear regression and simple linear correlation analyses with time, temperature and air flow rate, independently. Similar analyses were used to compare one method with another. Statistical significance for the study was taken as $P < 0{\cdot}05$, and the coefficient of variation (CV %), expressing the standard deviation as a per cent of the mean, is reported for all methods and conditions.

RESULTS AND DISCUSSION

The extent of lipid oxidation in foods, including meat systems, has been measured objectively by TBA, peroxide value and total carbonyl methods (Gray, 1978). This study evaluated these methods as indices of lipid oxidation using a simple lipid system. Mean values for total carbonyls, peroxide values and TBA numbers as a function of time, temperature and air flow rate are presented in Figs 1, 2 and 3, respectively. Analysis of variance reveals that time, temperature and air flow rate—and their interactions—are all significant at $P < 0{\cdot}05$. The results indicate that time, temperature and air flow rate are not independent of one another. Any simple effect of one factor such as temperature is dependent on the level of the other two factors. This is of importance and should be considered when using accelerated shelf-life tests to predict the oxidative stability of lipids. Coefficients of variation (CV%), which express the standard deviation as a per cent of the means, indicate that, in this study, highly precise measures were obtained for each method and over all physical conditions. Literature values for the methods in complex food systems generally indicate greater variability than obtained in this study (Gray, 1978).

Although methods for measuring lipid oxidation have been reviewed (Morton, 1967; Gray, 1978), virtually no statistical comparisons between the methods are found in the literature. Therefore, PV, TC and TBA values were evaluated as parameters for measuring lipid oxidation by means of simple linear correlation

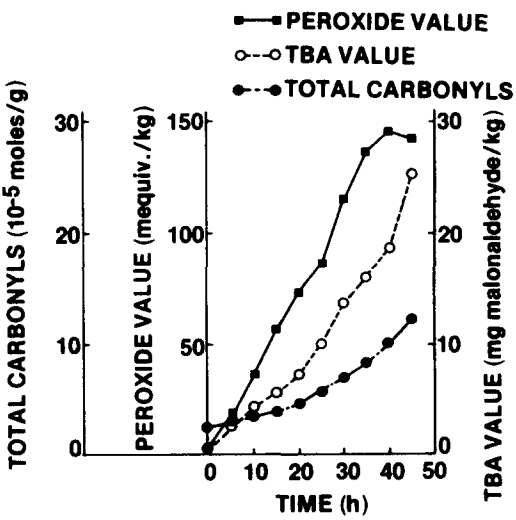


Fig. 1. Effect of time on the oxidation of linoleic acid as measured by total carbonyl, peroxide value and TBA methods. Regression analysis for methods (y) on time (x) are as follows: TBA, $y = 1{\cdot}373 + 0{\cdot}208\,x$ ($r = 0{\cdot}97^{**}$, CV% = 0·95); TC, $y = 3{\cdot}505 + 3{\cdot}456\,x$ ($r = 0{\cdot}99^{**}$, CV% = 0·82); PV, $y = -0{\cdot}988 + 0{\cdot}509\,x$ ($r = 0{\cdot}98^{**}$, CV% = 0·61).

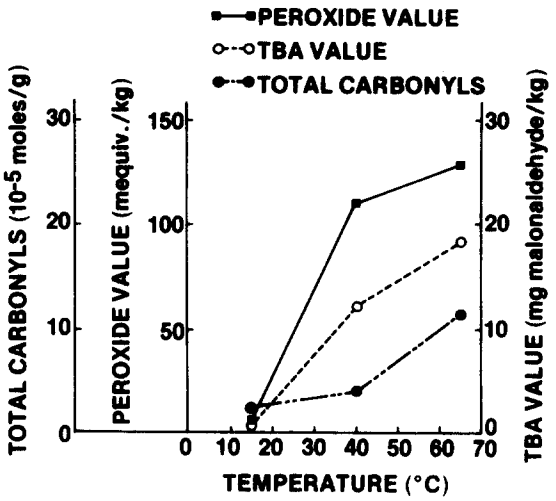


Fig. 2. Effect of temperature on the oxidation of linoleic acid as measured by total carbonyl, peroxide value and TBA methods. Regression analyses for methods (y) on temperature (x) are as follows: TBA, $y = -3{\cdot}33 + 0{\cdot}35x$ ($r = 0{\cdot}98^{**}$, CV% = 2·58); TC, $y = -1{\cdot}43 + 0{\cdot}19x$ ($r = 0{\cdot}93^{**}$, CV% = 0·29); PV, $y = -17{\cdot}67 + 2{\cdot}5x$ ($r = 0{\cdot}92^{**}$, CV% = 0·95).

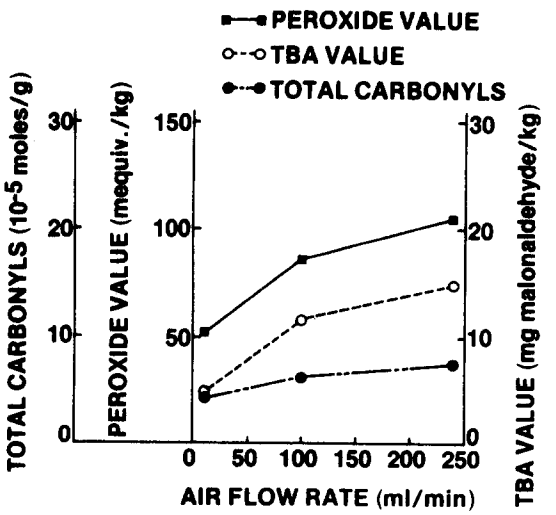


Fig. 3. Effect of air flow rate on the oxidation of linoleic acid as measured by the total carbonyl, peroxide value and TBA methods. Regression analyses for methods (y) on air flow rate (x) are as follows: TBA, $y = 5{\cdot}665 + 0{\cdot}041x$ ($r = 0{\cdot}95^{**}$, CV% = 0·29); TC, $y = 4{\cdot}4104 + 0{\cdot}015x$ ($r = 0{\cdot}98^{**}$, CV% = 1·39); PV, $y = 55{\cdot}068 + 0{\cdot}221x$ ($r = 0{\cdot}97^{**}$, CV% = 0·08).

analyses. Correlation coefficients, which measure how well the regression equations fit the data, are statistically significant ($P < 0.05$) and practically the same for each method over time. Significant correlation coefficients ($P < 0.05$) are also found between the TBA and PV methods ($r = 0.91$) as well as between the TC and TBA methods ($r = 0.87$). A smaller but still significant ($P < 0.05$) correlation coefficient ($r = 0.69$) is found between the TC and PV methods. Therefore, it appears that all three methods are suitable for monitoring oxidation within a simple lipid system at selected physical conditions.

It is interesting to observe the formation of malonaldehyde in the oxidising linoleic acid system at low peroxide values. This is in direct contrast to results of a study by Dahle *et al.* (1962) who reported that linoleate, even when oxidised to a peroxide value of 2000, failed to give a TBA reaction. The slight TBA colour which occurs in systems containing only oleate or linoleate has been explained as due to the further autoxidation of α, β-unsaturated aldehydes, initially formed from the decomposition of hydroperoxides (Patton & Kurtz, 1951; Lillard & Day, 1964). Clearly, our results may indicate that the malonaldehyde formed, which is a measure of oxidation, could arise by other mechanisms such as endoperoxide formation and decomposition.

REFERENCES

ALEXANDER, J. C. (1978). Biological effects due to changes in fats during heating. *J. Amer. Oil Chem. Soc.*, **55**, 711–17.

AOCS (1971). *Official and tentative methods*, Vol. 1. Champaign, Illinois, Amer. Oil Chem. Soc.

ARTMAN, N. R. (1969). The chemical and biological properties of heated and oxidized fats, *Adv. Lipid Res.*, **7**, 245–330.

CHANG, S. S., PETERSON, R. J. & HO, C-T. (1978). Chemical reactions involved in the deep-fat frying of foods. *J. Amer. Oil Chem. Soc.*, **55**, 718–27.

COCHRAN, W. & COX, G. M. (1957). *Experimental designs* (2nd ed.). Chapters 5 and 7, John Wiley and Sons, New York.

DAHLE, L. K., HILL, E. G. & HOLMAN, R. T. (1962). The thiobarbituric acid reaction and the autoxidation of polyunsaturated fatty acid methyl esters. *Arch. Biochem. Biophys.*, **98**, 253–6.

GRAY, J. I. (1978). Measurement of lipid oxidation: A review. *J. Amer. Oil Chem. Soc.*, **55**, 539–46.

GRAZIANO, V. J. (1979). Portable instrument rapidly measures quality of frying fat in food service operations. *Food Technol. (Champaign)*, **33**, (9), 50, 52, 56–7.

LAPPIN, G. R. & CLARK, L. C. (1951). Colorimetric method for determination of carbonyl compounds. *Anal. Chem.*, **23**, 541–2.

LILLARD, D. A. & DAY, E. A. (1964). Degradation of monocarbonyls from autoxidising lipids. *J. Amer. Oil Chem. Soc.*, **41**, 549–52.

MORTON, I. D. (1967). Peroxide changes in edible oils and keeping quality. *Food Processing and Marketing*, **36**, 303–9 and 319.

PATTON, S. & KURTZ, G. W. (1951). 2-Thiobarbituric acid as a reagent for detecting milk fat oxidation. *J. Dairy Sci.*, **34**, 669–74.

ROSAS-ROMERO, A. J. & MORTON, I. D. (1975). Competitive oxidation of fatty acids: Effect of the degree of unsaturation. *J. Sci. Food Agric.*, **28**, 916–20.

ROSAS-ROMERO, A. J. & MORTON, I. D. (1977). Competitive oxidation of fatty acids: Effect of the carboxylic group and of saturated fatty acids. *J. Sci. Food Agric.*, **28**, 921–6.

TARLADGIS, B. G., WATTS, B. M., YOUNATHAN, M. T. & DUGAN, L. R. (1960). A distillation method for the quantitative determination of malonaldehyde in rancid foods. *J. Amer. Oil Chem. Soc.*, **37**, 44–8.

Food Chemistry 8 (1982) 27–32

CHANGES IN THE COMPOSITION AND CARBOHYDRATE CONSTITUENTS OF OKRA (*ABELMOSCHUS ESCULENTUS*, LINN.) WITH AGE

OYEBIODUN G. LONGE,* B. L. FETUGA* & M. E. AKEN'OVA†

* *Division of Nutritional Biochemistry, Department of Animal Science.*
† *Department of Agronomy,*
University of Ibadan, Ibadan, Nigeria

(Received: 18 September, 1980)

ABSTRACT

Changes which occurred in the proximate composition, concentrations of free sugars, high molecular weight carbohydrates (water-soluble polysaccharides, starch hemicellulose and cellulose) and lignin in four varieties of okra when harvested at different ages have been studied. Samples were collected from the field at 1, 4, 7 and 10 days after flowering. Crude protein, which initially ranged from 19·9 to 24·7 g/100 g DM, decreased with age whilst crude fibre, 12·5–16·7 g/100 g DM, increased with age. Ether extract varied between 1·60 and 2·19 g/100 g DM whilst ash was 8·00–8·59 g/100 g DM. Available carbohydrates (starch + sugars) ranged from 11·2 to 13·1 g/100 g DM whilst unavailable carbohydrates increased from 33·9–42·0 g/100 g DM to 51·9–59·7 g/100 g DM.

Overall varietal differences were apparent for ethanol-soluble sugars and the structural carbohydrates while differences among the means due to age were significant ($P < 0·05$).

INTRODUCTION

Okra (*Hibiscus esculentus*, Linn., now classified as *Abelmoschus esculentus*, Linn.), or lady's finger, is self pollinating and, like a number of other commonly eaten green vegetables, is available almost throughout the year in Nigeria. It is usually cut into pieces for use in soup or boiled and drained to be served as vegetables with starchy foods such as rice and yams. The fruits of most cultivated local varieties are ready for harvest 2–3 months after sowing although there are varieties which are either early or late maturing. The first harvest for consumption takes place about 3 days after flowering and this can continue regularly for the next 7 days or more.

Food Chemistry 0308-8146/82/0008-0027/$02·75

Information is available on the chemical composition of mature okra fruits (FAO, 1968; Oyenuga, 1968; Longe, 1981). Apart from being appreciable in protein content, a major component of okra is carbohydrate, most of which is fibrous in nature. The seed, as distinct from the whole fruit, is also a potential source of oil varying between 15 and 22% (Oyenuga, 1968) and the residual protein after oil extraction was found to compare favourably with cottonseed cake when fed to rats. Mucilage isolated from the whole fruit has also been fed to rats by Woolfe (1977) to test its effect on plasma cholesterol level.

Knowledge of changes in the carbohydrate composition of okra is rudimentary. It is generally noted that older fruits lose their tenderness and the slimy texture possessed by young okra fruits when cooked disappears with advancing age. The purpose of this study is to contribute to a better knowledge of the changes which occur in the chemical composition of okra harvested at different ages with particular reference to the carbohydrate fractions. Information concerning varietal differences in chemical composition does not appear to be available even though differences in physical characteristics such as fruit colour, length, shape and seed number are well recognised (Van Epenhuijsen, 1974). Two imported and two local varieties, selected on the basis of fruit yield, have therefore been employed in the present analysis to show varietal contribution to the differences in nutrient content with advancing age.

MATERIALS AND METHODS

Description of materials

Four promising accessions in the okra world collection maintained by the Department of Agronomy, University of Ibadan, Nigeria, were used. Two of these, UI 92 and UI 104, were local accessions developed through selection from an accession collected from parts of Nigeria (Fatokun *et al.*, 1978). UI 92 exhibits facultative short-day response to daylength, and UI 104 is similar, but to a lesser degree. The remaining two varieties, UI 72-11 and UI 72-262, are introductions from Asia and are day-neutral.

Field experiment

The varieties described above were planted in May, 1978 at the University of Ibadan Teaching and Research Farm and fertiliser was uniformly applied to all treatments at the rate of 56 kg N/ha as urea, 25 kg K/ha as murate of potash and 15 kg P/ha as triple superphosphate. Each variety was randomly allocated a plot, 5·4 m long, within the experimental area. Each plot consisted of six rows and the spacing was 90 × 45 cm. Flowers were tagged as they opened with date of opening and pods were harvested at 1-, 4-, 7-, and 10-day intervals from the five inner of the seven plants in each row of each of the four inner rows. Harvested pods were freed of stalks and receptacles to obtain the edible portions. These were dried prior to milling.

Analytical procedure

Dry matter, crude protein, crude fibre, ether extract and total ash estimation was carried out according to the methods of AOAC (1970) and the nitrogen-free extractives estimated by difference. The total soluble sugars obtained by exhaustive extraction with hot 80 % (v/v) ethanol were determined by the method of DuBois *et al.* (1956). Starch was determined after extraction of the ethanol-soluble sugars by the method of Thivend *et al.* (1972) and ethanol-soluble sugars and starch were summated as available carbohydrates.

Unavailable carbohydrates of cell wall origin were estimated (after elimination of soluble sugars and starch) by acid hydrolysis (Southgate, 1969) after which total sugars in hydrolysates were estimated (Dubois *et al.*, 1956). Data obtained were analysed statistically (Steel & Torrie, 1960).

RESULTS AND DISCUSSION

Proximate composition

The proximate chemical composition of the four varieties harvested at different ages is presented in Table 1. Crude protein content decreased as harvesting

TABLE 1

EFFECT OF AGE AT HARVEST ON THE PROXIMATE COMPOSITION OF FOUR VARIETIES OF OKRA (g/100 g DM)

Variety	*Age in days*	*Crude fibre*	*Crude protein*	*Ether extract*	*Ash*	*NFE*
UI 92	1	15·0	24·7	1·60	8·59	49·9
	4	20·1	22·0	1·69	8·88	47·4
	7	23·0	18·2	1·77	9·02	48·1
	10	23·2	18·6	1·86	9·19	47·2
	Mean	20·3	20·9	1·73	8·92	48·1
	SD	±3·8	±3·1	±0·11	±0·25	±1·3
UI 72-11	1	12·5	20·0	1·96	8·00	57·6
	4	15·6	23·8	2·12	8·46	50·0
	7	20·7	16·6	2·07	9·23	51·3
	10	23·6	14·4	2·11	9·11	50·8
	Mean	18·1	18·7	2·07	8·70	52·4
	SD	±5·0	±4·1	±0·07	±0·58	±3·5
UI 104	1	16·7	19·9	2·19	8·10	53·1
	4	17·9	19·5	2·25	8·25	52·2
	7	20·0	18·4	2·37	8·54	50·7
	10	21·1	18·8	2·37	8·72	49·1
	Mean	18·9	19·2	2·30	8·40	51·3
	SD	±2·0	±0·7	±0·09	±0·28	±1·7
UI 72-262	1	15·93	24·5	1·67	8·27	49·7
	4	16·56	16·1	1·78	8·45	57·1
	7	18·62	18·7	1·79	8·58	52·3
	10	20·74	17·06	1·82	8·66	51·7
	Mean	17·96	19·1	1·77	8·49	52·7
	SD	±2·18	±3·8	±0·07	±0·17	±3·1

SD = standard deviation.

advanced whilst crude fibre content increased. At day 1 after flowering, fibre content varied between 12 and 17% but rose to 21–23% by the tenth day. Ash and ether extract increased gradually with time for all varieties. Analysis of variance gave no indication of real differences in crude protein and crude fibre between the four varieties although values varied significantly with age.

Available and unavailable carbohydrates

Among the four varieties studied, average values for available carbohydrates for the different ages were 11·2 to 13·1 g/100 g DM and, for unavailable carbohydrates, 44·2 to 50·9 g/100 g DM (Table 2). Significant age differences were found in all carbohydrate fractions studied but overall varietal differences were apparent for the cell wall carbohydrates only. A significant age × variety interaction for all carbohydrates, however, suggests varietal differences for the different ages. This was similar to the crude protein and crude fibre of the okra varieties for the different harvesting periods.

Free sugars declined gradually with age for UI 92 and about 40% of the sugars disappeared by the tenth day. Starch content was least among the carbohydrates and starch synthesis reached a peak by the seventh day. Apart from a rise in concentration between the first and fourth days, subsequent increases were very slight.

The water-soluble fraction followed the same trend as the free sugars. The water-soluble carbohydrate content progressively decreased except in two varieties, UI 92 and UI 72-262, where there was a slight increase within the first four days, after which the values declined. Despite the fall in the concentration of free sugars and water-soluble carbohydrates with advancing age, hemicellulose, cellulose and lignin values increased. With the exception of the cellulose in UI 72-262 and hemicellulose of UI 104, cell wall carbohydrates in all varieties increased sharply from the first to fourth day and thereafter slowly in an almost linear fashion.

Results obtained to date from the four varieties suggest that the carbohydrates of the fibre fraction, with the exception of the water-soluble fraction, are synthesised rapidly between days 1 and 4 so that they accumulate steadily and probably reach a maximum between the seventh and tenth days. It is possible, however, that the free sugars are degraded by an enzyme system before polysaccharide synthesis. The water-soluble polysaccharides may have been converted to insoluble structural carbohydrates, i.e. hemicellulose and cellulose. As maturation proceeds, lignification increases. All these observations may explain the 'woody' nature (Van Epehuijsen, 1974) and hard texture of late harvested okra fruits. The individual sugars of okra have already been quantified (Longe, 1981) but nothing is known concerning the metabolism of these sugars. Metabolic processes affecting change in carbohydrate fractions merit examination. Further studies are also necessary in order to understand fruit development. Studies of other parts of the plant, apart from the fruit alone, may have to be undertaken in order to obtain such a

TABLE 2

EFFECT OF AGE AT HARVEST ON THE CARBOHYDRATE CONSTITUENTS OF FOUR VARIETIES OF OKRA (g/100 g DM)

Variety	*Age in days*	*Free sugars*	*Starch*	*Available carbohydrates*	*Water-soluble carbohydrate**	*Hemicellulose*	*Cellulose*	*Lignin*	*Unavailable carbohydrate*
UI 92	1	9·21	2·33	11·5	19·6	8·05	2·05	7·10	36·8
	4	8·76	3·29	12·1	18·7	18·3	8·84	8·20	54·0
	7	8·07	3·62	11·7	10·8	22·8	10·7	8·40	52·7
	10	5·58	3·83	9·41	7·46	21·2	14·0	10·4	53·0
	Mean	7·91	3·27	11·2	14·2	17·6	8·90	8·56	49·1
	SD	±1·61	±0·66	±1·19	±6·05	±6·62	±5·05	±1·37	±8·27
UI 72-11	1	11·2	2·58	13·8	15·6	6·22	6·06	6·00	33·9
	4	11·0	3·27	14·3	13·5	12·2	17·8	6·52	49·6
	7	8·70	3·50	12·2	16·3	13·1	19·5	6·81	55·6
	10	8·10	3·69	11·8	10·9	14·5	24·3	10·0	59·7
	Mean	9·76	3·26	13·0	14·1	11·5	16·9	7·33	49·7
	SD	±1·59	±0·48	±1·21	±2·44	±3·65	±7·75	±7·75	±11·3
UI 104	1	10·8	2·49	13·3	23·7	10·4	3·85	4·00	42·0
	4	9·96	3·18	13·1	19·7	9·59	4·19	5·10	38·6
	7	9·48	3·81	13·3	16·1	11·8	18·3	8·10	44·3
	10	8·82	3·95	12·8	14·2	16·3	12·8	8·62	51·9
	Mean	9·76	3·36	13·1	18·4	12·0	7·29	6·46	44·2
	SD	±0·82	±0·67	±0·24	±4·19	±2·97	±4·20	±2·26	±5·65
UI 72-262	1	11·2	2·37	13·5	14·3	11·7‴	4·56	9·50	40·1
	4	11·8	2·44	14·2	16·6	14·7	6·84	11·2	49·4
	7	8·88	2·72	11·6	11·5	16·0	12·6	15·5	55·6
	10	8·01	3·93	11·9	11·2	16·3	14·8	16·3	58·6
	Mean	9·86	2·87	12·8	13·4	14·7	9·70	13·1	50·9
	SD	±1·30	±0·73	±1·25	±2·57	±2·08	±4·80	±3·28	±8.15

SD = standard deviation.

* Water-soluble carbohydrate determined after removal of starch and ethanol soluble free sugars.

comprehensive view. Only four of the numerous varieties of okra in existence have been examined; caution should therefore be exercised in drawing a general conclusion.

REFERENCES

AOAC (1970). *Official Methods of Analysis* (11th edn.), Association of Official Analytical Chemists, Washington, DC.

DuBois, M., Gilles, K., Hamilton, J. K., Rebers, P. A. & Smith, F. (1956). Colorimetric method for determinations of sugars and related substances. *Anal. Chem.*, **280**, 350–6.

Fatokun, C. A., Aken'ova, M. E. & Chheda, H. R. (1978). Two new varieties of okra. *Niger J. Genet.*, **2**, 110–11.

Food and Agriculture Organization of the United Nations (FAO) (1968). *Food composition for use in Africa*, FAO, Rome, p. 113.

Longe, O. G. (1981). Effect of boiling on the carbohydrate constituents of some non-leafy vegetables. *Fd. Chem.*, **7**, 1–6.

Oyenuga, V. A. (1968). *Nigeria's foods and feeding-stuffs. Their chemistry and nutritive value* (3rd edn.), Ibadan University Press, Ibadan, Nigeria, 90.

Southgate, D. A. T. (1969). Determination of carbohydrates in foods. II. Unavailable carbohydrates. *J. Sci. Fd. Agric.*, **20**, 331–5.

Steel, R. G. D. & Torrie, J. H. (1960). *Principles and procedures of statistics*. McGraw-Hill Book Co. Inc., London.

Thivend, P., Mercier, C. & Guilbot, A. (1972). Determination of starch with glucoamylase. VII. General carbohydrate methods. In: *Methods in carbohydrate chemistry*. (Whistler, R. L. & BeMiller, J. N. (Eds)), Academic Press, London and New York.

Van Epenhuijsen, C. W. (1974). *Growing native vegetables in Nigeria*. FAO, Rome, 65–7.

Woolfe, J. (1977). The effect of okra mucilage (*Hibiscus esculentus*, L.) on the plasma cholesterol level in rats. *Proc. Nutr. Soc.*, **36**, 59A.

Food Chemistry 8 (1982) 33–41

STUDIES IN THE USE OF RECOMBINED MILK FOR THE MANUFACTURE OF RAS CHEESE

M. M. OMAR & M. M. ASHOUR

Department of Food Science, Faculty of Agriculture, University of Zagazig, Egypt

(Received: 1 October, 1980)

ABSTRACT

Whey was employed as a reconstituting medium for dried milk used for cheese making.

Ras cheese was made from fresh milk; whey was collected and dried skim milk was used to prepare a reconstituted milk with 20% total solids. Ras cheese was made from it and this process was repeated a further three times.

The addition of whey was beneficial in reducing, by 50%, the time necessary to raise the acidity of milk to make it suitable for rennet action. The time necessary to make it suitable for whey removal was also reduced by 50%. Consequently, the time required for pressing was only 8 h, instead of 16 h. Generally, the use of whey is considered to be a better process for Ras cheese making. In addition to the utilisation of whey, it produced a good and acceptable cheese. The cheese was manufactured within a shorter time than cheese made with fresh milk.

INTRODUCTION

Ras cheese is a local Egyptian hard cheese, usually made from cow's and buffalo's milk in a 1:1 ratio. The cheese is marketed after four months' ripening, when it has a flavour close to that of Kashkawal cheese (Abdel-Tawab, 1963). Imported powdered milk, which is available in the local market, is already used in some dairy products after recombination in water. The reconstitution of the powdered milk in whey is considered to be a new approach.

In this study it was planned to use the reconstituted milk in whey in cheese making. This may shorten the manufacturing time of Ras cheese by shortening the time required to make the acidity suitable for rennet action, reducing the time for raising the curd's acidity after cutting and the time consumed in pressing.

Food Chemistry 0308-8146/82/0008-0033/$02·75

MATERIALS AND METHODS

As a source of materials, fresh cow's and buffalo's milk was obtained from the herd of the Faculty of Agriculture, Zagazig University. Imported skin milk powder was purchased from a local market.

Ras cheese preparation

Ras cheese was prepared from a mixture of equal proportions of cow's and buffalo's milk according to the method of Hofi *et al.* (1973). Lactic acid starter (*Str. lactis*, 1 %) was added to the pasteurised milk at 35 °C, mixed well and left to develop up to 0·19 % acidity. The rennet was added in the proportion 100 ml per 100 kg of milk. After coagulation, the curd was cut vertically and horizontally with cheese knives and the temperature was raised to 45 °C in 15 min. Curd was held at this temperature for 50 min and whey was later drained to the level of the curd (acidity, 0·14 %). Salt was then added at a rate of 2 kg per 100 litres of milk used and the curd was cooled, moulded and pressed with 160 lb for the first 2 h. Pressing was continued overnight after increasing the weight to 1000 lb. The cheese mould was then dried and resalted by rubbing dry salt on both sides. The salting process lasted for 12 days and the cheese was waxed and ripened at a temperature of 10–14 °C and a relative humidity of 80–90 % for 4 months. Cheese whey was collected and dried skim milk was used to prepare a reconstituted milk of 20 % total solids; cream was added to give 5·1 % to 5·2 % fat in the reconstituted milk which was used in Ras cheese making by the same method.

This was repeated three times.

The final products were:

(1) Ras cheese made from fresh milk.
(2) Ras cheese made from reconstituted milk in whey obtained by means of step (1).
(3) Ras cheese made from reconstituted milk in whey collected from step (2).
(4) Ras cheese made from reconstituted milk in whey collected from step (3).

Milk and whey were analysed for pH, °SH, fat, total nitrogen and non-protein nitrogen according to the AOAC official methods of analysis (AOAC, 1970; Budstawski, 1963) and total nitrogen recovery during cheese making was calculated.

Cheese was analysed when fresh, and after 2 and 4 months for pH, moisture, fat, total nitrogen, soluble protein nitrogen, non-protein nitrogen and amino acid nitrogen (Budstawski, 1963; Pearson, 1973).

The bacterial total count, lipolytic bacteria and proteolytic bacteria were counted according to the APHA methods (APHA, 1960) and those of Sharf (1970). Organoleptic tests were conducted after 4 months according to the method of Bruncke (1968).

RESULTS AND DISCUSSION

Data presented in Table 1 show the analysis of milk and whey used in Ras cheese making.

The pH values range from 6·1 for the milk reconstituted in whey for the third time to 6·7 for the fresh milk. Acidity, expressed as °SH values, is high in the case of reconstituted milk and this is due to the continuous reconstitution of dried milk in whey which allowed the acidity to increase during reconstitution. The acidity was 5·8 °SH in the whey resulting from the fresh milk; it was increased to 17·6 °SH when dried milk was reconstituted in it. The whey resulting from the second step had an °SH of 12·6; this was increased to 22·9 when dried milk was reconstituted in it. At the second stage acidity was 16·9 °SH and increased up to 24·1 after reconstitution of milk in it. This increase was due to the addition of the dry milk components which were present in whey. It is well known that the natural acidity arises from milk components; with each reconstitution of dried milk in whey, acidity was increased.

TABLE 1
ANALYSIS OF MILK AND WHEY USED IN 'RAS' CHEESE MAKING

No.	*Treatment*	*Milk*				*Whey*			
		pH	*°SH*	*Fat (%)*	*Protein (TN × 6·38)*	*pH*	*°SH*	*Fat (%)*	*Protein (TN × 6·38)*
1	Fresh milk	6·7	7·9	2·5	3·04	5·3	5·8	0·32	0·74
2	Milk reconstituted in whey No. 1 + cream	6·5	17·6	5·1	5·18	5·1	12·6	0·42	1·05
3	Milk reconstituted in whey No. 2 + cream	6·3	22·9	5·2	5·30	5·0	16·9	0·29	1·14
4	Milk reconstituted in whey No. 3 + cream	6·1	24·1	5·1	5·25	4·9	19·8	0·33	1·15

Fat and protein content were greater in the case of reconstituted milk than fresh milk. This is due to the high percentage of total solids (20%).

Data presented in Table 2 show the nitrogenous constituents of milk and whey used in Ras cheese making. The protein nitrogen represents 94% of the total nitrogen in fresh milk but in its whey it represents 59·50% of total nitrogen. The total nitrogen recovery is 77·6%. In all cases of reconstituted milk, total nitrogen recovery was higher than its value in fresh milk. This is due to the utilisation of the nitrogen components which are present in whey. During the manufacture of cheese, it was clear that the time required to raise the acidity of milk to make it suitable for rennet action was shortened by half. Also, the pressing time was reduced. This was due to the acidity obtained from whey.

Chemical analysis

The chemical analysis of Ras cheese is shown in Table 3. The pH values increased with the progress of ripening. Cheese made from fresh milk had a pH value of

TABLE 2
TOTAL NITROGEN RECOVERY DURING 'RAS' CHEESE MAKING

No.	Treatment	Milk						Whey			Total nitrogen recovery (%)
		Total nitrogen (TN)	Non-protein nitrogen (NPN)	NPN TN (%)	Protein nitrogen (PN)	PN TN (%)	TN	NPN	NPN TN %	PN	PN TN %	
1	Fresh milk	0·476	0·025	5·25	0·451	94·8	0·116	0·047	40·5	0·069	59·5	77·6
2	Milk reconstituted in whey No. 1	0·812	0·061	7·51	0·751	92·5	0·165	0·067	40·6	0·098	59·4	79·7
3	Milk reconstituted in whey No. 2	0·831	0·056	6·74	0·775	93·3	0·178	0·058	32·6	0·120	67·4	78·6
4	Milk reconstituted in whey No. 3	0·823	0·052	6·32	0·771	93·7	0·180	0·063	35·0	0·117	65·0	78·1

TABLE 3
CHEMICAL ANALYSIS OF 'RAS' CHEESE

Age of cheese	Treatment No.	pH	Moisture (%)	Fat (%)	N, soluble TN (%)	Non-protein nitrogen TN (%)	Non-protein nitrogen N soluble (%)	Amino acid nitrogen TN (%)	Amino acid nitrogen N soluble (%)
Fresh	1	5·30	49·16	19·50	12·70	5·47	47·69	0·32	2·56
	2	5·20	44·21	18·50	13·29	5·63	42·38	0·51	3·81
	3	5·12	40·35	19·50	12·57	3·71	29·69	0·47	3·77
	4	5·06	39·40	20·00	12·53	6·01	47·95	0·33	2·65
2 months	1	5·42	42·40	18·60	15·29	7·57	49·48	3·09	20·21
	2	5·28	40·81	17·90	17·08	9·61	59·29	3·17	18·57
	3	5·20	38·81	18·40	15·18	7·36	47·79	3·20	21·08
	4	5·16	36·19	19·30	15·25	8·51	55·77	3·31	21·72
4 months	1	5·51	41·21	19·10	19·34	10·22	52·71	5·77	29·83
	2	5·39	39·18	18·30	20·03	13·94	69·67	4·17	20·85
	3	5·22	37·25	19·40	17·53	8·66	49·44	4·90	27·78
	4	5·18	36·19	19·90	18·93	12·13	63·90	5·41	28·90

5·30 when fresh which increased to 5·42 after 2 months, reaching 5·51 after 4 months.

In cheese made from reconstituted milk it was clear that the pH values were less than those of cheeses made from fresh milk. It was also clear that the pH values decreased with every progressive reconstitution step. The pH values increased gradually during ripening of all types of cheese (Foster *et al.*, 1962). Generally, moisture was less in the cheese made from reconstituted milk than that made from fresh milk. In addition, it decreased with the progress of ripening in all types of cheese. This is the same trend as observed with the pH values.

Fat content ranged from 17·9% to 20·0% in all cheeses made in the study.

Ripening indices

Protein degradation of cheese was studied in all cheeses, when fresh and after 2 and 4 months.

Soluble nitrogen of total nitrogen was increased with the progress of ripening; this may be due to the breakdown of nitrogen components of cheese which are soluble in water as a result of enzymatic and bacterial activity. (Ledford *et al.*, 1966; Ohmiya & Sabo, 1972). It is also clear from Fig. 1 that the cheese which was made of fresh milk

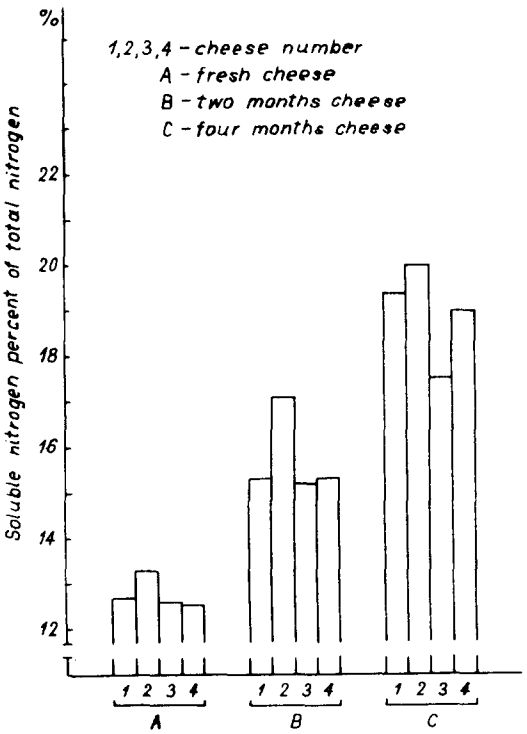


Fig. 1. Soluble nitrogen of Ras cheese during ripening.

started with 12·7% soluble nitrogen/total nitrogen (SN/TN) and ended at 19·9% after 4 months.

Cheese made from reconstituted milk in whey started with SN/TN values of 13·3, 12·6 and 12·5% and reached 20·0%, 17·5% and 18·9% after 4 months.

Non-protein nitrogen of total nitrogen (NPN/TN) values increased in all cheeses with the progress of the ripening period. In the meantime, non-protein nitrogen/soluble nitrogen values behaved in the same way (Fig. 2). Amino acid nitrogen values to total nitrogen and soluble nitrogen, in fresh cheese made of fresh milk, were less than the fresh cheese made of reconstituted milk. All values were increased with the progress of the ripening period (Fig. 2). Foster *et al.* (1967) reported that the extent of protein degradation could be obtained from measurements of amino nitrogen. The resulting values increase markedly with the liberation of free amino groups by hydrolysis to amino acids or simple peptides.

It was clear that the cheese made from reconstituted milk in the third whey had a lower moisture content, greater hardness, a lower pH value and a higher amino acid nitrogen content than cheese made from reconstituted milk in the first and second wheys. This was in agreement with the results of Stefanovic (1974) who reported

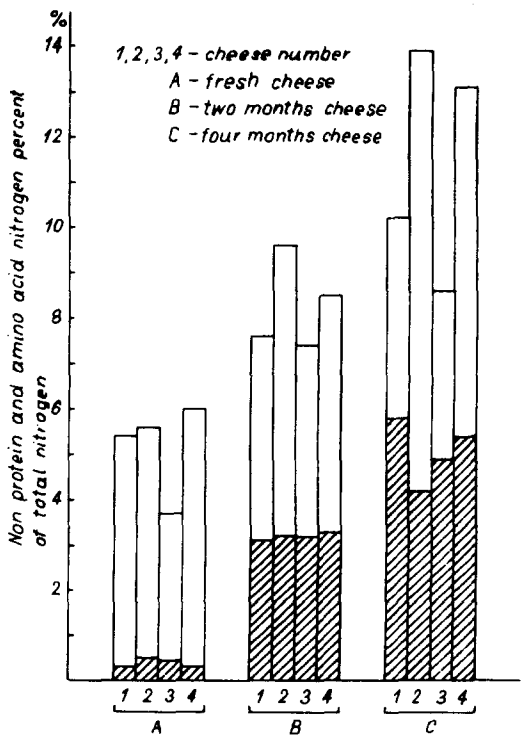


Fig. 2. Non-protein nitrogen □ and amino acid nitrogen ▨ content of Ras cheese during ripening.

that, during Kashkaval cheese making, cheese made from more acid curd was harder, with lower moisture content and more rapid proteolysis than cheeses made from less acid curd. These results are in agreement with those obtained by Hofi *et al.* (1973*a*,*b*; 1976).

Bacteriological analysis

Data presented in Table 4 show the bacteriological analysis of Ras cheese; it is clear that the total count of microorganisms in Ras cheese decreases with the progress of ripening. In addition, lactic acid bacteria show the same trend. Lipolytic and proteolytic bacteria increased with the progress of ripening. It is clear that the microorganisms undergo a regular succession during ripening. The lactic acid bacteria from the starter reach maximum numbers soon after the end of

TABLE 4
BACTERIOLOGICAL ANALYSIS OF 'RAS' CHEESE

No.	*Age of cheese*	*Total count (per gramme)*	*Lactic acid bacterial count (per gramme)*	*Lipolytic bacterial count (per gramme)*	*Proteolytic bacterial count (per gramme)*
1	Fresh	219×10^6	210×10^6	90×10^3	112×10^3
2		169×10^6	220×10^6	59×10^3	49×10^4
3		250×10^6	90×10^6	109×10^3	199×10^3
4		49×10^6	13×10^6	82×10^3	92×10^3
1	2	55×10^6	115×10^5	79×10^4	84×10^4
2	months	43×10^6	129×10^5	69×10^4	195×10^4
3		230×10^5	169×10^5	80×10^4	40×10^4
4		25×10^5	64×10^5	60×10^4	65×10^4
1	4	95×10^5	90×10^4	187×10^4	139×10^4
2	months	82×10^5	75×10^4	76×10^5	59×10^5
3		79×10^5	95×10^4	43×10^5	50×10^5
4		72×10^5	35×10^4	39×10^5	42×10^5

manufacturing. For the remainder of the ripening period their number decreases; fairly rapidly at first, then more slowly. The increase of proteolytic and lipolytic bacteria agrees with the observation made by Goranov (1972) who mentioned that, during the progressive period of ripening, proteolysis and lipolysis are always observed. These results are in agreement with those obtained by Glukhov (1965), Nassib (1974) and Youssef (1976).

Determinations of the organoleptic properties of Ras cheese were conducted after 4 months.

From Table 5 it is evident that the control cheese made from fresh milk is better than all those made from reconstituted milk in whey. This may be due to the slower degradation of the components in the cheese made of reconstituted milk. Such findings coincide with those reported by Czulak & Hammond (1974). Also, it was

TABLE 5
ORGANOLEPTIC SCORES OF 'RAS' CHEESE AFTER 4 MONTHS

Treatment No.	*Finish and appearance (20)*	*Body and texture (30)*			*Flavour (50)*		*Total (100)*
		Holes (5)	*Colour (10)*	*Consistency (15)*	*Aroma (25)*	*Taste (25)*	
1	20	5	10	15	21	24	95
2	20	5	10	12	21	21	89
3	20	5	10	10	19	20	84
4	20	5	10	10	18	19	82

clear that the scores obtained by cheese made from milk reconstituted in whey for the first time were higher than those made of milk reconstituted in whey for the second and the third times. There was no difference between the finish, appearance, holes and colour of all cheeses.

Differences are observed in the consistency, aroma and taste due to the lower pH values, lower acidity, lower moisture and lower protein degradation (Omar, 1977). The consistency of cheese made from reconstituted milk in whey for the second and third times was grainy; delay in the aroma was observed. Taste was not inferior but it was not comparable with that of the control cheese.

REFERENCES

ABDEL-TAWAB, G. (1963). *Manufacturing of Ras cheese from pasteurised milk.* MSc Thesis. Faculty of Agriculture, Ein Shams University, 1966.

AMERICAN PUBLIC HEALTH ASSOCIATION (APHA) (1960). *Standard methods for the examination of dairy products (Microbiological and chemical)* (11th edn.), New York.

AOAC (1970). *Official methods of analysis* (11th edn.), Association of Official Agricultural Chemists, Washington, DC, USA.

BRUNCKE, R. (1968). *Qualitats bewertung von wilch und milcherzeugnissen*, VER, Leipzig.

BUDSTAWSKI, J. (1963). *Chemia i analiza mleko oraz jego przetworow.* Pw. Ril-w-wa.

CZULAK, J. & HAMMOND, L. A. (1974). Manufacture of cheese from recombined milk. *19th International Dairy Congress, India*, IE. 781.

FOSTER, E. M., NELSON, F. E., SPECK, M. L., DOETSH, R. N. & OLSON, J. C. (1967). *Dairy microbiology*, Prentice-Hall, Inc., New Jersey.

GLUKHOW, G. (1965). Development of microflora in cheese from ewe's milk. *Mol. Prom.*, **26** (1), 17–19.

GORANOV, V. (1972). Method of manufacture and ripening of cows' milk Kashkaval cheese. 11. Microbiological changes, paracasein breakdown and accumulation of volatile fatty acids. *Zhivotnov dni Nauki*, **9**, 13. *C.F. Dairy Sci. Abstr.*, **35** (1462).

HOFI, A. A., MOHRAN, A. G., ABDEL SALAM, M. H. & RIFAAT, I. D. (1973*a*). Acceleration of cephalotyre 'Ras' cheese ripening by using trace elements. I. Pilot experiments. *Egyptian J. Dairy Sci.*, **1**, 33–44.

HOFI, A. A., MOHRAN, G. A., ABDEL SALAM, M. H. & RIFAAT, I. D. (1973*b*). Acceleration of cephalotyre 'Ras' cheese ripening by using trace elements. 11. Optimum conditions. *Egyptian J. Dairy Sci.*, (1), 45–55.

HOFI, A. A., EL-SAFTY, M. S., MAHRAN, G. A. & KHORSHID, M. A. (1976). Ripening changes in cephalotyre 'Ras' cheese as affected by microbial rennet. *Egyptian J. Dairy Sci.*, **4**(1), 63–70.

LEDFORD, R. A., O'SULLIVAN, A. C. & NATH, K. R. (1966). Residual casein fractions in ripened cheese determined by polyacrylamide gel electrophoresis. *J. Dairy Sci.*, **49**, 1098.

NASSIB, T. A. (1974). The effect of manufacturing processes on the microflora of Kashkaval cheese. *Assiut J. of Agric. Sci.*, **5**(1), 131–7.

OHMIYA, K. & SATO, Y. (1972). Studies on the proteolytic action of dairy lactic acid bacteria. XII. Significant contribution of intracellular protease of lactic acid bacteria to the casein hydrolysis in cheese ripening. *Milchwissenschaft*, **27**(7), 417–22.

OMAR, M. M. (1977). *Studies on the technology of whole dry milk manufacture and its suitability in producing cheese for tropical countries.* Ph.D. Thesis, ART, Olsztyn.

PEARSON, D. (Ed.) (1973). *Laboratory techniques in food analysis*, London, Butterworth.

SHARF, J. M. (1970). *Recommended methods for the microbiological examination of food* (2nd edn.), A.P.H.A. Inc., New York.

STEFANOVIC, R. (1974). Effect of curd acidity on the variation in soluble nitrogen and hardness during ripening of Kashkaval cheese. *XIX. Int. Dairy Congr.*, IE 726.

YOUSSEF, E. A. (1976). *Studies on hard cheese coating.* M.Sc. Thesis, Ain Shams University, Egypt.

Food Chemistry 8 (1982) 43–49

DETERMINATION OF β-SITOSTEROL IN MEATS, SOYA AND OTHER PROTEIN PRODUCTS

I. B. AGATER & J. W. LLEWELLYN

Laboratory of the Government Chemist, Cornwall House, Stamford Street, London SE1 9NQ, Great Britain

(Received: 7 October, 1980)

ABSTRACT

β-Sitosterol is shown to be present in a range of soya bean products, egg powder, several meats and wheatflour. Sterols were isolated and β-sitosterol in the resultant mixture was determined by GLC. Repeated analyses of individual samples displayed wide variance and the soya bean products themselves exhibited considerable interproduct variation in β-sitosterol content. A level of β-sitosterol similar to that found in soya products was found in wheatflour and somewhat lower levels in beef, pork, lamb and egg powder. The method cannot be used for the quantitative determination of soya in meat products but may be of some diagnostic value.

INTRODUCTION

The practice of including non-meat protein ingredients in meat products is now well established. These materials may be added for functional purposes or as meat substitutes and soya bean products have found increasing use in both these capacities.

The determination of non-meat protein in meat products poses a difficult analytical problem to which much effort has been devoted (Olsman & Krol, 1978; Llewellyn, 1979). As many of the analytical procedures currently in use are time-consuming and complicated, there is a need for a simple, robust method of determination, particularly for use with cooked products in which protein denaturation reduces solubility, thereby rendering the sample unamenable to many analytical techniques.

A straightforward method has been proposed by Lanzani *et al.* (1977) who demonstrated that sterols and triterpene alcohols which occur in soya bean oil were, surprisingly, still present in defatted soya flours and soya protein isolates.

Food Chemistry 0308-8146/82/0008-0043/$02·75

Hydrolysis of an ethanol extract of the soya product enabled determination of the sterols and triterpene alcohols by GLC after initial separation from non-saponifiable material by TLC. The same sterols and triterpene alcohols were shown to be present in meat and fish but at a very much lower concentration than in soya products, and thus the occurrence of relatively high levels of these compounds in a meat product was interpreted as evidence of the inclusion of soya bean ingredients. Quantitative determination of soya protein isolate in mixtures with meat and fish was possible even when the mixture had been cooked at 140 °C for 1 h. We have developed a method, based on that of Lanzani *et al.* (1977), and have examined several different meats and a range of soya protein products. We report our results below.

EXPERIMENTAL

Extraction and saponification of samples

Beef (shin), lamb (leg), chicken and cured ham obtained from a local butcher were deboned, hand minced, freeze dried overnight and defatted by Soxhlet extraction for 3 h in a mixture of chloroform and methanol, 2:1 (v/v). The residues were dried in air at room temperature and ground to fine powders in a domestic electric coffee grinder. Samples (3 g) of the freeze-dried and defatted meat powders and of soya bean products were extracted with hexane (100 ml) in a Soxhlet apparatus. The solvent was discarded, the residue allowed to dry in air and then re-extracted with aqueous ethanol solution (50 % v/v) for 2 h. The Soxhlet thimble was allowed to drain into the extraction flask and the extraction solution and drainings subsequently distilled under reflux for 1 h after the addition of potassium hydroxide solution (20 % w/v; 10 ml). On cooling to room temperature the solution was shaken twice with light petrolum (40–60 °C fraction, 50 ml); the aqueous fractions were discarded and the combined light petroleum layers washed twice with distilled water (100 ml). After desiccation by standing over anhydrous sodium sulphate, the solution was evaporated to dryness under reduced pressure at 30–40 °C.

Separation of the sterol fraction by thin layer chromatography (TLC)

The residue was dissolved in diethyl ether (1 ml) and applied as a narrow band to a 200 × 200 × 0·25 mm silica gel TLC plate (Merck; Kieselgel 60; glass backed). The plate was developed in a solution of diethyl ether and light petroleum (40–60 °C fraction; 3:2 v/v) and the bands were detected under ultraviolet light after spraying with a solution of 2,7-dichlorofluorescein in ethanol (0·2 % w/v). The band containing the sterol fraction, identified by comparison with β-sitosterol (Sigma) which was run on the same plate, was scraped off carefully into a beaker and 5-α-cholestan-3-one (Sigma) in chloroform solution (0·2 mg/ml; 200 μl) was added as an internal standard. The silica gel was suspended in a mixture of chloroform and methanol (7:3 v/v; 25 ml), filtered through Whatman No. 32 paper and washed with

chloroform: methanol (7:3 mixture; 25 ml). The combined filtrate and washings were evaporated to dryness under reduced pressure at 40 °C and the residue dissolved in diethyl ether (1 ml).

TLC plate recovery experiment

40, 60, 80, 100 and 200 μl aliquots of a solution of β-sitosterol in diethyl ether (1·02 μg/ml) were spotted onto a silica gel 200 × 200 × 0·25 mm plate which was developed in the manner described above. After detection, spots were removed from the plate, 5-α-cholestan-3-one internal standard solution (200 μl) was added to each and eluted as described above. The eluates were analysed by gas chromatography.

Measurement of β-sitosterol by gas chromatography

Gas chromatographic determinations were performed on a Pye 104 chromatograph, fitted with a 2 m × 2 mm inside diameter glass column packed with 3 % SE30 on Gas Chrom Q; column temperature was 250 °C. Components were detected by FID at 300 °C. 1–2 μl aliquots of both samples and standards were injected directly onto the column. The sterols were identified by comparison of retention time with authentic cholesterol (BDH), stigmasterol (Aldrich) and β-sitosterol (campesterol was not confirmed by this method), and the relative peak areas of 5-α-cholestan-3-one and β-sitosterol established by analysis of a standard solution containing 246 μg/ml β-sitosterol and 82 μg/ml 5-α-cholestan-3-one.

RESULTS

Typical gas chromatograms of an extract of a soya protein concentrate and of a similar extract from beef are shown in Figs 1 and 2, respectively. The absolute and relative retention times of the major sterols present are given in Table 1. The β-sitosterol contents, determined for a range of soya protein products, some meats and other protein sources, are presented in Table 2. The value quoted for each substance

TABLE 1
RETENTION TIMES OF STEROLS

	Retention time (mins)	*Relative retention time*
Cholesterol	25·2	0·9
5-α-cholestan-3-one (standard)	27·4	1·0
Stigmasterol	32·6	1·2
Campesterol	35·4	1·3
β-Sitosterol	40·8	1·5

Repeatability of GLC determinations was determined to be ±3·5 %.
The average recovery of β-sitosterol from the preparative TLC plate was 95 % (standard deviation, 5·5 %, coefficient of variation, 5·7 %).

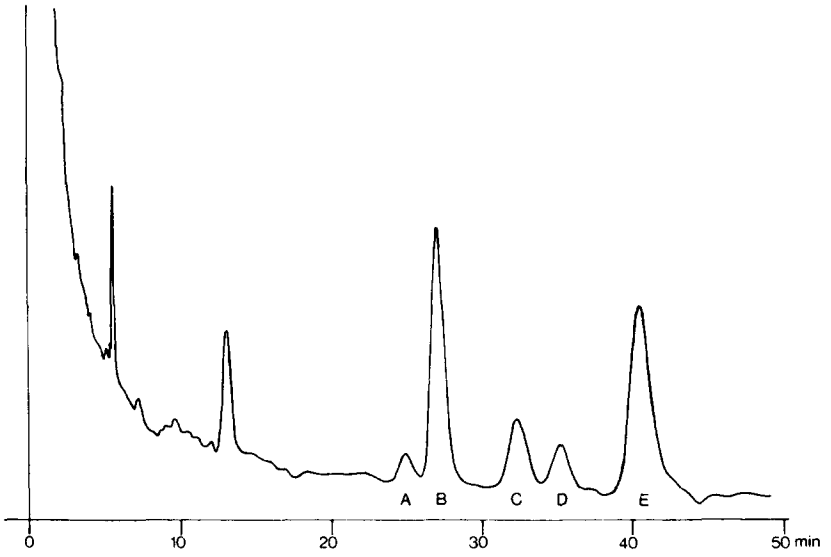


Fig. 1. Gas chromatogram of sterol fraction extracted from a soya protein concentrate. A = cholesterol. B = 5-α-cholestan-3-one (internal standard). C = stigmasterol. D = campesterol. E = β-sitosterol.

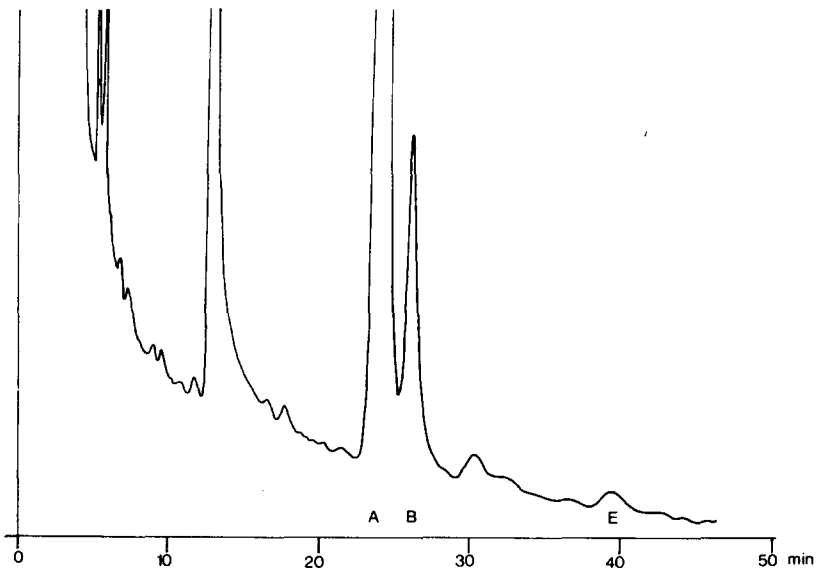


Fig. 2. Gas chromatogram of sterol fraction extracted from beef. A = cholesterol. B = 5-α-cholestan-3-one (internal standard). E = β-sitosterol.

TABLE 2
β-SITOSTEROL CONTENT OF VARIOUS SOYA PROTEIN PRODUCTS, MEATS AND OTHER PROTEIN SOURCES

Sample No.	*Type*	*β-sitosterol μg/g**	*σ*	*Coefficient of variation (%)*	*Number of detns.*
1	Soya protein isolate	7	2·3	33	5
2	Soya protein isolate	10	3·4	39	5
3	Soya protein isolate	9	2·8	34	7
4	Soya protein isolate	8			
5	Soya protein isolate	12			
6	Enzyme hydrolysed soya isolate	—			
7	Soya protein concentrate	4			
8	Soya protein concentrate	19			
9	Soya protein concentrate	8	3·0	35	4
10	Soya protein concentrate	18	3·3	18	6
11	Soya protein concentrate	26			
12	Soya flour	3			
13	Soya flour	9			
14	Soya flour	2	0·6	30	4
15	Soya flour	21			
16	Soya flour	5			
17	Textured soya flour	7			
18	Extracted soya flour	21			
19	Extracted soya flour	11			
20	Extracted soya flour	26			
21	Extracted soya flour	18			
22	Extracted soya flour	27			
23	Spun soya flour	13			
24	Beef	0·3			
25	Lamb	1·9			
26	Chicken	3·6			
27	Ham (pork)	1·0			
28	Wheatflour	10			
29	Egg powder	1·6			

* Mean of three determinations.

is the mean of three determinations, except where indicated, and the standard deviation and coefficient of variation have been calculated for several soya protein products.

DISCUSSION

Lanzani *et al.* (1977) have reported the total sterol content of soya protein isolate to be 0·001 % (10 μg/g sample) and of soya meals to be 0·011 % (110 μg/g sample). The main component of the mixture was β-sitosterol (4·8 μg/g sample in isolates, 55 μg/g in meals) and lesser quantities of cholesterol, campesterol and stigmasterol were also noted. The triterpene alcohol contents were lower (5 μg/g in isolates and 10 μg/g in meals) and cycloartenol was found to be the major constituent (2·9 μg/g in isolate,

6·2 μg/g in meal) and accordingly they therefore investigated the use of both β-sitosterol and cycloartenol as potential indicators of the amount of soya bean product in a meat product.

We have restricted our studies to the investigation of β-sitosterol in various soya products as it was the predominant component of the sterol and triterpene alcohol mixture.

Our experimental procedure was based on that of Lanzani *et al.* (1977). The sterols were isolated (after preliminary hexane extraction to remove any 'free' sterols) by saponification of an ethanol extract and separation from non-saponifiable material and triterpene alcohols by preparative TLC. β-sitosterol was detected in all soya products except an enzyme hydrolysed isolate. Cholesterol, campesterol and stigmasterol were also detected, although the relative proportions varied from product to product. Examination of Table 2 shows that the β-sitosterol content varied considerably from product to product and that, whereas the average content in isolates was approximately twice that noted by Lanzani *et al.*, the average content in flours (including extruded and spun forms) was less than half of their value. Contents ranged from 2 μg/g in soya flour 14 to 27 μg/g in extruded soya flour 22.

We were able to confirm Lanzani *et al.'s* observation of cholesterol, campesterol, stigmasterol and β-sitosterol in several meat extracts although identification of campesterol and stigmasterol in beef was tentative. They report a β-sitosterol content in meat of approximately 2×10^{-3} μg/g but in this study much higher contents were found, ranging from 0·3 μg/g in beef to 3·6 μg/g in chicken. Furthermore, β-sitosterol was also detected in an extract of wheat flour at a level similar to that found in the soya products, and also in egg powder at a concentration within the range found in the meats.

Repeated analyses of the same samples of three protein isolates, two concentrates and one flour indicated that the method lacked precision as coefficients of variation of the order of 35 % were noted. As recovery of β-sitosterol in a standard mixture of sterols from the TLC plate was nearly complete and of low variance, the saponification/extraction stage must give rise to the high variability, although the reason for this is not clear.

More serious than the lack of precision is the variation of β-sitosterol content from product to product. As can be seen from Table 2, this is considerable, and it is possible that the variation in sterol content of soya products reflects differences in defatting and other production processes. The variability of β-sitosterol content thus precluded its use as an indicator for quantitative analysis of soya products. β-sitosterol is not a specific indicator of soya products as substantial quantities were present in meats and other common protein foods which might well be included in a meat product. Hence, β-sitosterol content can only be used as a qualitative indicator of the possible inclusion of soya products in a meat product.

Even when considering the method as a diagnostic tool, it is difficult to assign a

level above which the presence of soya could be positively ascertained as this largely depends upon the meat species involved. An overall level of more than 4 μg/g of β-sitosterol could be interpreted either as evidence of the inclusion of a relatively high proportion (30–40 %) of a soya product with low β-sitosterol content in a pork or lamb product, or a lower content (10–20 %) of a product such as an extruded soya flour with higher β-sitosterol content. For beef products a lower overall level (1 μg/g) might indicate the inclusion of soya, but, conversely, for chicken products a much higher level (6 μg/g) would be more appropriate. Again, quantitation would be impossible. A low level of β-sitosterol cannot be interpreted as a low level of inclusion of soya products.

The method is therefore not a reliable screening test for low levels of soya in meat products. Although the final GLC analysis is fairly rapid, the preceding stages of hexane and ethanol extraction, saponification and TLC separation are slow and thus the overall procedure is relatively time-consuming. In laboratories which lack the sophisticated equipment required by some other techniques, the detection of high levels of β-sitosterol in a meat product could furnish some supportive evidence of the inclusion of relatively large amounts of soya products.

ACKNOWLEDGEMENT

The authors wish to thank the Government Chemist for permission to publish this paper.

REFERENCES

LANZANI, A., CAROLA, C., JACINI, G. & CARDILLO, M. (1977). Minor lipidic constituents typical of certain seed meals and of their proteic derivatives. *Riv. Ital. del. Sos. Grasse.*, **54**, 448–50.

LLEWELLYN, J. W. (1979). Determination of non-meat proteins in meat products. *International Flavours and Food Additives*, **10**, 115, 117–18, 128.

OLSMAN, W. J. & KROL, B. (1978). Appendix VII. Methods for detection and determination of vegetable proteins in meat products. *Report of the Study Group on Vegetable Protein in Foodstuffs for Human Consumption, in particular in Meat Products EUR. 6026.* Commission of the European Communities, Luxembourg, 117–45.

Food Chemistry 8 (1982) 51–60

DISTRIBUTION OF HYDROCARBONS AND FATTY ACIDS IN MEATS IMPORTED INTO ITALY

M. P. BERNARDINI, L. BONIFORTI, G. CITTI & V. MOSINI

Laboratorio di Tossicologia, Istituto Superiore di Sanità, Viale Regina Elena, 299 00161 Rome, Italy

(Received: 11 November, 1980)

ABSTRACT

Samples of the pork, veal and chicken produce imported into Italy from other EEC countries were taken and analysed to ascertain saturated and unsaturated hydrocarbon levels.

Gas chromatography–mass spectrometry analysis showed overall n-*alkane levels to be in the range of 0·3 to 10·5 ppm.* N-*alkanes ranged from* C_{12} *to* C_{33}. *Phytenes were only found in bovine tissue. No alkenes were present in any of the samples analysed.*

INTRODUCTION

Small amounts of paraffinic hydrocarbons in human and animal tissue have been reported by several authors (Nicholas & Bombaugh, 1965; Tagliamonte & Tomassi, 1976; Bandurski & Nagy, 1975; Lintas *et al.*, 1979). Normal biosynthesis processes are only thought to be limitedly responsible for the presence of paraffinic hydrocarbons in man. Environmental pollution, which accounts for their presence in human and animal food chains, is considered to be the main source.

Furthermore, it has recently been shown that single-cell protein feeds lead to an accumulation of *n*-paraffins in swine adipose tissue and various organs (Di Muccio *et al.*, 1979; Tagliamonte & Tomassi, 1976; Bingham & Falk, 1969; Boniforti *et al.*, 1979; Alimenti *et al.*, 1979).

From a biological and toxicological standpoint, paraffinic hydrocarbons, particularly those in the C_{18}–C_{30} range, are regarded with suspicion because of the potential carcinogenic and cocarcinogenic activity they exhibit (Bingham & Falk, 1969).

Food Chemistry 0308-8146/82/0008-0051/$02·75

In collaboration with Italy's Ministry of Health, the investigation described in this paper was undertaken to evaluate paraffinic hydrocarbon levels in meat imported from other EEC countries (France, Belgium and The Netherlands).

MATERIALS AND METHOD

Specimens of adipose tissue (subcutaneous, perirenal and muscular) taken from bovine, pork and chicken samples seized at Italian frontier checkpoints from stocks of fresh or frozen edible meats originating in Belgium, France and The Netherlands were examined (see Table 1).

TABLE 1
SAMPLE TYPES, ORIGIN AND IDENTIFICATION

	Sample	*Origin*
Bovine muscular tissue	A	France
	B	France
	C	France
	D	The Netherlands
	E	The Netherlands
	F	France
	G	France
	H	France
	C (+)	France
	F (+)	France
Swine muscular tissue	I	Belgium
	L	France
	M	France
	N	Belgium
	O	The Netherlands
	P	France
	I (+)	Belgium
	N (+)	Belgium
Swine gc = subcutaneous tissue gp = perirenal tissue	L_{gc}	France
	M_{gc}	France
	N_{gc}	Belgium
	N_{gp}	Belgium
	O_{gc}	The Netherlands
	P_{gc}	France
	P_{gc} (+)	France
	P_{gp}	France
Bovine	A_{gc}	France
	E_{gc}	The Netherlands
	E_{gp}	The Netherlands
Chicken	Q	France
	R	France
	S	France
	T	France
	Q (+)	France
	Q (+ +)	France
	S (+)	France
	T (+)	France

(+) (+ +): Different samples from same place.

Lipid extraction and fatty acid identification

A minced, homogenised sample (5 g) was extracted with a chloroform/methanol solution (2:1 v/v) according to the Folch *et al.* (1957) procedure.

After evaporation of the organic phase in a rotary evaporator, the residue was dissolved in *n*-hexane and the resulting solution filtered. After removal of the solvent, the residue was oven-dried to constant weight at 80 °C.

Fatty acids were converted to their methyl esters and then determined by GLC, as reported previously (Bernardini *et al.*, 1975). All samples were analysed either in a 15 % DEGS column or in a 15 % NPGS column (Bernardini *et al.*, 1975) in order to detect the possible presence of heptadecadienoic acid ($C_{17:2}$).

Hydrocarbon fraction extraction

The minced, homogenised sample (50 g) was refluxed for 2 h in 100 ml of 2N KOH ethanol/water solution (9:1) (Di Muccio *et al.*, 1979). The warm solution was transferred to a separating funnel and the refluxing vessel sequentially washed with small quantities of water, with 30 ml of *n*-pentane to extract hydrocarbons. These three aliquots were combined, washed with water and eluted with *n*-pentane through a 20 × 200 × 30 mm column containing silica gel and surmounted by a layer of anhydrous sodium sulphate (80 mm). The eluate was concentrated to about 1 ml under reduced pressure at approximately 35 °C. The eluate was then applied as a thin band of spots on a 20 × 20 cm chromatographic plate coated with a 0·5 mm layer of silica gel (Merck 60 F_{254}) previously activated for 1 h at 105 °C. Hexadecane was spot-added to the sample and used as a reference. The plate was developed with *n*-pentane for a 14-cm run and then sprayed with a 0·2 % solution of 2',7'-dichlorofluorescein in ethanol. The band with R_f corresponding to *n*-C_{16} was scraped from the plate, transferred to a small glass column and eluted with 15 ml of an *n*-pentane/ether solution (1:1 v/v). One millilitre of isooctane was added to the eluate to prevent the sample from drying completely. The solution was then concentrated to about 1 ml in a reduced pressure evaporator.

Hydrocarbon assays

The solution investigated was subjected to GC analysis using both packed and open tubular columns. A packed 3 m × 3 mm (inside diameter) glass coil column was used with a gas chromatograph (Carlo Erba Model 2200) equipped with a hydrogen flame-ionisation detector. Column packing was 5 % OV-61 on 80-100 mesh Chromosorb W HP. The column was programmed from 100 to 250 °C at 6 °C/min and operated with a nitrogen carrier gas flow rate of 30 ml/min. The injector operated at 250 °C.

Open tubular column analysis was performed using a gas chromatograph (Perkin Elmer Model 226) equipped with a hydrogen flame ionisation detector and a stainless steel 90 m × 0·25 mm (inside diameter) open tubular column coated with Apiezon L. The column was operated isothermally at 175 °C and with a helium

carrier gas flow rate of 2 ml/min. Injection port temperature was 280 °C and splitter ratio at the entrance, 1:50.

Hydrocarbons were detected by comparison with a standard mixture containing a homologous series of even number carbon atom *n*-alkanes (hereinafter referred to as enca *n*-alcanes) from C_{12} to C_{32}. Quantitative determination was performed by adding a known amount of *n*-alkane which was not present in the gas chromatogram of the sample being examined. Hydrocarbon identity was confirmed using a GC–MS system (LKB Model 9000 S MS) with ionising energy set at 20 eV. The GC column was a 2 m × 3·5 mm (inside diameter) glass coil packed with Chromosorb W HP (80–100 mesh) at 5% OV-61 programmed from 100 to 250 °C at 4 °C/min. Helium carrier gas flow rate was 20 ml/min.

Solvents were frequently checked to ensure the absence of contaminating hydrocarbons.

RESULTS AND DISCUSSION

Tables 2, 3 and 4 show the percentage of fatty acids in muscular, subcutaneous and perirenal tissue from the eight calves and eight pigs examined. Adipose tissue samples were not always available and consequently the relative data are missing from the Tables.

Table 5 shows the percentage of fatty acids in the eight samples of chicken examined. In all the samples oleic acid ($C_{18:1}$) is the most abundant. Palmitic ($C_{16:0}$) and stearic ($C_{18:0}$) acids prevail among saturated acids. Linoleic acid ($C_{18:2}$) is also

TABLE 2
TOTAL FATTY ACIDS FOUND IN BOVINE MUSCULAR TISSUE*

	A	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*C* (+)	*F* (+)
Lipids %	26	10	20	6	24	15	36	48	26	14
Fatty acids										
$C_{12:0}$	tr	tr	tr	tr	tr	tr	0·8	0·8	0·4	0·5
$C_{14:0}$	4·7	3·2	4·2	5·0	4·2	1·6	5·8	10·5	5·4	6·3
$C_{16:0}$	26·0	23·4	23·3	21·3	21·0	24·5	21·9	24·4	24·2	25·4
$C_{16:1}$	3·9	4·6	5·5	5·4	3·9	2·6	3·9	6·2	5·8	4·9
$C_{17:0}$	1·3	1·5	1·0	0·9	0·4	0·5	1·0	0·9	1·1	1·0
$C_{17:1}$	1·2	1·4	0·9	1·2	0·8	0·4	0·7	0·9	1·0	1·0
$C_{17:2}$	—	—	—	—	—	—	—	—	—	—
$C_{18:0}$	20·1	21·2	14·9	15·2	15·3	16·8	19·4	11·7	14·1	12·0
$C_{18:1}$	34·9	35·9	45·1	43·8	42·5	36·3	39·2	39·3	42·8	42·2
$C_{18:2}$	5·4	5·6	3·4	2·9	10·8	13·9	4·5	3·8	3·3	5·2
$C_{18:3}$	1·3	1·7	1·0	1·1	0·5	1·1	0·8	0·9	0·9	0·5
$C_{20:0}$	tr	1·0	0·3	0·3	0·4	1·2	0·4	0·3	0·4	0·4
Total onca fatty acids	2·5	2·9	1·9	2·1	1·2	0·9	1·7	1·8	2·1	2·0

(+): Different samples from same place.
* Per cent of total fatty acids. Each value is the mean of at least two assays.
onca: odd number carbon atom.

TABLE 3
TOTAL FATTY ACIDS FOUND IN SWINE MUSCULAR TISSUE*

	I	*L*	*M*	*N*	*O*	*P*	*I* (+)	*N* (+)
Lipids %	35	12	9	5	23	5	18	16
Fatty acids								
$C_{14:0}$	1·0	1·4	1·3	1·7	1·3	1·4	1·3	1·5
$C_{16:0}$	23·9	26·8	24·9	22·4	25·1	25·9	22·8	25·3
$C_{16:1}$	2·7	3·3	3·2	3·0	2·0	2·4	2·6	2·4
$C_{17:0}$	0·2	0·3	0·4	0·3	0·6	0·2	0·4	0·4
$C_{17:1}$	0·4	0·4	0·6	0·3	0·3	0·7	0·3	0·4
$C_{17:2}$	—	—	—	—	—	—	—	—
$C_{18:0}$	9·1	16·2	14·0	14·7	17·2	16·4	15·5	15·8
$C_{18:1}$	41·1	52·2	43·4	45·2	42·9	39·1	43·1	43·0
$C_{18:2}$	20·4	9·3	10·7	10·2	8·0	12·2	11·4	9·8
$C_{18:3}$	0·3	0·4	0·8	0·9	0·8	0·3	0·9	0·9
$C_{20:0}$	0·8	0·2	0·3	0·7	1·3	0·8	1·0	1·2
Total onca fatty acids	0·6	0·7	1·0	0·6	0·9	0·9	0·7	0·8

(+): Different samples from same place.
* Per cent of total fatty acids. Each value is the mean of at least two assays.
onca: odd number carbon atom.

present in appreciable quantities. Heptadecadienoic acid ($C_{17:2}$), the presence of which may be an indicator of single-cell protein assimilation (Boniforti *et al.*, 1979; Alimenti *et al.*, 1979), was never found in our samples. The total percentage of odd number carbon atom fatty acids (hereinafter referred to as onca fatty acids) is less than 5% of total fatty acids and therefore agrees with the data in the available literature (Alimenti *et al.*, 1979).

TABLE 4
TOTAL FATTY ACIDS FOUND IN BOVINE AND SWINE SUBCUTANEOUS AND PERIRENAL ADIPOSE TISSUE*

Fatty acids	L_{gc}	M_{gc}	N_{gc}	N_{gp}	O_{gc}	P_{gc}	P_{gc} (+)	P_{gp}	A_{gc}	E_{gc}	E_{gp}
$C_{12:0}$	tr	tr	tr	tr	tr	tr	tr	tr	tr	1·6	1·6
$C_{14:0}$	1·1	1·2	1·2	1·5	1·4	1·2	1·4	1·5	1·3	8·9	8·2
$C_{16:0}$	25·4	22·8	23·3	27·1	25·3	23·0	21·9	27·0	25·6	26·3	24·1
$C_{16:1}$	2·7	3·2	2·4	3·8	2·5	2·3	3·1	2·8	2·3	4·4	3·6
$C_{17:0}$	0·2	0·2	0·3	2·3	0·5	0·4	0·4	0·4	0·3	0·6	0·8
$C_{17:1}$	0·1	0·2	0·3	1·2	0·4	0·3	0·3	0·3	0·3	0·5	0·5
$C_{17:2}$	—	—	—	—	—	—	—	—	—	—	—
$C_{18:0}$	14·8	14·2	14·6	17·7	14·8	14·2	17·1	17·0	16·1	11·4	14·7
$C_{18:1}$	42·8	39·9	44·9	36·6	43·9	45·4	43·2	36·5	32·8	37·7	37·5
$C_{18:2}$	9·8	10·1	10·0	7·9	8·7	10·1	11·8	12·1	11·9	5·9	6·4
$C_{18:3}$	0·8	0·6	0·9	0·8	0·7	0·8	0·6	0·9	0·7	0·5	0·6
$C_{20:0}$	0·9	1·1	1·3	0·6	1·1	1·3	1·1	0·8	0·2	0·5	0·6
Total onca fatty acids	0·3	0·4	0·6	3·5	0·9	0·7	0·7	0·7	0·6	1·1	1·3

(+): Different samples from same place.
* Per cent of total fatty acids. Each value is the mean of at least two assays.
onca: odd number carbon atom.
tr = trace.

TABLE 5

TOTAL FATTY ACIDS FOUND IN CHICKEN TISSUE*

	Q	*R*	*S*	*T*	*Q* (+)	*Q* (++)	*S* (+)	*T* (+)
Lipids %	10	4	11	4·5	7	3	6	—
Fatty acids								
$C_{12:0}$	tr	tr	tr	tr	tr	0·2	tr	tr
$C_{14:0}$	1·3	0·2	0·8	0·2	0·3	2·1	1·0	1·6
$C_{16:0}$	19·7	22·9	21·8	22·3	22·9	21·6	22·1	23·2
$C_{16:1}$	3·9	5·3	3·7	5·8	4·6	3·5	4·2	3·7
$C_{17:0}$	0·4	0·4	0·4	0·7	0·3	0·5	0·2	0·3
$C_{17:1}$	0·5	0·5	1·1	0·5	0·4	0·3	0·5	0·4
$C_{17:2}$	—	—	—	—	—	—	—	—
$C_{18:0}$	11·8	8·6	11·7	8·6	10·6	11·9	10·9	11·5
$C_{18:1}$	37·4	40·9	32·4	38·8	37·0	38·1	33·8	47·1
$C_{18:2}$	22·7	18·6	20·5	19·9	21·9	18·8	21·8	9·4
$C_{18:3}$	0·6	0·9	0·4	0·9	0·3	1·8	1·0	1·0
$C_{20:0}$	0·1	0·7	0·4	0·3	0·8	0·6	0·4	1·0
Total onca fatty acids	0·9	0·9	1·5	1·2	0·7	0·8	0·7	0·7

(+) (++): Different samples from same place.
* Per cent of total fatty acids. Each value is the mean of at least two assays.
onca: odd number carbon atom.
tr = trace.

TABLE 6

HYDROCARBONS PRESENT IN BOVINE MUSCULAR TISSUE*

	A	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*C* (+)	*F* (+)
Hydrocarbons										
C_{12}	0·09	0·57	0·07	0·10	0·07	0·13	0·54	0·28	0·20	0·07
C_{13}	0·01	0·04	0·03	tr	tr	0·02	0·06	0·04	tr	0·03
C_{14}	0·25	1·21	0·23	0·27	0·16	0·38	1·82	0·85	0·50	0·26
C_{15}	0·02	0·05	0·04	0·04	0·02	0·04	0·10	0·06	0·02	0·04
C_{16}	0·20	0·82	0·19	0·24	0·07	0·22	1·20	0·71	0·22	0·16
C_{17}	0·06	0·09	0·15	0·09	tr	0·05	0·08	0·12	tr	0·05
C_{18}	0·06	0·41	0·15	0·06	0·02	0·08	0·55	0·41	0·06	0·04
C_{19}	0·08	tr	0·05	tr	—	0·02	0·04	0·04	tr	0·03
C_{20}	tr	0·24	tr	tr	—	0·08	0·19	0·14	tr	tr
C_{21}	tr	tr	0·06	0·01	—	0·01	—	—	—	tr
C_{22}	0·10	tr	0·11	0·04	—	tr	0·07	0·10	tr	0·02
C_{23}	0·02	—	0·05	—	—	0·02	—	—	—	0·03
C_{24}	0·02	tr	0·06	—	—	0·02	tr	tr	—	0·03
C_{25}	0·03	0·06	0·05	—	—	0·03	—	—	—	0·02
C_{26}	0·01	—	0·03	—	—	0·02	—	—	—	0·02
C_{27}	0·04	0·07	0·04	—	—	0·02	—	—	—	0·02
C_{28}	—	—	0·03	—	—	0·1	—	—	—	0·01
C_{29}	0·15	0·10	0·05	—	—	0·01	—	—	—	0·02
C_{30}	—	—	0·03	—	—	—	—	—	—	—
C_{31}	0·06	—	—	—	—	—	—	—	—	—
C_{32}	—	—	—	—	—	—	—	—	—	—
C_{33}	0·50	—	—	—	—	—	—	—	—	—
Total	1·70	3·66	1·42	0·85	0·34	1·16	4·65	2·75	1·00	0·85
phyt-1-ene	0·03	0·26	0·15	—	0·05	tr	tr	0·07	—	tr
phytane	0·03	0·19	tr	—	—	—	tr	—	—	—
phyt-2-ene	0·20	0·41	0·20	—	—	tr	0·04	0·03	—	tr

(+): Different samples from same place.
* Values expressed in ppm (mg/kg). Each value is the mean of at least two assays.
tr = trace.

Table 6 shows hydrocarbon distribution in the muscular tissue of the calves examined. Although hydrocarbon composition in these samples is neither qualitatively nor quantitatively the same, linear enca hydrocarbons in the C_{12}–C_{20} range prevail over onca *n*-alkanes, of which *n*-tetradecane is the most abundant. The following branched hydrocarbons were occasionally identified: 3,7,11,15-tetramethylhexadec-1-ene (phyt-1-ene), 3,7,11,15-tetramethylhexadec-2-ene (phyt-2-ene) and 3,7,11,15-tetramethylhexadecane (phytane), of which phyt-2-ene prevailed. Octadecane was separated from phytane in an open tubular column, coated with Apiezon L because both components exhibited the same retention times on packed columns. Both enca (even number carbon atom) and onca *n*-alkenes up to C_{33} were sometimes identified. Data obtained with the open tubular column show that small quantities of *n*-alkenes are present with their corresponding, more abundant, alkanes; hydrocarbon concentration in bovine muscular tissue was in the range of 0·34–4·65 ppm.

As regards hydrocarbon distribution in pork muscle (Table 7), onca and enca *n*-alkanes from C_{12} to C_{33} were found, low number enca components being the most abundant. Quantities ranged from 0·41 to 4·05 ppm. Open tubular column analysis showed that *n*-alkenes were either absent or present at trace level quantities.

TABLE 7
HYDROCARBONS FOUND IN SWINE MUSCULAR TISSUE*

Hydrocarbons	*I*	*L*	*M*	*N*	*O*	*P*	*I* (+)	*N* (+)
C_{12}	0·06	0·65	0·10	0·09	0·03	0·10	0·05	0·07
C_{13}	0·02	0·02	0·02	tr	tr	tr	tr	tr
C_{14}	0·21	1·48	0·26	0·17	0·25	0·28	0·15	0·17
C_{15}	0·02	0·10	0·03	0·02	0·02	0·02	0·02	0·04
C_{16}	0·11	0·91	0·13	0·15	0·02	0·14	0·10	0·29
C_{17}	0·08	0·16	0·05	0·01	0·31	tr	0·04	0·05
C_{18}	0·07	0·55	0·05	0·05	0·06	0·04	0·03	0·08
C_{19}	0·02	tr	tr	tr	0·21	tr	0·01	0·02
C_{20}	tr	0·18	tr	0·01	0·02	tr	tr	0·05
C_{21}	0·05	tr	0·02	0·02	0·10	tr	0·01	0·02
C_{22}	0·07	tr	0·01	0·01	—	—	—	0·09
C_{23}	0·12	—	0·02	0·04	—	—	—	0·01
C_{24}	0·16	—	0·01	0·05	0·08	—	—	0·01
C_{25}	0·19	—	0·02	0·06	0·02	—	—	0·02
C_{26}	0·21	—	0·02	0·09	—	—	—	—
C_{27}	0·21	—	0·02	0·01	0·02	—	—	—
C_{28}	0·18	—	0·02	0·02	0·02	—	—	—
C_{29}	0·18	—	—	0·02	0·02	—	—	—
C_{30}	0·14	—	—	—	—	—	—	—
C_{31}	0·12	—	—	—	0·02	—	—	—
C_{32}	0·09	—	—	—	—	—	—	—
C_{33}	0·06	—	—	—	—	—	—	—
Total	2·37	4·05	0·78	0·82	1·20	0·58	0·41	0·92

(+): Different samples from same place.
* Values expressed in ppm (mg/kg). Each value is the mean of at least two assays.
tr = trace.

Table 8 shows hydrocarbon distribution in the adipose tissue of some of the animals examined. All *n*-alkanes in the C_{12}–C_{31} range were present, the distribution being more homogeneous in the subcutaneous and perirenal adipose tissue of pork; again, low number enca components being the most abundant. Phytenes were present only in bovine fat. Hydrocarbon concentration in the adipose tissue ranged from 0·78 to 6·63 ppm for pork tissue and from 4·46 to 10·54 for bovine tissue.

TABLE 8

HYDROCARBONS FOUND IN SWINE AND BOVINE SUBCUTANEOUS AND PERIRENAL ADIPOSE TISSUE*

	L_{gc}	M_{gc}	N_{gc}	N_{gp}	O_{gc}	P_{gc}	P_{gc} (+)	P_{gp}	A_{gc}	E_{gc}	E_{gp}
Hydrocarbons											
C_{12}	0·45	0·20	0·12	0·17	0·95	0·10	0·09	0·14	0·71	0·58	1·34
C_{13}	0·03	0·05	tr	tr	0·02	0·01	0·05	0·02	0·04	0·03	0·04
C_{14}	1·34	0·57	0·22	0·36	2·60	0·22	0·26	0·24	1·40	1·53	3·36
C_{15}	0·07	0·05	0·03	0·06	0·08	0·02	0·05	0·06	0·06	0·05	0·04
C_{16}	0·84	1·94	0·14	0·23	2·48	0·13	0·13	0·20	1·42	1·16	3·04
C_{17}	0·07	0·07	0·11	0·11	0·17	0·04	0·05	0·09	0·06	0·02	0·04
C_{18}	0·40	1·92	0·08	0·12	1·41	0·06	0·06	0·07	0·44	0·59	1·73
C_{19}	0·04	0·08	0·04	0·05	0·06	tr	0·04	0·03	0·46	0·01	tr
C_{20}	0·16	1·10	tr	tr	0·57	tr	tr	tr	0·42	0·23	0·69
C_{21}	0·04	0·06	0·07	0·08	0·04	0·03	0·04	0·05	0·31	0·03	0·05
C_{22}	tr	0·46	0·07	0·06	0·21	tr	0·07	0·01	0·07	0·09	0·21
C_{23}	—	0·07	0·11	0·07	tr	0·02	0·13	0·05	tr	tr	—
C_{24}	—	tr	0·16	0·09	0·15	0·01	0·23	0·05	tr	0·14	—
C_{25}	—	—	0·21	0·11	tr	0·05	0·28	0·08	0·11	—	—
C_{26}	—	—	0·19	0·09	—	0·01	0·28	0·05	—	—	—
C_{27}	—	—	0·18	0·12	—	0·04	0·27	0·07	—	—	—
C_{28}	—	—	0·11	0·03	—	—	0·16	0·04	—	—	—
C_{29}	—	0·04	0·10	0·04	—	0·04	0·15	0·09	—	—	—
C_{30}	—	—	0·04	0·02	—	—	0·09	tr	—	—	—
C_{31}	—	0·02	tr	tr	—	tr	0·07	tr	—	—	—
Total	3·44	6·63	1·98	1·81	8·74	0·78	2·50	1·34	5·50	4·46	10·54
phyt-1-ene	—	—	—	—	—	—	—	—	0·05	—	—
phytane	—	—	—	—	—	—	—	—	0·20	—	—
phyt-2-ene	—	—	—	—	—	—	—	—	0·18	—	—

(+): Different samples from same place.
* Values expressed in ppm (mg/kg). Each value is the mean of at least two assays.
tr = trace.

Table 9 shows that all enca and onca *n*-alkanes from C_{12} to C_{33} were present in chicken tissue with a typical bell distribution between C_{12} and C_{18}, the peak being at C_{14}. Corresponding *n*-alkenes were found in small quantities. Branched hydrocarbons (phytenes) were absent. Concentration of hydrocarbons in chicken tissue range from 0·85 to 4·77 ppm. These quantities agree with those reported in the literature (Lintas *et al.*, 1979; Di Muccio *et al.*, 1979).

TABLE 9
HYDROCARBONS FOUND IN CHICKEN TISSUES*

	Q	*R*	*S*	*T*	*Q* (+)	*Q* (++)	*S* (+)	*T* (+)
Hydrocarbons								
C_{12}	0·10	0·09	0·17	0·37	0·18	0·18	0·12	0·02
C_{13}	0·01	0·01	0·02	0·03	tr	tr	tr	0·02
C_{14}	0·40	0·18	0·43	1·16	0·52	0·51	0·27	0·05
C_{15}	0·02	0·02	0·02	0·06	0·03	0·03	0·02	0·03
C_{16}	0·23	0·17	0·22	1·24	0·28	0·30	0·29	0·06
C_{17}	0·03	0·02	0·03	0·03	0·04	0·05	0·04	0·07
C_{18}	0·07	0·05	0·07	0·98	0·10	0·11	0·05	0·06
C_{19}	0·01	0·01	0·02	tr	0·01	0·01	0·01	0·02
C_{20}	0·03	tr	tr	0·58	0·03	0·06	0·03	0·01
C_{21}	0·02	0·02	0·02	0·04	0·02	0·02	tr	0·04
C_{22}	0·11	0·05	tr	0·28	0·02	0·04	tr	0·10
C_{23}	0·06	0·07	0·03	tr	0·02	0·05	tr	0·09
C_{24}	0·09	0·10	0·03	tr	0·01	tr	tr	0·10
C_{25}	0·12	0·18	0·03	—	0·02	0·03	0·01	0·12
C_{26}	0·10	0·15	0·03	—	0·02	0·04	0·01	0·12
C_{27}	0·11	0·15	0·01	—	0·03	0·05	tr	0·11
C_{28}	0·09	0·10	tr	—	—	tr	—	0·08
C_{29}	0·09	0·09	—	—	—	—	—	0·10
C_{30}	0·06	0·08	—	—	—	—	—	0·07
C_{31}	0·05	0·06	—	—	—	—	—	0·23
C_{32}	tr	0·02	—	—	—	—	—	0·05
C_{33}	—	—	—	—	—	—	—	0·04
Total	1·80	1·62	1·13	4·77	1·33	1·48	0·85	1·59

(+) (++): Different samples from same place.
* Values expressed in ppm (mg/kg). Each value is the mean of at least two assays.
tr = trace.

REFERENCES

ALIMENTI, R., ANTONUCCI, G., BERNARDINI, M. P., BERGONZINI, E., BIANCHI, W., BONIFORTI, L., BORGHESE, A., CACCIAPUOTI, B., CAVINA, G., CITTI, G., COTTA RAMUSINO, F., D'AGNOLO, G., DELLE FEMMINE, P., FABBRI, R., FATTORI, I., GALLI, G., GALLINELLA, B., GATTI, L., GIORDANO, R., GIOVENALE, E., MACRI', A., MALOSSINI, F., MARTILLOTTI, F., MASTRANTONIO, P., MAZZIOTTI DI CELSO, M. P., MORETTI, G., MORISI, G., NANNI, A., NUSDORFI, G., PACE, W., PALLIOLA, E., PAOLETTI, E., PICCHIANI, C., PICCININNO, G., RICCI, A., SALVATI, S., SAMPAOLO, A., SERLUPI CRESCENZI, G., SILANO, V., SPADONI, M. A., TAGGI, F., TAGLIAMONTE, B., TOMASSI, G., TIECCO, G., TONOLO, A., TUTTOBELLO, L., VALFRE', F. & VENDRAMIN, C. (1979). Indagini nutrizionali sulla toprina (lieviti coltivati su *n*-paraffine). *Ann. Ist. sup. Sanità*, **15**(3), 649–89.

BANDURSKI, E. L. & NAGY, B. (1975). Nature of alkanes in beef heart lipids. *Lipids*, **10**(2), 67–9.

BERNARDINI, M. P., BONIFORTI, L., MERLI, F. & MOSINI, V. (1975). Studio mediante gas cromatografia e spettrometria di massa della composizione e della struttura degli acidi grassi presenti nella toprina (lieviti su *n*-paraffine). *Riv. Soc. ital. sci. Alimentaz.*, **10**, 391–4.

BINGHAM, E. & FALK, H. L. (1969). Environmental carcinogens. *Archs envir. Hlth*, **19**, 779–83.

BONIFORTI, L., CAVINA, G., CITTI, G., MORETTI, G., ALIMENTI, R., BERNARDINI, M. P., FATTORI, I., GALLINELLA, B. & GIORDANO, R. (1979). Studio dei costituenti lipidici di lieviti coltivati su *n*-paraffine (Toprina e Liquipron). Metodologie per la loro determinazione in tessuti ed in liquidi biologici di animali alimentati con tali lieviti. *Ann. Ist. sup. Sanità*, **15**(3), 473–89.

DI MUCCIO, A., BONIFORTI, L., PALOMBA, A., BERNARDINI, M. P. & DELISE, M. (1979). Idrocarburi saturi negli alimenti. Metodo di analisi e valori riscontrati in alcuni alimenti per uso umano e in campioni di organismi unicellulari. *Ann. Ist. sup. Sanità*, **15**(3), 525–40.

FOLCH, J., LEES, M. & SLOANE STANLEY, G. M. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J. biol. Chem.*, **226**, 497–509.

LINTAS, C., BALDUZZI, A. M., BERNARDINI, M. P. & DI MUCCIO, A. (1979). Distribution of hydrocarbons in bovine tissues. *Lipids*, **14**(3), 298–303.

NICHOLAS, H. J. & BOMBAUGH, K. J. (1965). Constituents of the hydrocarbon fraction of beef brains. *Biochim. biophys. Acta*, **98**, 372–9.

TAGLIAMONTE, B. & TOMASSI, G. (1976). Gli acidi grassi a numero dispari di atomi di carbonio: aspetti metabolici e nutrizionali. *S.T.A.N.U.*, **1**, 7–12.

Food Chemistry 8 (1982) 61–70

CHEMICAL CHARACTERISATION OF THE VITELLINE MEMBRANE OF HENS' EGGS

T. TRZISZKA & T. SMOLIŃSKA

Institute of Storage and Food Technology,
The Agricultural Academy, Norwida 25/28, 50-375 Wrocław, Poland

(Received: 1 December 1980)

ABSTRACT

The chemical composition of the vitelline membrane of hens' eggs, both fresh and stored (± 1°C) for 6 months, has been studied. The results indicate that the vitelline membrane contains mainly protein compounds with some carbohydrates and lipids. The amounts of particular components depend on the method of preparation. During cold storage there is a loss of nitrogen and changes in the chemical composition of the vitelline membrane should first be considered in respect of the protein part of the membrane.

INTRODUCTION

The vitelline membrane plays an important role in the metabolic processes of an egg (Doran & Mueller, 1961; Fromm & Matrone, 1962; Bellairs *et al.*, 1963; Britton & Hale, 1973; Kido *et al.*, 1975; Heath, 1976; Kido *et al.*, 1976; Trziszka, 1977; Smolinska & Trziszka, 1979, 1980). The membrane serves to separate the two phases of white and yolk with their different chemical compositions and functions. It is also a diffusion barrier with a varied degree of selectivity for electrolytes and non-electrolytes, and maintains the osmotic balance between the different media of the white and the yolk. (Fromm, 1964; Trziszka, 1977; Smolinska & Trziszka, 1979, 1980). Earlier studies (Smolińska & Trziszka, 1979, 1980; Trziszka, 1977) have shown that the vitelline membrane loses its properties after separation from the egg which suggests that the properties of the membrane are dependent on the presence of egg white and yolk. Studies on the vitelline membrane constitute a complex problem, mainly due to the great difficulties in separating and purifying the membrane. Such studies are not very numerous and, in particular, studies of the chemical composition of the vitelline membrane are both scarce and controversial.

Food Chemistry 0308-8146/82/0008-0061/$02·75

The chemical changes which take place in the process of ageing undoubtedly influence changes of a functional nature, leading to worsening or complete loss of the biological properties of the vitelline membrane.

The objective of the study described in this paper was to determine the basic chemical composition of the vitelline membrane of hens' eggs, both fresh and stored in the cold.

Some initial relationships between the chemical changes of the vitelline membrane and its function in the egg are described with the aid of mathematical analysis.

MATERIALS AND METHODS

The eggs (820) used in the investigation were from NX × Sussex hens of an 11 month old flock. The eggs weighed 60–65 g each. To ensure equality of freshness the eggs were all collected 3–5 h after laying. A sample (100) was used immediately for investigation and the remainder (720) were left in cold storage for 6 months at $-1\,^{\circ}C$ ($\pm 0{\cdot}5\,^{\circ}$) and 84% ($\pm 4\%$) relative humidity. The chemical tests were carried out at 30-day intervals. They were of a model character, based on Polish Standard (Polska Norma PN 67/A–07005, 1967) storage parameters. Two methods of vitelline membrane preparation were employed and the analyses for chemical composition were replicated ten times for each method. The storage period and use of eggs for analysis is shown in Table 1.

Preparation of the vitelline membrane consisted of separation from the remainder of the egg and purification as shown in Fig. 1.

Purification method A (employing salines) was according to the techniques of Kido *et al.* (1975) with modifications to the concentration of solutions and the time of purification. Purification by method B (employing an alcohol–ether mixture

TABLE 1
STORAGE PERIOD AND USE OF EGGS FOR ANALYSIS

Month of storage	*Number of eggs used in the experiment* *Method of separation*		*Number of membranes used in one analysis* *Method of separation*		*Number of repetitions*
	A	*B*	*A*	*B*	
0	50	50	5	5	10
1	50	50	5	5	10
2	50	50	5	5	10
3	60	60	6	6	10
4	60	60	6	6	10
5	70	70	7	7	10
6	70	70	7	7	10

0 Fresh eggs.
1–6 Eggs stored in the cold for 1–6 months.

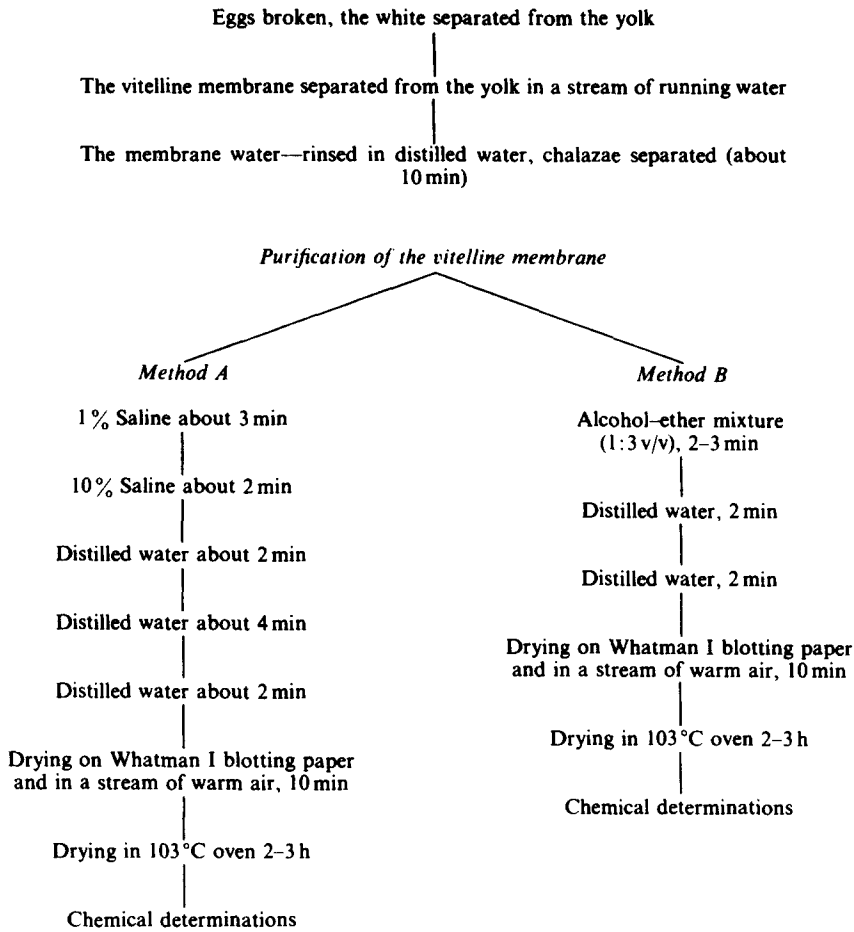


Fig. 1. Diagram of the vitelline membrane preparation.

(1:3 v/v)) was carried out according to the technique of Bellairs *et al.* (1963) with modifications to the time and sequence of solutions.

CHEMICAL ANALYSIS OF THE VITELLINE MEMBRANE

Individual chemical determinations were carried out on the dried samples of the vitelline membrane. Drying to constant weight was undertaken at 103 °C according to the accepted method (Mejbaum–Katzenellenbogen & Mochnacka, 1968; Ostrowski, 1974). Determinations of the parameters in question were repeated ten

times and each time about 20 mg of the dry matter of the vitelline membrane was used. The following components were identified in the dry matter.

(i) Total nitrogen by the micro-Kjeldahl procedure (Mejbaum–Katzenellenbogen & Mochnacka, 1968). For individual investigations about 10 mg of the vitelline membrane (dry matter) were used. The values of nitrogen were multiplied by the factor 6·25 (suggested by Bellairs *et al.*, 1963) and the percentage of the total protein was obtained.

(ii) True protein was determined by a colorimetric method according to Folin–Ciocalteau's modification of Lowry's method (Gorsuch & Norton, 1969; Ślopek, 1970). The method consists in the reduction of phosphomolybdic phosphotungstic reagent by residues of tyrosine and tryptophan present in proteins. The standards of proteins were prepared from ovalbumin dissolved in 0·15 M NaCl. The basic solution contained 2 mg of protein per millilitre. The spectrophotometric measurement was taken at $\lambda = 750$ nm.

(iii) Total lipid was determined by the Zölner–Kirsch method (Ostrowski, 1974). Determination is based on the formation of complexes of coloured lipids during heating with concentrated sulphuric acid in the presence of phosphoric acid and vanillin. Determination was carried out spectrophotometrically at $\lambda = 530$ nm. The standards used were cholesterol and triolein.

(iv) Total carbohydrates were identified by the method of Dubois *et al.* (1956) and Kido *et al.* (1975). The principle of this method is to hydrolyse the sample with concentrated sulphuric acid in the presence of concentrated phenol (80 %) which gives a coloured complex with carbohydrates. The intensity of the colour is proportional to the content of carbohydrates in the sample.
The determination was carried out spectrophotometrically at $\lambda = 490$ nm. The standards were made of 1 % solutions of glucose.

(v) Ash was determined by burning the dry matter of the vitelline membrane at 600 °C. Next, on the basis of the weight analysis with an accuracy of 0·001 g, the percentage of ash in the dry matter of the membrane was established.
The results were processed statistically as the mean value and variability coefficient. In addition, the method of variance analysis was used to examine the dynamics of change in the various parameters during storage. Correlation coefficients were calculated to assess the relationships between some of the vitelline membrane components.

DISCUSSION OF RESULTS

The two methods of vitelline membrane preparation were useful for different analytical purposes. Method A, using saline as the solvent, is useful for total

nitrogen determination as the vitelline membrane is likely to be free from contamination by proteins from the chalazae.

Method B allows thorough purification of the vitelline membrane from the lipid fractions of the yolk which stick to the inner side of the membrane. It is also useful for the determination of the lipid fractions which constitute an integral part of the membrane structure (Trziszka, 1977). In the process of storage, due to some biochemical processes occurring, the functional properties of the membrane change and it loses some of its mass. As the results were considered in relation to the dry matter, in order to obtain the right quantity of sample for the analysis (20 mg of dry matter for one complete analysis) it was necessary, in the period of storage, to increase the number of prepared membranes from five to seven pieces. The results are shown in Tables 2, 3 and 4. As Table 2 shows, the initial quantity of total nitrogen in the dry matter of the membranes isolated by method A is lower, on average, by 1 % than the results of the total nitrogen quantity determined by method B. Thus, the quantity of total protein (with factor 6·25 used) in the membranes isolated by method A is lower by about 6 % than that in the membranes isolated by method B. The course of changes in the quantity of total nitrogen determined by both methods is nevertheless similar, as the correlation value shows ($r = 0{\cdot}86$) (Table 3).

Statistically significant losses of total nitrogen for both methods of isolation occur between the third and fourth months of storage. It can be suggested, then, that some essential quantity changes in the chemical composition of the vitelline membrane take place in this period. The analysis of true protein, carried out exclusively on the membranes isolated by method A, was to confirm the actual content of proteins connected structurally with the vitelline membrane.

Statistical calculations point to significant losses of true protein, starting from the fourth month of storage. The actual correlation ($r = 0{\cdot}71$) found for the content of total nitrogen and true protein (Table 3) is a true reflection of the changes occurring in the structure of the vitelline membrane. The calculated correlation also shows that the loss of total nitrogen refers not only to protein fractions but also to nitrogen of non-protein origin. As Table 2 shows, the lipids constitute a large part of the dry matter of the vitelline membrane. Their content depends on the method of preparation. For method A their content is between 13·26 % and 15·92 % whereas, for method B, it is between 5·2 % and 6·06 %. The quantity changes of lipid compounds are positively correlated ($r = 0{\cdot}82$) in both methods of preparation. In the course of cold storage of eggs there is a negative correlation between the content of lipids and total nitrogen. Carbohydrates have a very important chemical function in the vitelline membrane and their content in the process of cold storage decreases by a statistically significant value (Tables 2 and 3). This loss of carbohydrate is highly correlated with the loss of total nitrogen ($r = 0{\cdot}82$–$0{\cdot}84$), suggesting the presence of nitrogen–carbohydrate compounds being involved in metabolic processes and changes during the ageing of the eggs in cold storage.

The content of ash in the dry matter of the vitelline membrane was between 0·61 % and 0·65 % and no changes caused by storage have been found.

TABLE 2

THE CHEMICAL CHARACTERISATION OF THE VITELLINE MEMBRANES FROM FRESH AND STORED (IN THE COLD FOR 6 MONTHS) EGGS. ISOLATION BY SALINES (METHOD A) AND ALCOHOL–ETHER MIXTURE (METHOD B) (MEAN AT $n = 10$)

Length of storage (months)		*Percentage of the components in the dry matter of the vitelline membrane*											
		Purification by method A						*Purification by method B*					
		Total nitrogen	*Total protein*	*True protein**	*Total lipids*	*Total carbo-hydrates*	*Proportion of nitrogen to lipids*	*Total nitrogen*	*Total protein*	*Total lipids*	*Total carbo-hydrates*	*Proportion of nitrogen to lipids*	*Minerals*
1	2	3	4	5	6	7	8	9	10	11	12	13	14
0	$\bar{x}$	13·65	85·31	70·05	13·26	8·35	1·03	14·64	91·50	5·20	9·12	2·82	0·62
	w	4·65		5·87	8·11	7·46		2·59		7·84	8·46		
1	$\bar{x}$	13·59	84·94	70·08	13·23	8·04	1·03	14·62	91·38	5·19	8·86	2·82	0·64
	w	3·78		5·82	6·22	8·32		2·36		5·89	7·11		
2	$\bar{x}$	13·55	84·69	69·96	13·29	7·60	1·02	14·57	91·06	5·27	8·72	2·76	0·61
	w	5·11		5·12	8·95	10·03		2·36		7·14	5·46		
3	$\bar{x}$	13·19	82·44	69·42	14·19	7·06	0·93	14·46	90·37	5·43	8·40	2·66	0·65
	w	2·92		4·53	4·94	6·17		2·52		4·65	9·13		
4	$\bar{x}$	12·90	80·63	68·70	14·94	6·50	0·86	14·30	89·37	5·64	7·96	2·53	0·63
	w	3·85		7·80	5·22	9·66		4·80		8·11	8·49		
5	$\bar{x}$	12·67	79·19	68·04	15·58	5·98	0·81	14·17	88·56	5·85	7·58	2·42	0·61
	w	4·72		4·75	8·76	8·76		2·35		5·14	5·12		
6	$\bar{x}$	12·58	78·63	67·39	15·92	5·64	0·79	14·06	87·88	6·06	7·25	2·32	0·64
	w	3·81		4·94	8·20	9·48		4·87		4·70	5·87		

* Identification by Lowry's method.

0 Fresh eggs.

1–6 Eggs stored in cold in the period of 1–6 months.

$w = \frac{s}{\bar{x}} . 100$

TABLE 3

STATISTICAL ANALYSIS OF THE QUANTITATIVE CHANGES OF CERTAIN CHEMICAL COMPONENTS OF THE VITELLINE MEMBRANE WITH RESPECT TO THE PERIOD OF STORAGE (METHOD OF THE MULTIFACTOR VARIANCE ANALYSIS)

Method / *Components*	*Statistical differences*	*Period of storage in months*[a]						
		0	1	2	3	4	5	6
Method A								
N %	n	1, 2	0, 2	0, 1	—	—	6	5
	*	3	3	3	0, 1, 2, 4	3, 5	4	—
	**	4, 5, 6	4, 5, 6	4, 5, 6	5, 6	0, 1, 2, 6	0, 1, 2, 3	0, 1, 2, 3, 4
Total protein[b]	n	1, 2, 3	0, 2, 3	0, 1, 3, 4	0, 1, 2, 4	3, 5	4, 6	5
	*	4	4	4	5	0, 1, 2, 6	3	4
	**	5, 6	5, 6	5, 6	6	—	0, 1, 2	0, 1, 2, 3
Lipids	n	1, 2	0, 2	0, 1	—	—	6	5
	*	3	3	3	0, 1, 2, 4	3, 5, 6	4	4
	**	4, 5, 6	4, 5, 6	4, 5, 6	5, 6	0, 1, 2	0, 1, 2, 3	0, 1, 2, 3
Carbohydrates	n	—	—	—	—	—	—	—
	*	—	—	—	—	—	—	—
	**	1, 2, 3, 4, 5, 6	0, 2, 3, 4, 5, 6	0, 1, 3, 4, 5, 6	0, 1, 2, 4, 5, 6	0, 1, 2, 3, 5, 6	0, 1, 2, 3, 4, 6	0, 1, 2, 3, 4, 5
Method B								
N %	n	1, 2, 3	0, 2, 3	0, 1, 3	0, 1, 2, 4	3, 5	4, 6	5
	*	4, 5	4, 5	4, 5	5, 6	0, 1, 2, 6	0, 1, 2, 3	3, 4
	**	6	6	6	—	—	—	0, 1, 2
Lipids	n	1, 2, 3	0, 2, 3	0, 1, 3	0, 1, 2, 4	3, 5	4, 6	5
	*	4, 5	4, 5	4, 5	5, 6	0, 1, 2, 6	0, 1, 2, 3	3, 4
	**	6	6	6	—	—	—	0, 1, 2
Carbohydrates	n	—	—	—	—	—	—	—
	*	—	—	—	—	—	—	—
	**	1, 2, 3, 4, 5, 6	0, 2, 3, 4, 5, 6	0, 1, 3, 4, 5, 6	0, 1, 2, 4, 5, 6	0, 1, 2, 3, 5, 6	0, 1, 2, 3, 4, 6	0, 1, 2, 3, 4, 5

[a] Statistical relationship between the periods of storage (months).
[b] In relation to total nitrogen.
n No statistical differences.
* Statistical differences. ($p < 0{\cdot}05$).
** Substantial statistical differences ($p < 0{\cdot}01$).

The vitelline membrane undergoes some physical and chemical changes at the same time as other components of the egg's contents. In the course of the natural process of ageing in the storage period, several changes take place which, in macroscopic observations, manifest themselves as, for example, decreased strength of the membrane, which was the subject of our own and other studies (Fromm & Matrone, 1962; Bellairs *et al.*, 1963; Fromm, 1967; Trziszka, 1977; Smolińska & Trziszka, 1979).

TABLE 4
CORRELATIONS BETWEEN SOME CHEMICAL COMPONENTS OF THE VITELLINE MEMBRANE DURING THE PERIOD OF COLD STORAGE

No.	*Compared values*	*Correlation value* (r)
1	% N (method A) × % N (method B)	0·86**
2	% Lipids (method A) × % lipids (method B)	0·82**
3	Method A: % N × % carbohydrates	0·84**
4	Method A: % N × % total lipids	−0·91**
5	Method A: Total protein (N × 6·25)* true protein	0·71*
6	Method B: % N × % carbohydrates	0·82**
7	Method B: % N × % lipids	−0·88**
8	Total protein (method B) × true protein (method A)	0·93**

*, $\alpha = 0{\cdot}05$.
**, $\alpha = 0{\cdot}01$.

Our own studies indicate, however, that the physical changes in the vitelline membrane may be considered as secondary effects conditioned by advanced changes of a chemical and biochemical nature. The analysis of the chemical composition of the vitelline membrane isolated by two different methods of preparation has allowed detailed analysis of quantitative changes of proteins, as well as lipids, due to the selective nature of both those methods.

The values of total nitrogen obtained initially in the present study are close to the results obtained by Bellairs *et al.* (1963) and Britton (1973). It has been found that cold storage of eggs resulted in some losses of the total nitrogen. These losses may be caused by transformation of nitrogen into other forms; for example, soluble compounds which can migrate beyond the structure of the vitelline membrane. It seems that calculations of the protein content in the vitelline membrane may be controversial because of the application of the factor 6·25.

Many researchers have employed this factor for recalculations of the total nitrogen into protein and it has also been used in this study. On the basis of results obtained in the present study it must be stressed that this method of protein estimation gives an incorrect total for the components of the dry matter of the vitelline membrane. This indicates that the total nitrogen value is not only of protein origin but may also arise from phospholipids, nucleic acids and aminosaccharides

which constitute a considerable proportion of the dry matter of the vitelline membrane. This suggestion is confirmed by the estimation of true protein by the method of Lowry which gives a lower value for protein than that calculated from the nitrogen value multiplied by the factor 6·25. According to the studies by Gorsuch & Norton (1969), true protein determination by Lowry's method is particularly advisable for biological material. As the total nitrogen is a dominant component of the vitelline membrane and its chemical components are of different forms, the chemical changes in the vitelline membrane should be considered not only on the basis of quantitative changes in the total nitrogen but also on the basis of the changes in the remaining components. Only then will it be possible for the real picture of the metabolic transformations, which take place in the vitelline membrane in the course of the natural process of ageing, to emerge.

The changes relating to the lipids of the vitelline membrane of eggs stored for 6 months are closely correlated with other components of the membrane dry matter. In terms of absolute value the amount of lipid in the membrane increases due to the relative increases of its dry matter content. It is possible that, in the process of storage, the lipid compounds from the yolk are transferred to the vitelline membrane. At the same time some lipid compounds (e.g. cholesterol) are freed from the structural elements of the vitelline membrane (Trziszka, 1977; Smolińska & Trziszka, 1977).

The differences shown here are mainly the result of the accepted method of membrane preparation. The data on the lipid content, in comparison with the studies by Bellairs *et al.* (1963) and Britton (1975), are higher—a fact which can be attributed to the different method of preparation as well as the different material used. In biological membranes in general carbohydrates appear as heteropolysaccharide complexes and glycoproteins (Trziszka, 1977; Sawicka & Piasek, 1979). Similarly, high content of glycoproteins has been shown to occur in the vitelline membrane (Kido *et al.*, 1975; Trziszka, 1977). They probably play a major role in the structural synthesis of the vitelline membrane and in its functional properties. It has been observed that lipids have a high rate of degradation in the period of storage, which is confirmed by the studies of Kido *et al.* (1975). Furthermore, the changes in the lipids are positively correlated with the losses of total nitrogen, indicating that they are mainly aminosaccharides or lipid derivatives containing nitrogen—a fact which was confirmed in earlier studies (Kido *et al.*, 1975; Trziszka, 1977). The constant content of the mineral compounds in the vitelline membrane indicates their important function in the processes of exchange.

Our earlier investigations have demonstrated that the biological selectivity of the vitelline membrane depends on the balance of concentrations of particular elements (Trziszka, 1977; Smolińska & Trziszka, 1979; Smolińska & Trziszka, 1980). The vitelline membrane as a structural element in eggs is closely related to the white of the chalazae layer and the yolk. This suggests the possibility of interaction between these components mediated by the vitelline membrane and demands further study.

CONCLUSIONS

On the basis of the studies carried out the following conclusions have been drawn:

(1) The vitelline membrane of hens' eggs is chemically composed of proteins, carbohydrates and lipids.
(2) The proportions of these components depend, to a considerable extent, on the method of vitelline membrane preparation.
(3) In the process of cold storage of eggs the nitrogen of the vitelline membrane is continuously degraded; therefore, the changes in chemical composition of the membrane should be considered in relation to the protein content.

REFERENCES

BELLAIRS, R., HARKNESS, M. & HARKNESS, R. D. (1963). The vitelline membrane of the hen's egg: A chemical and electron microscopical study. *J. Ultrastructure Research*, **8**, 339–59.

BRITTON, W. M. (1973). Vitelline membrane chemical composition in natural and induced yolk mottling. *Poultry Sci.*, **52**, 459–64.

BRITTON, W. M. & HALE, K. K. (1973). Stepwise discriminant analysis of amino acid composition of vitelline membranes from yolks exhibiting various degrees of mottling. *Poultry Sci.*, **53**, 64–69.

DORAN, B. M. & MUELLER, W. J. (1961). The development and structure of the vitelline membrane and their relationship to yolk mottling. *Poultry Sci.*, **40**, 474–8.

DUBOIS, M., GILLES, K. A., HAMILTON, J. K., REBERS, P. A. & SMITH, F. (1956). Colorimetric method for determination of sugars and related substances. *Anal. Chem.*, **28**, 350–6.

FROMM, D. (1964). Strength distribution, weight and some histological aspects of the vitelline membrane of the hen's egg yolk. *Poultry Sci.*, **43**, 1240.

FROMM, D. (1967). Some physical and chemical changes in the vitelline membrane of the hen's egg during storage. *J. Food Sci.*, **32**, 52–6.

FROMM & MATRONE, G. (1962). A rapid method for evaluating the strength of the vitelline membrane of the hen's egg yolk. *Poultry Sci.*, **41**, 1516–21.

GORSUCH, T. T. & NORTON, R. L. (1969). The determination of protein in biological materials and foodstuffs, *J. Food Technol.*, **4**, 1–6.

HEATH, J. L. (1976). Factors affecting the vitelline membrane of the hen's egg. *Poultry Sci.*, **55**, 936–42.

KIDO, S., JANADO, M. & NUNOURA, H. (1975). Macromolecular components of the vitelline membrane of hen's egg. I. Membrane structure and its deterioration with age. *J. Biochem.*, **78**, 261–8.

KIDO, S., JANADO, M. & NUNOURA, H. (1976). Macromolecular components of the vitelline membrane of hen's egg. II. Physicochemical properties of glycoprotein, *J. Biochem.*, **79**, 1351–6.

MEJBAUM-KATZENELLENBOGEN W. & MOCHNACKA, I. (1968). *Kurs praktyczny z biochemii.* PWN Warsaw.

OSTROWSKI, W. (1974). *Wybrane metody z chemii Klinicznej.* PZWL, Warsaw.

POLSKA NORMA PN 67/A-07005 (1967).

SAWICKA, T., PISEK, A. (1971). Heteropolysaccharides of plasma membranes. *Postępy Bioch.*, **17**, 275–80.

ŚLOPEK, S. (1970). *Immunologia praktyczna*, PZWL, Warsaw.

SMOLIŃSKA, T. & TRZISZKA, T. (1979). Examination on selection of vitelline membrane for some electrolytes. *Zesz. Nauk. AR Wrocław. Technologia Żywności* I., **124**, 65–73.

SMOLIŃSKA, T. & TRZISZKA, T. (1980). The vitelline membrane: The dynamics of cholesterol metabolism in the egg (unpublished).

TRZISZKA, T. (1977). *Zmiany w strukturze błony witelinowej jaj kurzych pochodzących z różnych okresów chłodniczego przechowywania.* Dissertation, Wrocław, AR.

Food Chemistry 8 (1982) 71–73

BOOK REVIEWS

Condensed Chemical Dictionary. By G. G. Hawley. Van Nostrand Reinhold, 1981. xi + 1135 pp. Price: £27·50.

The tenth edition of this excellent book contains much extra valuable information for food chemists including manufacturing equipment, its components, waste control, raw materials, etc. It is interesting that the term 'food additive' still evidently embraces pesticide residues as 'unintentional additives' and the term 'glucose syrup' is confined to products obtained from corn starch (maize starch), rather than the more widely accepted Codex Alimentarius definition. However, the dictionary is still a substantial reference text and is modestly priced.

G. G. BIRCH

Polymers in Nature. By E. A. MacGregor and C. T. Greenwood. John Wiley and Sons, Chichester, New York, Brisbane, Toronto, 1980, ix + 391 pp. Price: £19·50.

In the Preface, the authors remind us that the polymers in Nature impinge on every aspect of our existence, and propose to give an outline of the *present* state of knowledge of the structure, properties and functions of the whole spectrum of different natural macromolecules—both organic and inorganic.

They have indeed set themselves a Herculean task. The seven main chapters (numbers of pages in parentheses) deal with extraction and purification (30), molecular size and shape (38), proteins (92), nucleic acids (74), polysaccharides (89), rubber and lignin (24) and inorganic polymers (20). As this book is intended as a text for undergraduates in chemistry, etc., and as the chemistry of natural polymers is often combined in a single course, I appreciate the authors' plan to combine examples of each different class of macromolecule in a single treatise. I cannot review critically the chapters on proteins, nucleic acids, rubber, lignins and inorganic

Food Chemistry (8) (1982)—
Printed in Great Britain

Food Chemistry 8 (1982) 71–73

BOOK REVIEWS

Condensed Chemical Dictionary. By G. G. Hawley. Van Nostrand Reinhold, 1981. xi + 1135 pp. Price: £27·50.

The tenth edition of this excellent book contains much extra valuable information for food chemists including manufacturing equipment, its components, waste control, raw materials, etc. It is interesting that the term 'food additive' still evidently embraces pesticide residues as 'unintentional additives' and the term 'glucose syrup' is confined to products obtained from corn starch (maize starch), rather than the more widely accepted Codex Alimentarius definition. However, the dictionary is still a substantial reference text and is modestly priced.

G. G. BIRCH

Polymers in Nature. By E. A. MacGregor and C. T. Greenwood. John Wiley and Sons, Chichester, New York, Brisbane, Toronto, 1980, ix + 391 pp. Price: £19·50.

In the Preface, the authors remind us that the polymers in Nature impinge on every aspect of our existence, and propose to give an outline of the *present* state of knowledge of the structure, properties and functions of the whole spectrum of different natural macromolecules—both organic and inorganic.

They have indeed set themselves a Herculean task. The seven main chapters (numbers of pages in parentheses) deal with extraction and purification (30), molecular size and shape (38), proteins (92), nucleic acids (74), polysaccharides (89), rubber and lignin (24) and inorganic polymers (20). As this book is intended as a text for undergraduates in chemistry, etc., and as the chemistry of natural polymers is often combined in a single course, I appreciate the authors' plan to combine examples of each different class of macromolecule in a single treatise. I cannot review critically the chapters on proteins, nucleic acids, rubber, lignins and inorganic

Food Chemistry (8) (1982)—
Printed in Great Britain

polymers, but having read most of the book and all of the general chapters and the chapter on polysaccharides, I must conclude that their plan was ill-conceived. The promise to give an outline of the *present* state of knowledge in the field of polysaccharides is not fulfilled. The authors are surely aware of monographs—*some* are cited in the list of additional reading material—which are also suitable for reading in connection with advanced undergraduate courses in chemistry. However, I have looked in vain for an outline of the 'existing advances' (see the Preface) made in this area during the last decade, and have found little which would make this chapter more recommendable than an earlier treatise of similar size (ASPINALL, G. O. (1970). *Polysaccharides*, Pergamon Press, Oxford and New York). It must be granted that the selection of topics reflects the interest of the authors, but a mere description of primary structures cannot, in the 1980s, make stimulating reading. The no-longer 'new' methods, to mention only NMR and mass spectrometry, are discussed in a few sentences. For example: 'Comparative magnetic resonance studies carried out on methylated or acetylated oligosaccharides and polysaccharides in solution have indicated that this is indeed the case—chair conformations occurring commonly in oligosaccharide crystals also occur in polysaccharide chains in solution' (p. 272), and 'some information on disaccharide structure may often be obtained by mass spectrometry, i.e. it is often possible to confirm the presence of pyranose rings or, for example (1 → 6)-linked hexose sugars', (p. 264) are the only reference to these techniques. Equally, reagents and specific chemical degradation reactions which have been developed and discovered, respectively, during the last decade, are not mentioned.

The secondary and tertiary structures of polysaccharides are fields which are no longer in their infancies. Whilst it is true to say that the functions of *all* polysaccharides in Nature are not yet fully understood, if they ever will be, it is unforgiveable that the 'three-dimensional structure' is allocated no more than one and a half pages (pp. 272–3). In few instances are details of structure correlated with function, e.g. in the discussion of κ-carrageenan, but in most cases functions like 'food-reserve material', 'main structural material', etc., are just listed.

We do not know the answer to all problems, but even when a polysaccharide contains several types of monomer units, glycosidic linkages, sequences of units and linkages, the function of the polysaccharide can often be understood by considering the properties of each individual type of sequence separately. A book, which is supposedly an outline of the *present* state of knowledge, must also give an account of the relationship between primary and three-dimensional structure, between three-dimensional structure and function. Even the monographs which have appeared on this subject (for example: REES, D. A. (1977). *Polysaccharide shapes*, Chapman and Hall, London) are missing from the recommended reading list.

It might now seem superfluous to make more detailed comments, but some must be made to indicate that the book is not free of errors. The authors think (p. 244) that the Haworth projection formulae 'should not be considered to represent the

absolute stereochemistry of the monosaccharides'. This was true before Bijvoet, Peerdeman and van Bommel published the results of their X-ray work in 1951! On p. 245, views of the chair conformation of cyclohexane from the two different angles are designated 'chair form C1' and 'chair form 1C'. Are these two examples simply errors? It is also incorrect to state that glycosides are alkali-stable (p. 249), as this will depend on the structure of the aglycon. All the reaction schemes with periodate, depicted on p. 252, are pH dependent and may not proceed as the authors indicate. The definition (pp. 254–55) of an α-glycosidic bond as one formed in a 'hypothetical reaction between an α-hydroxyl on the anomeric carbon atom of one residue and the hydroxyl of a second residue' is incorrect, and also ignores our present knowledge of the mechanism and stereochemistry of glycosylation reactions.

I cannot recommend the chapter on polysaccharides as a text for undergraduates, particularly as several better and more up to date monographs are available.

HELMUT WEIGEL

Human Nutrition. A Comprehensive Treatise. Vol. 3A Nutrition and the Adult. Macronutrients. General Editors: R. B. Alfin-Slater and D. Kritchevsky. Plenum. 1980. viii + 290 pp. Price: £25·00.

This authoritative volume contains both research and field applications covering the present state of knowledge and ten substantial chapters as follows.

'Nutrient Requirements: What They Are and Bases for Recommendations' (R. B. Alfin-Slater and R. Mirenda). 'Energy: Caloric Requirements' (E. R. Buskirk and J. Mendez). 'Suppliers of Energy: Carbohydrates' (I. Macdonald). 'Suppliers of Energy: Fat' (R. B. Alfin-Salter and L. Aftergood). 'Suppliers of Energy: Carbohydrate–Fat Interrelationships' (D. R. Romsos and S. D. Clarke). 'Energetics and the Demands for Maintenance' (K. A. Crist, R. L. Baldwin and J. S. Stern). 'Nutrients with Special Functions: Proteins and Amino Acids in Tissue Maintenance' (A. J. Clifford). 'Nutrients with Special Functions: Essential Fatty Acids' (J. F. Mead). 'Nutrients with Special Functions: Cholesterol' (D. Kritchevsky and S. K. Czarnecki). 'Nutrients with Special Functions: Dietary Fibre'. (J. A. Story and D. Kritchevsky).

The book is undoubtedly aimed at the specialist but food chemists will find the contents invaluable for reference purposes and their substantial bibliographies. Inevitably, covering a vast field in one chapter gives an impression to the reader of dense factual matter. However, all the authors are to be congratulated on the delicacy with which they have handled this problem. The chapter by Macdonald on carbohydrates is especially valuable in its clarity of presentation, and includes an interesting reference to the effect of dietary carbohydrate on brain neurotransmitters. The book is reasonably priced and recommendable.

G. G. BIRCH

absolute stereochemistry of the monosaccharides'. This was true before Bijvoet, Peerdeman and van Bommel published the results of their X-ray work in 1951! On p. 245, views of the chair conformation of cyclohexane from the two different angles are designated 'chair form C1' and 'chair form 1C'. Are these two examples simply errors? It is also incorrect to state that glycosides are alkali-stable (p. 249), as this will depend on the structure of the aglycon. All the reaction schemes with periodate, depicted on p. 252, are pH dependent and may not proceed as the authors indicate. The definition (pp. 254–55) of an α-glycosidic bond as one formed in a 'hypothetical reaction between an α-hydroxyl on the anomeric carbon atom of one residue and the hydroxyl of a second residue' is incorrect, and also ignores our present knowledge of the mechanism and stereochemistry of glycosylation reactions.

I cannot recommend the chapter on polysaccharides as a text for undergraduates, particularly as several better and more up to date monographs are available.

HELMUT WEIGEL

Human Nutrition. A Comprehensive Treatise. Vol. 3A Nutrition and the Adult. Macronutrients. General Editors: R. B. Alfin-Slater and D. Kritchevsky. Plenum. 1980. viii + 290 pp. Price: £25·00.

This authoritative volume contains both research and field applications covering the present state of knowledge and ten substantial chapters as follows.

'Nutrient Requirements: What They Are and Bases for Recommendations' (R. B. Alfin-Slater and R. Mirenda). 'Energy: Caloric Requirements' (E. R. Buskirk and J. Mendez). 'Suppliers of Energy: Carbohydrates' (I. Macdonald). 'Suppliers of Energy: Fat' (R. B. Alfin-Salter and L. Aftergood). 'Suppliers of Energy: Carbohydrate–Fat Interrelationships' (D. R. Romsos and S. D. Clarke). 'Energetics and the Demands for Maintenance' (K. A. Crist, R. L. Baldwin and J. S. Stern). 'Nutrients with Special Functions: Proteins and Amino Acids in Tissue Maintenance' (A. J. Clifford). 'Nutrients with Special Functions: Essential Fatty Acids' (J. F. Mead). 'Nutrients with Special Functions: Cholesterol' (D. Kritchevsky and S. K. Czarnecki). 'Nutrients with Special Functions: Dietary Fibre'. (J. A. Story and D. Kritchevsky).

The book is undoubtedly aimed at the specialist but food chemists will find the contents invaluable for reference purposes and their substantial bibliographies. Inevitably, covering a vast field in one chapter gives an impression to the reader of dense factual matter. However, all the authors are to be congratulated on the delicacy with which they have handled this problem. The chapter by Macdonald on carbohydrates is especially valuable in its clarity of presentation, and includes an interesting reference to the effect of dietary carbohydrate on brain neurotransmitters. The book is reasonably priced and recommendable.

G. G. BIRCH

ANNOUNCEMENTS

SEVENTH WORLD CEREAL AND BREAD CONGRESS

'Cereals' 82: Bread and Peace for all Nations' is the theme of the Seventh World Cereal and Bread Congress, to take place in Prague, Czechoslovakia, from 28 June to 2 July 1982.

Held under the auspices of the Czechoslovak Government and organised in co-operation with the International Association for Cereal Chemistry (ICC) and other professional organisations, the Congress will include two plenary sessions, technical sessions, symposia and a number of round table discussions.

Topics to be covered include cereal production (plant breeding, biochemistry, genetics and crop physiology); storage and preservation of cereal grains; milling; cereals and nutrition; future trends in cereal science, technology and nutrition; and cereals in animal nutrition.

Further information may be obtained from: The Secretariat, Seventh World Cereal and Bread Congress, Na Pankráci, 30, 140 04 Prague 4, Czechoslovakia.

INTERNATIONAL WORKSHOP ON CEREAL CARBOHYDRATES

An international workshop on cereal carbohydrates will be held from 15 to 17 August 1982, in Winnipeg, Canada. Invited speakers will present papers on starch formation and breakdown in cereals, starch biotechnology, the properties of cereal endosperm cell walls and the industrial utilisation of cell wall polysaccharides.

Further information may be obtained from: Dr R. D. Hill, Department of Plant Science, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2.

Food Chemistry (8) (1982)—
Printed in Great Britain

Food Chemistry **8** (1982) 77

EDITORIAL

The papers making up this issue of *Food Chemistry* and presented as the fifth in a series of Symposia papers, were read at a Symposium entitled 'The Chemistry of Food Packaging Materials'. It was held on 4 June, 1981, at The Scientific Societies Lecture Theatre under the auspices of the Food Chemistry Group of the Royal Society of Chemistry. There are obvious physical problems of convenience in having packages which ought to be easy to open; but many of the papers that follow show that there are other problems which are not so obvious, such as possible hazards to health.

The subject matter of the papers has been adequately summed up in a Foreword by Mr F. A. Paine, who chaired the Symposium, but it could, perhaps, just be mentioned that, although it is strictly correct to use the singular word 'plastic' as an adjective, and the plural word 'plastics' as the noun, modern, colloquial usage seems to be using the former both ways. In these papers, an attempt has been made to conform to this former practice; any misinterpretation of the authors' intention must be forgiven.

L. F. GREEN

Food Chemistry (**8**) (1982)—
Printed in Great Britain

Food Chemistry 8 (1982) 79

CHAIRMAN'S FOREWORD

Food must be available wherever there are people and with modern population patterns this is seldom where it is grown: food in interesting variety must be provided all the year round, irrespective of when it is harvested. It must be presented in a manner that is convenient to purchase and to use. In almost every instance this means it must be packaged.

The choice of suitable packaging involves a number of decisions. For most foods the most important consideration is that the package provides optimum protection to keep the product in good condition for the required shelf-life. The packaging technique and choice of pack with appropriate protective qualities must prevent destruction of the food by microbial or insect attack, and preserve the quality and nutritive value by excluding oxygen and controlling moisture loss or gain.

Much of the development in recent years stems from plastics packaging materials; not from plastics alone—metal, glass, paper and board are used in conjunction with plastics for many of them, but without the plastics component they would be far less effective.

Few materials are completely inert towards food, and those that are often have others which are not, used with them to make the most effective pack. We must therefore know the extent of any interactions between foods and their containers, and clearly these must be small, or the package itself would be affected physically. Many so-called 'natural foods' contain toxic materials to a greater extent than some food additives or potential migrants from packaging. Few 'natural foods' contain a zero quantity of toxic material. We must, therefore, never assume that zero is the base line from which we work.

The food industries are the largest group using packaging at the consumer level and if we draw a balance between the way in which the public interest has been served by this heavy involvement in packaging it is evident that the advances in hygiene and the preservation of food weigh heavily on the positive side.

Packaging must be regarded as a benefit to be optimised, not just a cost to be minimised.

F. A. PAINE
Chiddingford, Surrey, Great Britain

Food Chemistry (8) (1982)—
Printed in Great Britain

Food Chemistry 8 (1982) 81–84

PLASTICS USED IN FOOD PACKAGING AND THE RÔLE OF ADDITIVES

A. W. BIRLEY

Institute of Polymer Technology, Loughborough University, Loughborough, Leics LE11 3TU, Great Britain

(Received: 8 September, 1981)

ABSTRACT

Whilst paper and derived products still dominate packaging and large quantities of glass are still used in containers, the usage of plastics is significant, particularly for food packaging. Plastics are advantageous in providing good barrier properties in packages of low density (low weight) and high durability at low cost.

Primary packaging of foodstuffs utilises film and containers principally; secondary packaging involves a wider variety of forms, including tote boxes and crates. In a cost-sensitive market, the commodity thermoplastics—polyolefins, poly(vinyl chloride) and styrene plastics—are widely employed in food packaging but there is considerable application of more expensive polymers in more advanced forms for special requirements.

Satisfactory plastics packaging should not adversely affect the food in contact with it. Whilst pure high polymers might be expected to be inert, residual monomer, residues from polymerisation ancillaries, adventitious impurities and deliberate additives, all of lower molecular weight than the polymer, might be migratory and require particular consideration.

INTRODUCTION

It is the purpose of this paper to set the scene for consideration of the use of plastics in food packaging, to which end basic properties will first be summarised, followed by a discussion of features which might seem to be surprising to those not familiar with the behaviour of polymers. Further, whilst plastics technology might appear to be well established, new developments likely to have a considerable effect on the packaging scene are taking place: these will be reviewed briefly.

Food Chemistry 0308-8146/82/0008-0081/$02·75

BASIC PROPERTIES OF PLASTICS

In general, plastics are organic compounds; that is, they are based on the element, carbon. They are also long chain or network structures. These two factors are the bases for a large number of properties of plastics; in respect of the former, low density, behaviour towards solvents, susceptibility to oxidation and flammability, low thermal conductivity and low electrical conductivity. High permeability to fluids, compared with metals and glasses, is a further consequence of organic structure. Plastics are inherently transparent, although this quality may be impaired by structural features or by additives; they also have low softening temperatures and thus require low energy for conversion. Properties dependent on the chain structure include high reversible extensibility—a factor contributing to toughness and the high viscosity of plastics melts and polymer solutions which dominates the manufacture of plastics articles.

A further feature of the structure of many plastics is that they are partially crystalline; that is, a part of the polymer molecules—frequently 50–70%—is arranged in regions of high order, entirely comparable with the crystals of low molecular weight compounds. Such polymer crystals have regular structures which can be analysed by classical techniques, including X-ray diffraction; they have identifiable melting points and appreciable latent heats of crystallisation. Crystals are, therefore, structural features of considerable importance and have a significant effect on the properties of partially crystalline plastics.

First, crystalline regions are of appreciably higher density than the non-crystalline material and so offer greater resistance to the diffusion of small molecules through the material. In fact, crystals are usually considered to be impenetrable. Secondly, the crystals of polymers, which are very small and frequently termed crystallites, aggregate into larger systems known as spherulites, which are comparable with, or larger than, the wavelength of light. Spherulites scatter some of the light transmitted through the plastic, giving the visual effect of 'haze' or 'opacity'. Crystallinity also has a significant effect on product manufacture from partially crystalline plastics.

The influence of crystallinity on the solution properties of plastics is of considerable practical importance, not least in packaging. Amorphous, or non-crystalline, plastics are frequently soluble in solvents of similar character—'like dissolves like'—i.e. polar solvents dissolve polar polymers and non-polar solvents attack non-polar polymers. As an example, poly(methyl methacrylate) dissolves in chloroform, acetone and ester solvents, but is resistant to hydrocarbons such as petrol. Polyethylene is a hydrocarbon polymer with a repeat unit of $—CH_2—$ and might, therefore, be expected to be susceptible to hydrocarbon solvents whilst being resistant to polar solvents. However, polyethylene is partially crystalline and this restricts solution even in the most suitable and obvious solvents. In fact, solution can only be effected by heating polyethylene–hydrocarbon solvent systems to high

temperatures when the stability resulting from crystal binding energy is considerably reduced. Polyethylene can be dissolved in xylene at its boiling point, but high density polyethylene (HDPE) is used for automobile petrol tanks at ordinary temperatures. The stabilising effect of crystals is thus of great significance.

THE IMPORTANCE OF ADDITIVES IN PLASTICS TECHNOLOGY

The terms 'plastics' and 'polymer' have been used indiscriminately, since we have been concerned with general matters so far. 'Polymer' is a precise term implying that the material has a repetitive character giving chain or network structures.

'Plastics' has a very different connotation, implying shapeability at some stage in the history, and, by common usage, a material which is comparatively rigid, in contradistinction to a rubber. Plastics are the raw materials from whence films, bottles and other products are manufactured. Plastics raw material manufacturers have significant, but limited, scope for tailoring their products to the requirements of the many applications for which plastics are now used; additives further extend the usefulness of plastics in many areas.

Plastics compounds in commercial use, therefore, almost invariably contain additives, including pigments and stabilisers. This latter class of additive is very important in plastics used for packaging, protecting the material during processing and use.

Polymers are unlikely to be affected by liquids, unless such liquids are recognised as solvents for the material. Solubility is dependent on molecular weight, species of low molecular weight being the more soluble, but commercial products usually contain little such material. Thus, reaction between a polymer and a liquid which is not a solvent is minimal. However, stabilisers are frequently low molecular weight organic compounds and their inclusion in a plastics compound may affect the interaction with liquids quite markedly: the stabiliser might be extracted. This has the double effect of polluting the liquid and leaving the plastics material susceptible to degradation which might lead to harmful products. In plastics for food packaging, therefore, it is desirable that stabilisers should preferably be non-extractable but, more usually, they are extracted and should then be non-toxic.

OTHER ASPECTS OF PLASTICS TECHNOLOGY RELEVANT TO PACKAGING

To those who are not experts in the field of plastics, there is a bewildering variety of materials available, seemingly with an almost infinite range of properties. Happily, for the packaging technologist, there is some limitation by price; indeed, this is well exemplified by the subject matter of the principal papers in this Symposium.

However, there may be some temptation to modify a particular plastics material

with a second polymer. It must be appreciated that polymer–polymer systems are only rarely homogeneous, i.e. polymers are generally incompatible one with another. This is true—perhaps not unexpectedly—for very different polymers, e.g. polyethylene and poly(vinyl chloride), but, equally, polyethylene and propylene are not compatible. Perfect mixing of two partially crystalline polymers must be a very rare occurrence, implying, as it does, that co-crystallisation takes place. On this strict interpretation of compatibility, even high density polyethylene and low density polyethylene are not miscible, since crystals appropriate to the two species can readily be identified when such a mixture is cooled from the melt.

It has been stated above that crystallinity provides a barrier to the penetration of plastics materials by permeating species. The disposition of the crystallites is also extremely important for, if they can be arranged in the plane of the film or container surface, they are particularly effective in impeding the passage of diffusing materials. Such morphological features can be obtained by orientation under controlled conditions, and since these same features lead to enhanced strength, this allows the use of very thin packaging with obvious cost implications. There are disadvantages to the use of highly oriented films, in that they cannot be heat sealed, as heating above the melting point, necessary to effect a heat seal, has disastrous consequences.

It is now common practice to provide for heat sealing of oriented films by layering a lower softening temperature polymer on them. Indeed, the manufacture of multi-ply films is carried out increasingly at the behest of the packaging technologist. Such multi-ply films, however, make it very difficult to recycle the plastics materials economically.

NEW DEVELOPMENTS

Mention has been made in the previous section of improvement in barrier properties consequent on orientation; biaxially oriented films based on poly(ethylene terephthalate) and on polypropylene have been in common use for a number of years. It is only recently, however, that a manufacturing process has been developed for biaxially oriented bottles, allowing further progress in plastics containers for potable liquids.

Although it seemed, a few years ago, that the range of commodity plastics suitable for food packaging was unlikely to change, the development of a new polymerisation process for ethylene has resulted in the appearance of olefin-copolymers with characteristics and properties different from those of traditional low density polyethylene, LDPE. These linear low density polyethylenes, LLDPE, are likely to become increasingly available at a favourable price and seem certain to feature in films and other packaging.

Food Chemistry **8** (1982) 85–96

PVC AS A FOOD PACKAGING MATERIAL

R. B. PEARSON

Imperial Chemical Industries Limited, Petrochemicals and Plastics Division, PO Box No. 6, Bessemer Road, Welwyn Garden City, Herts, Great Britain

(Received: 8 September, 1981)

ABSTRACT

Manufacture of polyvinyl chloride (PVC) in western Europe for all applications is now almost 4 m tonnes and the UK accounts for about 400 kt of this.

The aim of this paper is to illustrate the relative importance of PVC as a packaging material where, in the UK for example, for food packaging it accounts for almost 40 kt (10%) of the PVC used.

The different types of PVC packaging material—bottles, foil, cling film and cap sealing—and their method of manufacture are described, together with the range of food packaging applications in which they are used. The physical properties of PVC are described and, additionally, its cost and competitiveness with other materials are compared. The chemical nature of PVC compositions arising from the addition of heat stabilisers and plasticisers to achieve the desired properties is also described, with particular reference to heat stability.

With the VCM toxicity issue now behind us, and PVC polymer with a maximum level of 1 ppm vinyl chloride monomer (VCM) being supplied for food packaging, PVC must be the most stringently controlled and thoroughly researched material for food packaging. Recent developments in polymerisation technology and polymer manufacture, which have enabled this level to be achieved, are briefly described.

The current situation on the global migration issue, on which attention is currently focused, not only for plasticised PVC, but also other packaging materials as well, is briefly reviewed.

INTRODUCTION

Polyvinyl chloride (PVC) is one of the major thermoplastic materials used in foodstuffs packaging in western Europe and, indeed, throughout the world.

Food Chemistry 0308-8146/82/0008-0085/$02·75

Total manufacture for all applications in western Europe is now almost 4 m tonnes and its use in foodstuffs packaging accounts for an important tonnage.

There are, however, significant differences in emphasis in its use between the major countries and this is illustrated in Table 1.

TABLE 1
MAJOR APPLICATION AREAS IN WESTERN EUROPE FOR PVC AS A FOOD PACKAGING MATERIAL

	kt
France, PVC bottles	150
Germany, rigid thermoformed PVC foil	50
Italy and the UK, PVC cling film	20

The total tonnage of PVC used throughout western Europe for foodstuffs packaging is not known with any precision but is conservatively estimated at about 300 kt.

Turning our attention now to the UK, where manufacture of PVC for all applications is of the order of 400 kt, there are four basic types of PVC material used for packaging a wide range of foodstuffs. These four basic types, together with an estimate of the tonnage used for packaging in a wide range of foodstuffs, are given in Table 2.

TABLE 2
PVC AS A FOOD PACKAGING MATERIAL IN THE UK

Type	*Tonnage (kt)*	*Principal foodstuffs*
Bottles	12	Fruit squash, cooking oil, mineral water
Rigid thermoformed foil	12	Confectionery, biscuits, cakes and dairy products
Cling film	10	Fresh meat, poultry, fruit, vegetables, cheese
Cap sealing	3	Carbonated beverages, preserves

As an application area, the use of PVC in foodstuffs packaging in tonnage terms ranks with cables, 50 kt, flexible calendered sheeting, 30 kt, and floor coverings, 30 kt.

Having briefly mentioned the four basic PVC packaging types, I would now like to describe in a little more detail the various polymers used, and the manufacture of the different packaging materials and also comment, in passing, on the range of foodstuffs they are used to package, paying particular attention to the chemical aspects of the different stages. (I do not intend to deal with migration from the packaging material to the foodstuff since this will be covered in an accompanying paper in this issue.)

PVC POLYMERS USED FOR PACKAGING

The most widely used route for manufacturing PVC polymer is by polymerisation of the vinyl chloride monomer (VCM), in aqueous dispersion in the presence of a catalyst and a dispersing agent. The polymer is isolated at the end of the reaction, either by mechanical filtration followed by drying or, in the case of emulsion polymer, drying all the ingredients of the reaction. The most commonly used comonomer employed in copolymer manufacture is vinyl acetate monomer (VAM) to give vinyl chloride/vinyl acetate (VC/VA) copolymers of 10–15 % acetate content.

The only other commercially used route for PVC manufacture is the mass process but this, in tonnage terms, is very much less than the suspension route and is limited to homopolymer manufacture. The remainder of this paper is therefore devoted to PVC made by the aqueous route.

The three basic types of aqueous polymerised PVC used for the manufacture of the different packaging materials are listed in Table 3, together with an indication of the polymerisation residues remaining in the polymer after isolation.

TABLE 3
BASIC PVC POLYMER TYPES AND THEIR COMPOSITION

Type	*Composition*	*Packaging application*
Suspension PVC homopolymer	Very pure, trace polymerisation residues	Bottles, thermoformed foil, cling film, cap sealing
Suspension PVC copolymer	10 % acetate copolymer, trace polymerisation residues	Thermoformed foil
Emulsion PVC homopolymer	Up to 1 % polymerisation residues, mainly emulsifier	Cap sealing

However, even the trace polymerisation residues can confer advantages in both suspension and emulsion PVC during subsequent processing. With the increasing use of calcium/zinc stabiliser systems the response of different PVC's to zinc stabilisers, so-called zinc sensitivity, is becoming more important. In particular, trace quantities of hydroxyl-containing dispersing agents, such as cellulose or poly vinyl alcohol, present in aqueous polymerised suspension PVC, show advantages over PVC made by other routes.

The relatively high level of polymerisation residue, emulsifier soap, in emulsion PVC is dictated by the need to produce a very fine particle size PVC, of the order of 0·1 micron, in the latex for subsequent plastisol or paste manufacture.

Before the polymer can be processed into a packaging material, it has to be blended with the appropriate additives such as heat stabiliser, lubricant, processing aid, impact improving modifier or plasticiser according to the different application.

I propose next briefly to outline in turn the different types of formulation used for

each application, the subsequent conversion process into the packaging material and, finally, its physical properties, together with some indication of its competitiveness against other materials.

Bottles

The most commonly used PVC for bottle manufacture is a low molecular weight suspension PVC, K value, 57–58; polymerisation degree, about 650, with a porous open structure.

The principal ingredients in the formulation would be as follows:

PVC
Impact modifier
Processing aid
Stabiliser
Lubricant

The impact modifier generally used is of the methacrylate butadiene styrene (MBS) type which enables the PVC impact strength to be considerably improved without hardly affecting its clarity. The modifiers and processing aids now used have such good organoleptic properties that they can be used in the manufacture of bottles to package even that most sensitive of products—mineral water.

Two basic types of heat stabiliser systems are used for bottle manufacture, tin and calcium/zinc salts. While octyl thio tin stabilisers remain the most widely used; the newer calcium/zinc systems now available are capable of approaching the quality in terms of sparkle, clarity and colour, only believed possible with the tin stabilised systems of a few years ago.

The lubricant system is generally a combination of fatty acid ester and a polyethylene or similar type wax.

All these ingredients are blended together in a high speed mixer and then converted directly, or via a pre-compounding route, into a bottle in an extrusion blow moulding machine designed specifically for handling PVC. More recent machinery developments now permit both extrusion stretch blow moulding and injection stretch blow moulding, both of which give bottles with improved physical and chemical properties.

The range of foodstuffs packed in PVC bottles is wide, from fruit squash, with its content of aggressive natural oil, through the various edible oils to that most sensitive of products, mineral water. That these can all be safely packed in PVC is a tribute to the skill of the formulation chemist and the inertness of PVC.

Rigid thermoformed foil

The most widely used PVC employed for foil manufacture is either a low molecular weight suspension homopolymer, K 57–60; polymerisation degree, 650–750, for shallow forming or a similar polymer containing 10% copolymerised

acetate for deeper thermoformings. Alternatively, for many general purpose applications, a blend of the two in differing proportions is increasingly used.

The formulation ingredients used are very similar to those described above for bottle manufacture, an application with which it has many similarities.

The foil for thermoforming can be produced by either calendering or direct foil extrusion. The resulting foil, typically in the range 125–375 microns, is then fed in a separate operation to a thermoforming unit where it is heated and drawn into the mould, usually by vacuum. Other forming techniques such as plug assisted or drape forming can also be used.

The thermoformed foil is used for packaging a wide range of confectionery, biscuits and also some dairy products such as trifle portions. The great advantage of PVC is again its clarity, inertness and toughness.

Cling film

The most commonly used PVC for cling film manufacture is a medium to high molecular weight suspension PVC, K 67–71, polymerisation degree, 1050–1350, with a porous open structure. The principal ingredients in such a formulation would typically be as follows:

PVC
Adipate plasticiser
Epoxised soya bean oil
Heat stabiliser
Lubricant
Anti-fogging agent

The overall level and balance between the two plasticisers is carefully chosen to enable an appropriate transmission of oxygen, carbon dioxide and water vapour to be achieved and so allow the packaged foodstuff to breathe. This is particularly essential in the packaging of red meat to enable its colour and freshness to be maintained.

The heat stabiliser that has always been used in this application is the calcium/zinc type whose efficiency is considerably boosted by the relatively high levels of epoxised soya bean oil necessary in the formulation for other reasons.

The lubricant is of the conventional fatty acid ester type while the anti-fogging agent, usually of the polyoxyethylene sorbitan mono oleate chemical type, enables the fine droplets of condensed water vapour to coalesce and maintain the high clarity of the packaging material.

As with bottles, the ingredients are blended together and then blow extruded directly into tubular film. The film so produced is very thin, typically in the 10–20 micron range, and is used for packaging red meat, poultry, fruit, vegetables and cheese. In addition to the controlled barrier properties, the clarity and sparkle of the film enhances the presentation of the product and enables it to be readily inspected.

Cap sealing

This is the wad insert, in a screw, crown or lever top, usually aluminium or tin plate, used to seal a container. It can be made either from calendered plasticised sheeting and stamped out or, more usually, from a paste composition.

The type of PVC used for the paste manufacture is made by emulsion polymerisation and the route chosen is such as to enable it to give a low viscosity when mixed with plasticiser.

The principal ingredients used in the formulation would be as follows and would be broadly similar for both a suspension and an emulsion polymer-based formulation:

PVC
Plasticiser
Stabiliser
Blowing agent

The plasticiser is carefully selected to have a low odour and taint. The stabiliser would be of the calcium/zinc type, while the blowing agent would be of the azo dicarbonamide type.

The formulation ingredients are blended up in a slow speed mixer. A metered amount of paste is then applied to the inside face of the cap and gelled in an oven at 200 °C, when the composition also expands due to the decomposition of the blowing agent.

PVC-faced cap seals are used for closing a wide range of products, mainly carbonated beverages but also preserves. They have the advantage of giving a very good gas-tight seal.

Having described the various routes by which the four different packaging materials are manufactured, it is appropriate now to outline the main physical properties which single out PVC for foodstuffs packaging from the other materials available.

PHYSICAL PROPERTIES OF PVC

The major physical properties of PVC bottles and rigid thermoformed foil which make it a preferred material for packaging a wide range of foodstuffs are as follows:

Rigidity
Glass-like clarity
Good impact performance
Good gas barrier properties
Good chemical resistance

In the cling film area the properties which single it out over the other potential thermoplastic materials are as follows:

Sparkling clarity
Controlled gas barrier properties
Self blocking and sealing

The principal property of PVC cap sealing material, where clarity is of no importance, is to give a very good gas-tight seal, which is particularly important for carbonated drinks.

Having now described the manufacture of the different PVC packaging materials, it is appropriate to consider how they compare with other materials which are, or could be, used.

THE COMPETITIVENESS OF PVC

The greatest single in-built advantage of PVC must be that it is more than half inorganic—i.e. 57% chlorine—and therefore potentially less vulnerable to oil feedstock fluctuations.

In this context, the comparison is usually expressed as tonnes of oil equivalent required both for manufacturing the raw material and for converting it to the end

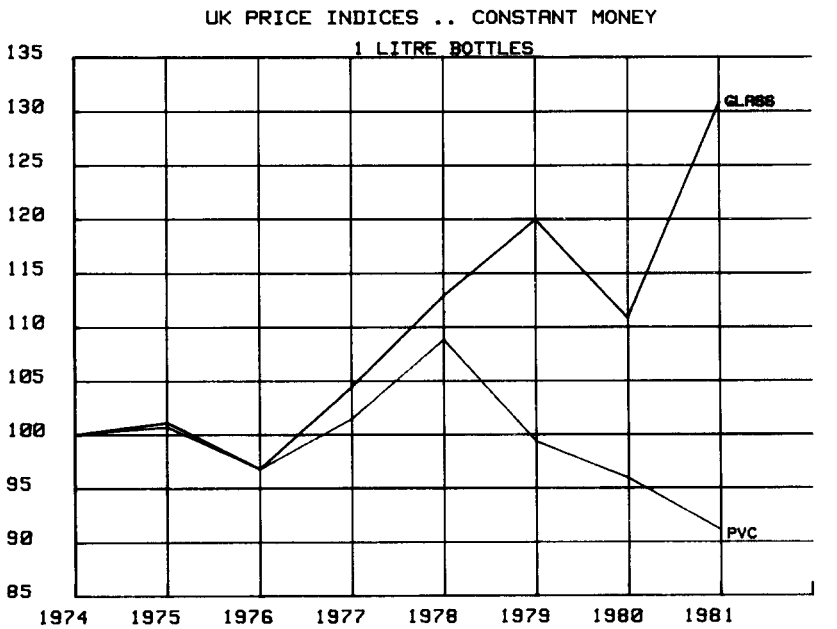


Fig. 1. Price movement of PVC against glass.

TABLE 4
ENERGY CONTENT OF SOME CLEAR PACKAGING MATERIALS

Material	*TOE for 1 million 1 litre bottles*
PVC	66
PET (polyester terephthalate)	150–175
Glass (non-returnable bottles)	230–300

product, tonnes of oil equivalent (TOE). In this comparison PVC is particularly well favoured, as Table 4 shows. This table is based on the latest data agreed between a number of western European chemical companies.

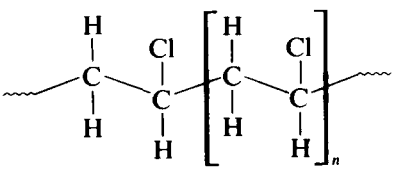
The competitiveness of PVC with other materials, such as glass, under present market conditions is best illustrated by Fig. 1 which charts the price movement of PVC and glass over the past 15 years or so.

Comparisons on the same basis with other traditional packaging materials, such as paper and board, aluminium and tinplate, give a very similar picture.

THE HEAT STABILITY OF PVC

Because of its sensitivity to thermal treatment, no description of PVC would be complete without a reference to heat stability. Indeed, in many ways the response of PVC to heat, i.e. its heat stability, is the most interesting aspect of its chemistry and has attracted the interest of chemists for many decades.

PVC is formed in a regular head to tail manner as illustrated below:



However, this is an idealised structure which, in practice, is never achieved, since such a polymer would have remarkable inherent stability based on studies of similar model compounds. Various sites for instability have been identified in commercial PVC, including internal allylic chlorides, tertiary chlorides, terminal allylic chlorides and secondary chlorides.

PVC decomposes by progressive dehydrochlorination and develops the characteristic colours ranging from clear to yellow to orange to brown and, finally, black. The mechanism of decomposition of PVC is still not completely understood, established or agreed. Both ionic and free radical mechanisms have been proposed to explain the progressive elimination of hydrogen chloride from PVC and both processes could be at work.

In practical terms, however, it is the initial colour which determines the appearance of the packaging material and its long-term heat stability which influences the economics of the polymer to package conversion process.

It is now accepted that, in order to produce the most stable type of PVC material, the greatest care should be exercised in selecting polymerisation and stabilising ingredients and minimising thermal treatment from the polymer manufacturing stage onwards.

It is an understanding of all these factors by polymer chemists and others that has led to the successful development of modern PVC and stabilising systems.

In the packaging area the two most important stabiliser systems used are di-*n*-octyl tin thio glycollate and combinations of calcium and zinc salts, the latter often boosted with complex organic materials.

The advances made with calcium/zinc systems particularly are now such that they are capable of approaching the performance of tin systems to an extent not believed possible a few years ago.

VCM TOXICITY

Another aspect of PVC which has taxed the skill and ingenuity of chemists and others in more recent years is the VCM toxicity issue. No mention of PVC in foodstuffs packaging nowadays would be complete without some reference to it, and much has been written about this important aspect over recent years.

In 1974 PVC faced the biggest challenge in its history when a link between inhalation of VCM, and angioscarcoma in man was confirmed. As a result, over succeeding years, the level in all polymer for food packaging was rapidly reduced to the current level of 1 ppm maximum.

By way of illustration, I will briefly describe how this was achieved in PVC for bottle manufacture by a combination of engineering and chemical modifications, firstly at the blending mixer stage and then later at the polymer manufacturing, isolation and drying stages.

Table 5 summarises the steps involved in terms of VCM level and polymer

TABLE 5
PROGRESSIVE REDUCTION IN VCM LEVEL IN PVC FOR BOTTLE MANUFACTURE

Date	*Polymer porosity* (%)	*VCM level (ppm)* *polymer*	*blend*	*Comments*
1974	7	1000 max	100 max	Heating to 135 °C Aspiration of mixer
1975	7	250 max	5 max	Improved stripping of VCM during polymer isolation
1977	12	30 max	1 max	Improved porosity at polymerisation stage
1980	20	1 max	1 max	Further improved porosity at polymerisation stage

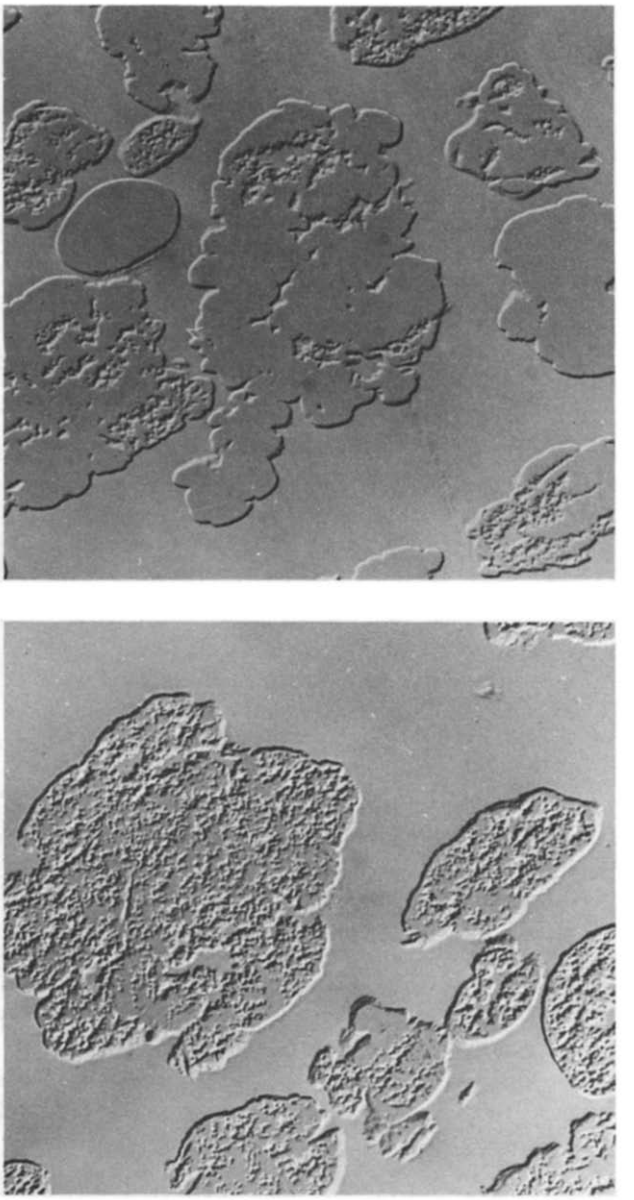


Fig. 2. Microphotograph section of 1974 and current K57 polymer. Top: 1974 polymer. Bottom: 1981 polymer. PVC suspension polymer: Sectioned granules (transmitted differential contrast microscopy. × 320).

porosity. A fuller description was given at the recent Plastics and Rubber Institute Conference on Advances on Blow Moulding II, held in London, on 20 and 21 May, 1981, in the paper entitled 'Recent PVC Material Advances in Extrusion Blow Moulding'.

The microphotograph sections in Fig. 2, of 1974 polymer compared with current material, illustrates the visible improvement in porosity.

In practice, the level of VCM in the packaging material is now very much less than 1 ppm and the migration of VCM into foodstuffs in contact with it is below the limit of detection, currently 0·010 mg/kgm (10 ppb w/w), but can be even lower, 2 ppb w/w, depending on the foodstuff. That this has been achieved is a tribute both to the polymer chemists and skill of the analytical chemists involved.

With the VCM toxicity issue now behind us, therefore, PVC must be the most stringently controlled and thoroughly researched material for food packaging. However, one problem still remains to be resolved, although it affects all thermoplastic—and even traditional—packaging materials, but is of particular concern for plasticised PVC for cling wrap and cap sealing, and that is global migration.

GLOBAL MIGRATION

A formal EEC proposal for a directive on an overall migration limit for plastics materials or articles intended for contact with foodstuff, COM (78) 115 Final, dated Brussels, 29 May, 1978, was put forward in 1978 after many years of discussion at the Commission. It limits the migration into foodstuffs to 60 mg/kg or its nominal equivalent of 10 mg/dm^2.

The proposed directive has been the subject of a very great deal of discussion within the Council and has not yet been adopted.

The UK and German Government authorities continue strongly to oppose the directive for the following reasons:

Overall limit has no toxicological significance.
Arbitrary limits.
No classification of foodstuffs.
No list of approved additives.
No agreed test methods to police it.

A number of thermoplastic materials, including PVC cling film and cap sealing wads, could be excluded by the directive as currently drafted. Discussions at the Council and Commission levels are continuing.

The German Federal Health Office has also been examining PVC cling film following its proposal in 1979 to withdraw approval on PVC film for meat wrap. As a result, the use of cling film in Germany is now controlled to the extent that a

maximum level of plasticiser of 22 % must not be exceeded and the migration limit to foodstuff must not exceed 100 mg/kg.

The film must also be suitably labelled to limit its use solely to wrapping fresh meat.

CONCLUSION

In conclusion, I hope I have been able to demonstrate the importance, scale and versatility of PVC as a food packaging material. In particular, I also hope I have been able to highlight the areas where the chemist can practice his craft, ranging from the polymer manufacturing stage, to development of improved PVC packaging material through to the development of analytical techniques to satisfy the various legislative authorities of the safety and suitability of PVC as a food packaging material.

Food Chemistry 8 (1982) 97–107

STYRENE POLYMERS AND FOOD PACKAGING

C. A. BRIGHTON

1 Gatesdene Close, Little Gaddesden,
Berkhamsted, Herts HP4 1PB, Great Britain

(Received: 8 September, 1981)

ABSTRACT

Polymers based on styrene monomer can be produced with a wide range of properties by co-polymerisation and the incorporation of rubber impact improvers. In some areas of food applications, polystyrene is under strong competition from polypropylene—the price is obviously an important factor.

For margarine packaging, thermoformed tubs based on acrylonitrile–butadiene–styrene still dominate the market.

The usual considerations of possible toxic hazard by migration of low molecular components of the polymers apply; the two most likely materials which will be in the polymers are styrene with ethylbenzene and acrylonitrile. Styrene is now considered to be fairly safe and the organoleptic effect is the controlling factor: acrylonitrile presents a more serious problem and is under very critical study—it is necessary to impose strict limits on migration into the food.

INTRODUCTION

The polymers of styrene continue to be used extensively for packaging—and particularly food packaging—in spite of fierce competition from other thermoplastics such as polypropylene. This has led to the development of improved grades of both the homopolymer and the copolymers so that the use of this class of thermoplastics continues to expand.

The normal general purpose polystyrene gained early acceptance in the packaging field because of its easy processing, good transparency and rigidity, but these properties are offset by its poor impact strength, the ease of stress cracking and other characteristics such as the tainting of food by residual traces of monomeric styrene.

Food Chemistry 0308-8146/82/0008-0097/$02·75

Before considering the material developments which have led to the extensive use of styrene-containing polymers for food packaging, it is helpful to list the property requirements for an acceptable performance:

(a) Easy processing to packs at a speed to make them competitive in price.
(b) Mechanical properties adequate for modern handling techniques at a wide range of temperatures by the food processor.
(c) A pack giving the required storage life for the food—this raises such issues as tainting, toxicological hazard and the permeability to water and oxygen.
(d) Attractive sales presentation, i.e. shape, print design, quality and colour.

Polystyrene met these requirements for certain food applications but a major step forward was the development of high impact polystyrene. The improvement in impact strength—65–80 J/m as compared with 13–19 J/m for normal polystyrene—is obtained by inclusion of a synthetic rubber, either SBR or polybutadiene, into the polymerisation stage of manufacture. There are basic methods of doing this, by mass or single phase and suspension polymerisation.

SINGLE PHASE (MASS) POLYMERISATION

In the first method, the reaction is carried out in two stages; the styrene, containing about 5 % of dispersed rubber and, possibly, ethylbenzene as diluent, is polymerised to a syrup containing about 25–30 % polymer in a prepolymeriser, which is a stirred reactor operating under reduced pressure and equipped with a reflux condenser. The operating temperature varies from about 80 °C, if peroxidic initiators are used, and up to about 130 °C when the polymerisation is initiated thermally. The use of a reflux condenser facilitates the removal of the heat of polymerisation by boiling off

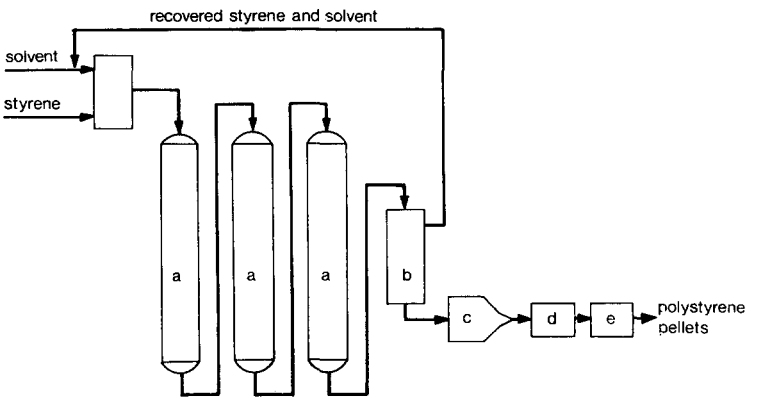


Fig. 1. Single phase (mass) polymerisation. a, Reactor; b, devolatiser; c, extruder; d, cooler; e, cutter.

part of the unreacted monomer, condensing it and returning the cooled liquid to the reactor. The syrup passes continuously into a series of reactors for the second stage in which the temperature is raised progressively from about 120 °C to 170 °C. The reactors are provided with Dowtherm tubes and cooling is necessary to remove the heat of reaction at the lower temperatures but as the degree of polymerisation reaches the final stages, heat is applied. The polymer containing some residual monomer and the diluent then passes to a devolatiser, operating at 225–250 °C under reduced pressure, where the volatile residuals are flashed off, condensed and recycled to the monomer feed. Finally, the polymer is extruded into strands and granulated. This technique is used for both the general purpose and high impact grades of polystyrene (Fig. 1).

SUSPENSION POLYMERISATION

Suspension polymerisation has some advantages over single phase techniques inasmuch as heat removal can be controlled more easily, but there are disadvantages such as the need for a dispersing agent. The suspension method is carried out in large jacketed reactors fitted with agitators, the styrene monomer being maintained as droplets in the aqueous phase, with diameters varying between 0·4 and 1 mm, by the use of a dispersing agent such as partially hydrolysed poly(vinyl acetate) or inorganic phosphates. Oil-soluble initiators are dissolved in the monomer phase; it is usual to use two different initiators so as to maintain a steady rate of polymerisation and to achieve a low residual monomer content in the final product. A high level of technological expertise is required to maintain the dispersion of the styrene during the reaction and avoid agglomeration into a single mass. After polymerisation, the beads are centrifuged and then the volatile residues are removed by passage through a devolatilising extruder. When this technique is used for rubber modified polymers, a mass type prepolymerisation step is included. At this initial stage, the rubber solution in styrene is subjected to polymerisation with shearing agitation until phase inversion of the two-phase polystyrene in styrene and rubber in styrene has taken place. This will occur normally at about 30 % solids level, when the prepolymer may be converted into a suspension by the addition of water and suspension stabilisers. (Fig. 2).

The improvement of impact strength is obtained only when the rubber appears in the final polymer as discrete particles with a diameter of about 5 microns. Although the rubber needs to be substantially insoluble in the styrene, some degree of swelling is required so as to improve the opportunity for grafting between the elastomer and the matrix to produce improved adhesion. This is achieved by using a rubber which is partially cross-linked, and it has been found that a medium gel content (5–20 % insoluble in toluene) is most effective. The effect of the amount of a styrene–butadiene (SBR) rubber on the impact strength of high impact polystyrene is shown in Fig. 3.

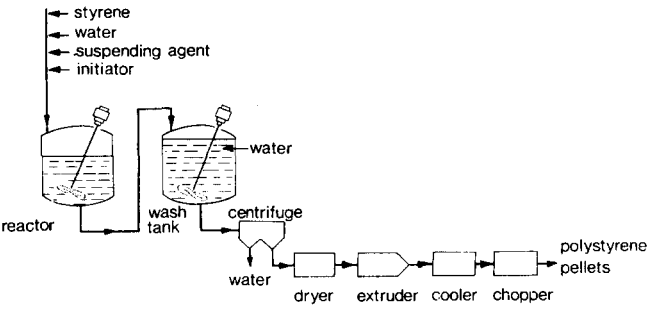


Fig. 2. Suspension polymerisation.

The improved impact strength is obtained at the expense of a reduced tensile strength, lower hardness and a reduced softening point. Because the rubber has a different refractive index from that of the polystyrene, the high impact (HI) grade is translucent instead of having the high clarity of general purpose polystyrene. There are now, however, grades of HI polystyrene with much better clarity, although still not as good as the unmodified material.

This can be achieved by using a styrene–butadiene block copolymer as the reinforcing agent in a single phase mass polymerisation system. The block copolymers are fundamentally different in structure to the random distribution of normal styrene–butadiene copolymers in that the styrene units form one or two blocks, and the diene units form a further block; these blocks are joined to form a single molecule with the general form ABA, where A and B represent the blocks of styrene and diene molecules, respectively. These polymers are transparent and enable high impact polystyrene to be produced with improved contact transparency.

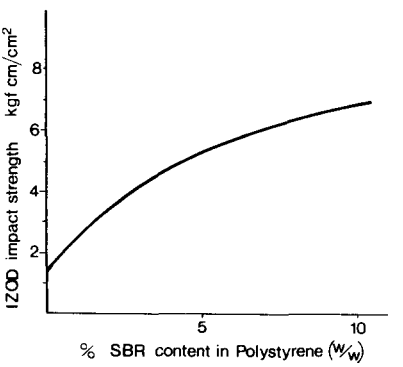


Fig. 3. Variation of impact strength with rubber content.

By variation of the polymerisation conditions, it is possible to produce the different types of polymer with a range of properties and processing characteristics so that a number of grades are offered by the manufacturers to meet the differing requirements of the conversion process and the final product.

POLYMERS OF POLYSTYRENE

The important differences between the general purpose and high impact (rubber modified) grades is not only the relative impact strengths but also the much higher ductility which is indicated by the elongation at break; the value for the normal polymer is between 1 and 2·5 %, while this increases to 20–30 % for the high impact grades.

For food packaging applications, in addition to the mechanical properties, the permeability to moisture and atmospheric gases is important. The values for both the general-purpose (GP) and high impact grades are:

	GP	*HI*
Oxygen[a]	300–350	380–410
Carbon dioxide[a]	900–1250	2570
Water vapour[b]	13	13

[a] cm^3—nil/100 in^2, day-atm at 73 °F.
[b] g—nil/100 in^2, day at 100 °F (100 % RH).

The polymers always contain small amounts of residual monomer and diluents such as ethylbenzene. The monomer can arise because of incomplete removal during the devolatilisation process and also because of breakdown of the polymer during processing. When polystyrene is heated to about 300 °C, breakdown occurs and results substantially in monomeric styrene. The level of this monomer in commercial grades of polymer has been gradually reduced over recent years and is now generally guaranteed at less than 0·1 %, with a working level of about 0·05 %, which ensures that the materials are suitable for food packaging.

USAGE FOR FOOD PACKAGING

At the present time, the total usage of polystyrene in the UK is about 130,000 tons/year; this is broken down to 60 % of high impact and 40 % of normal general purpose polystyrene. About half of the material goes into food packaging with vending cups accounting for about a third of this quantity. These cups are made by thermo-forming using extruded sheet which includes the formed material.

The recession has adversely affected the usage of vending cups—it is a good

barometer of the economic climate—because of the reduced hours of working. In order to produce the cups at the lowest price, it is normal to use a blend of high impact and normal impact grades. This is not a critical application, the thickness of the cup is just sufficient to give adequate rigidity and the beverage is in contact with the plastics material for a limited time. Under such circumstances there is no problem of possible migration or extraction of any of the low molecular compounds in the polymer, these include the residual monomer and additives such as lubricants.

The situation is rather more complex with containers for dairy products, which account for a large proportion of the polymer used for food packaging. There are limitations to the type of products which can be packed; essential oils cause rapid stress cracking of polystyrene and products containing fruit can cause problems. In addition to the possible migration into the food of low molecular weight compounds from the polymer, the walls of the package are permeable to some extent to atmospheric gases and moisture vapour, so that account must be taken of the effect of these—and particularly oxygen—on food under storage. Also, it is possible that the food may contain certain volatile ingredients which can be lost through the sides of the container.

All monomers for the plastics used in food packaging have come under close scrutiny since the serious toxicological effects of vinyl chloride became known. Styrene has been examined very thoroughly and toxicologists are satisfied that the monomer does not present a hazard if there is migration into the food; there remains, however, possible infringement of the Food and Drugs Act and the more difficult problem of tainting of the food. The detection of the styrene monomer depends largely on the smell, rather than the taste, so that styrene is detectable at much lower concentrations in aqueous foods at levels of about 1 ppm, whereas, in oils and fats, the level of detection increases to about 10 ppm. Experience has shown that styrene is much more objectionable than its main impurity, ethylbenzene.

EXPANDED POLYSTYRENE

The ease with which polystyrene is affected by organic solvents enables expanded materials to be produced relatively easily. The advantage of having a polymer in a cellular form is twofold: first, the rigidity of a moulded shape is several times greater than that of a similar shape of the same total weight in normal material and, secondly, the thermal conductivity is very much reduced—this can be exploited in food packaging for making containers for retaining steady thermal conditions such as packs for hot food from a Chinese take-away or boxes for transporting fish, packed in ice, over long distances. Expandable polystyrene beads are produced by polymerisation with about 5% to 7% pentane; when these are heated to temperatures above 95 °C, the polystyrene begins to soften and the hydrocarbon is released from the solid solution and the beads expand by the formation of a large

number of small cells. This stage is normally carried out with steam because it is an efficient heat transfer medium. The individual cells in the beads after expansion contain water vapour and pentane and, as the material cools, these latter condense to create a partial vacuum inside the cells. Air permeates into the individual cells and slowly replaces the remaining pentane; this process takes many hours and it is essential that it is complete before the expanded beads are used in the moulding process for making boxes and containers: the final density of such items produced in this manner is in the range 20–30 kg/m^3.

An alternative method for making expanded material in sheet form relies on extrusion of polystyrene incorporating a blowing agent so that, at the extrusion temperature (130 °C–150 °C), the polymer is in a fairly viscous state which prevents the material expanding too rapidly as the blowing agent vaporises and promotes the formation of consistent cells. The material is extruded in the form of a tube, which is then slit and opened out to form a sheet; the density of such a product is of the order of 80 kg/m^3. The sheet is stored for at least 24 h to allow any pentane remaining in the cells to migrate to the atmosphere; it is then formed mainly for shallow trays for packaging food items such as fish and meat and also for egg boxes.

WEATHERABILITY AND AGEING

Styrene-containing polymers are susceptible to degradation by the action of sunlight, due mainly to the uv radiation in the 350–400 nm waveband. The action of the uv radiation is accompanied by oxidation so that the overall degradation reaction is one of photodegradation. The effect can be detrimental, particularly to transparent polystyrene packs which are left in bright light for long periods; the high impact grades containing rubber modifiers are more sensitive to degradation because of the attack on the rubber unless antioxidants and uv stabilisers are incorporated in the materials. This effect is shown in Fig. 4 where the reduction in impact strength is followed, using accelerated ageing methods, for an unstabilised polymer and also for one containing a phenolic antioxidant. The ageing characteristics are also improved by pigmenting the material white and making it more reflective to uv radiation. The addition of uv absorbers such as the benzotriazoles is a very effective way of improving ageing, but such compounds might give rise to a toxic hazard when polymers stabilised in this way are used for food packs; fortunately, food packs are generally short term and are not stored in conditions of bright light.

To the environmentalists, plastics materials in the form of discarded packs and cups are creating a new litter problem: cellulose-based articles are no less offensive but, under the action of weather and bacteria, they break down and are absorbed in the soil.

There have been moves to introduce, for certain applications, styrene polymers with in-built self-destructive characteristics so that when a pack is discarded in the

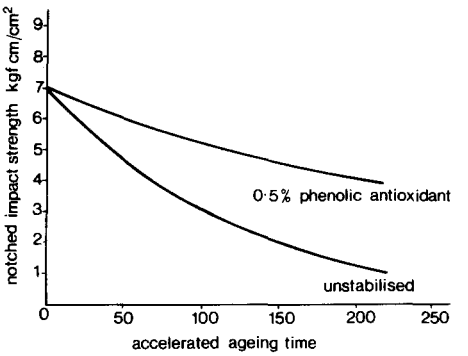


Fig. 4. Effect of antioxidant on the impact strength of high impact polystyrene during accelerated ageing.

open it very quickly degrades and disintegrates into very small pieces which soon become unnoticeable. This can be achieved by two different routes—either by the addition of certain compounds which act as photo-initiators or by the introduction of 'weak links' into the polystyrene molecule by copolymerisation with reactive vinyl carbonyls such as phenylvinyl ketone. The carbonyl groups in the polymer chain offer sites for very ready ultra-violet excitation leading to chain scission. The rate of degradation depends on the concentration of carbonyl groups and generally 10 mole % of a vinyl ketone is sufficient.

The normal practice is to blend a special reactive degradable polymer with the normal grades to achieve the desired breakdown characteristics. In this way, the processor can adjust the proportions to suit the application. For example, 20 % of a styrene/vinyl ketone copolymer offered by one manufacturer in a blend with normal polystyrene will embrittle after twenty days of outdoor exposure. Unfortunately, this increases the material cost and now that the price of plastics has increased so considerably over recent years, the competitive situation has made this approach to disposal of less interest.

STYRENE COPOLYMERS

The relatively poor chemical resistance and high permeability impose limits on the use of polystyrene for certain types of foods—reference has already been made to the problem of stress cracking in the presence of essential oils. This has been largely overcome by copolymerisation and the styrene/acrylonitrile (SAN) and acrylonitrile–butadiene–styrene (ABS) polymers are now widely used.

There is now a range of materials based on styrene and acrylonitrile, but the application for these materials has become limited over recent years because of the

toxicological hazards associated with acrylonitrile which will always be present to some extent in the copolymer. The SAN copolymers retain most of the transparency of general purpose polystyrene but possess other physical properties which are markedly superior. Stress cracking is much reduced, particularly as the amount of acrylonitrile in the copolymer increases; the commercially available polymers have an acrylonitrile content in the range of 10 % to 37 %. The change in gas permeability with change of composition is shown in Fig. 5. Considerable development work has been carried out on the possible use of the high acrylonitrile content (70 %–80 %) copolymers for the manufacture of bottles for carbonated beverages because of the low permeability and high barrier effect which is an essential requirement. Unfortunately, the toxicological problems associated with acrylonitrile have severely restricted this use and it would seem that polyethylene terephthalate (PET) bottles are fulfilling the technical requirements without the attendant toxic risks. The SAN polymers—that is, those containing a minor proportion of acrylonitrile—do meet special requirements and the residual acrylonitrile content has, for the moment, been reduced to an acceptable level.

The ABS polymers have very much better physical properties than high impact polystyrene and certain grades are extensively used for thermoforming tubs for packaging margarine; last year 10,000 tons was used in the UK for this application. A tub designed to hold 250 g of margarine weighs only 10 to 11 grammes and has a wall thickness of 120 to 200 microns.

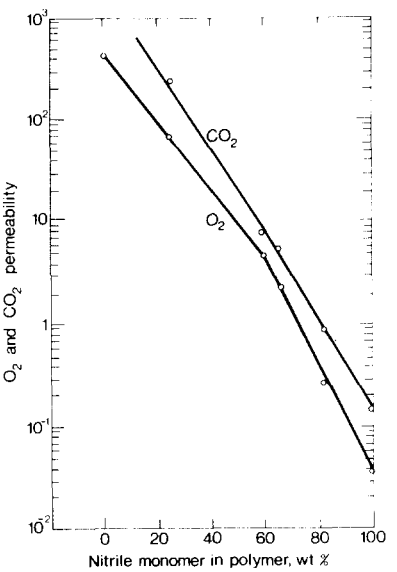


Fig. 5. Gas permeability ($cm^3/100 cm^2/day/atm$ at 23 °C) of acrylonitrile–styrene copolymers as a function of nitrile monomer content.

TABLE 1
PROPERTIES OF SAN AND ABS POLYMERS

Property	*Units*	*Test method*	*Styrene–AN (70–30)*	*ABS resins*			
				Medium impact	*Extra-high impact*	*Low-temperature impact*	*Heat resistant*
Tensile strength	lb/in^2	D 638	8300–11000	8800	4500–5500	3000–4000	7500–8000
	kgf/cm^2		583–773	618	316–386	211–283	527–562
Elongation at break	%	D 638	2·1–3·7	20	—	—	—
Modulus in tension	lb/in^2	D 638	$5·2–5·4 \times 10^5$	$4·0 \times 10^5$	$2–3 \times 10^5$	$1·0–2·6 \times 10^5$	$3·5 \times 10^5$
	kgf/cm^2		$0·37–0·38 \times 10^5$	$0·27 \times 10^5$	$0·14–0·21 \times 10^5$	$0·07–0·18 \times 10^5$	$0·25 \times 10^5$
Impact strength							
73 °F	ft lb/in	D 256	0·30–0·45	1·5	5–9	5·9	3–6
−40 °F of notch			—	0·4	0·6–2·5	1·5–3·5	0·3–1·0
Flexural strength	lb/in^2	D 790	—	13500	7600–8000	3400–6500	12000
Heat distortion temperature	0 °C	D 648					
At 264 psi			99–104	93·5–110	86–91	78–85	96–110
At 66 psi			—	101·5	97·5–98·0	98·5	104·5–115·5

There are now many different techniques for manufacturing the polymer using the single phase mass process and also suspension and emulsion methods. The first materials, however, were produced by mechanical blending of a butadiene–acrylonitrile rubber with styrene–acrylonitrile resins, the process being carried out under conditions such that some grafting occurs between the two polymers. This technique has now been superseded by chemical processes in which the styrene–acrylonitrile polymer is formed in a polymerisation system in the presence of a polybutadiene rubber; the final product is a complex mixture consisting of a styrene–acrylonitrile copolymer, a graft polymer of styrene–acrylonitrile with polybutadiene and some unchanged polybutadiene rubber. The proportions of the three components generally fall within the following limits:

Acrylonitrile	25%
Butadiene	15%–25%
Styrene	50%–65%

There are many variables in the process, besides the different proportions of the starting materials, so that polymers with a wide range of properties can be produced. This is illustrated in Table 1. ABS polymers possess an excellent combination of mechanical, thermal and electrical properties, as well as good chemical resistance, so that they can be used for packaging certain foods where polystyrene is unsuitable. Further advantages are ease of processing and the possibility of designing grades to meet the requirements of particular applications.

Food Chemistry 8 (1982) 109–119

POLYOLEFINS FOR FOOD PACKAGING

D. W. SHORTEN

BP Chemicals (Suisse) SA, Geneva, Switzerland

(Received: 8 September, 1981)

ABSTRACT

Polyolefins are the products of polymerisation of unsaturated aliphatic hydrocarbons. The most important are low density polyethylene, using oxygen or peroxides as catalyst, and high density polyethylene, using Ziegler or Phillips catalysts, respectively in their manufacture.

In most food packaging applications, the incorporation of such additives as antioxidants, slip agents, anti-block—and sometimes anti-static—agents as well as colorants, is necessary.

Some details of the legislation and Codes of Practice in European countries, and its effect on polyolefins for packaging applications are given.

The effects of changes during fabrication by, for example, tainting, are considered.

INTRODUCTION

Polyolefins are a family of plastics materials widely used in the packaging of all types of foodstuffs, being approved for food contact usage worldwide. The most important polyolefins are high and low density polyethylene, polypropylene and various copolymers, and these materials have been subjected to the most critical scrutiny and testing by many laboratories.

It can be stated that polyolefins rank amongst the safest of food packaging materials, being very pure and clean and manufactured by well proven and documented technology.

THE STRUCTURE OF POLYOLEFINS

Polyolefins are the products of polymerisation of unsaturated aliphatic hydrocarbons.

Food Chemistry 0308-8146/82/0008-0109/$02·75

POLYETHYLENE

This is the term used for a family of polymers derived from ethylene, optionally with minor amounts of unsaturated higher alpha olefins or functional co-monomers.

Simply, we can consider polyethylenes to have the general formula $(CH_2)_n$, the ethylene units being linked with varying levels of both short and long chain branching and pendant functional groups derived from non-hydrocarbon co-monomers where these have been used.

All the polymers contain a high proportion of unsubstituted or linear polymer molecule segments which can form organised arrays which crystallise and account for the familiar waxy appearance and the high toughness in comparison with non-crystalline polyolefins, which are of a rubbery nature.

Low density polyethylene (LDPE) was the first polyethylene of significant commercial importance and was produced by a free radical polymerisation process in continuous tube or stirred autoclave reactors. The pressure used was in the range 1000–2500 ats—hence the product is sometimes referred to as high pressure polyethylene.

LDPE molecules have relatively high levels of short chain branching (mainly ethyl and butyl), and the concentration of these is a function of the polymerisation conditions used. The amount of branching controls the level of crystallinity and of the density (0·910–0·935 g/cc). Some long chain branching is also present which has an influence on the rheological properties of the melt.

For the polymerisation process, oxygen or peroxides are used as catalysts (initiators), and consequently it is possible to have a very few oxygen-containing groups present (e.g. carboxylic, carbonyl). Co-monomers used in the high pressure process can be vinyl acetate, ethyl acrylate and butyl acrylate.

High density polyethylene (HDPE), developed commercially several years after LDPE, is different in that it has very little long chain branching in comparison with LDPE. Short chain branchings occur in a controlled manner by copolymerisation with alpha olefins such as propylene, butene-1, etc.

The density of HDPE is in the range 0·940–0·970 g/cc.

During recent years, linear low density polyethylene (LLDPE) has become available commercially. These are really copolymers of a lower density than HDPE and, as—like HDPE—they are produced by a low pressure process, it is convenient to deal with these polymers together.

It should be noted that the use of functional monomers such as vinyl acetate is not possible in the manufacture of HDPE or LLDPE, as they would deactivate the polymerisation catalysts used.

HDPE processes were initially carried out in purified hydrocarbon media at relatively low pressures (1–20 ats) either at temperatures in excess of the crystalline melting point, in which case polymerisation occurred in solution, or at low temperatures when the polymer precipitated out. Over the last decade or so, much

higher productivity processes, using either conventional slurry or high temperature solution or gas phase systems, have been developed. Catalysts used are primarily of two types: supported transition metal oxides (e.g. Phillips CrO_3 on silica or silica alumina) and Ziegler (co-ordination complexes, e.g. $TiCl_3$ and aluminium alkyls).

A line diagram showing the molecular structure of various polyethylenes is given in Fig. 1.

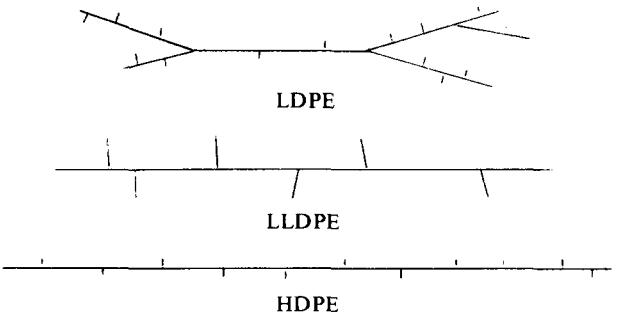
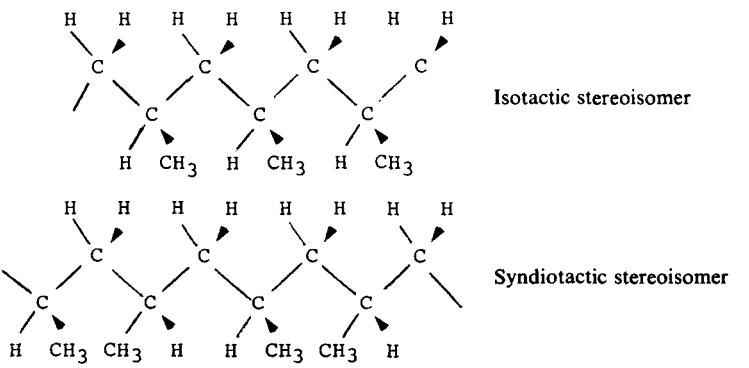


Fig. 1. Molecular structure of various polyethylenes.

POLYPROPYLENE

The polypropylene thermoplastics of today were discovered by Professor Natta in 1954 when he polymerised propylene on a modified form of Ziegler's co-ordination complex catalyst. Like polyethylenes, polypropylenes are highly crystalline materials as a consequence of the stereo control (stereo-specificity) exerted by the catalyst on the mode of addition of propylene units to the growing chain. Two forms of stereo structure are possible—the isotactic, in which the methyl groups attached to alternate carbon atoms on the backbone chain have the same stereo configuration, i.e. are on the same side of the chain, and syndioactic, in which the configurations alternate, i.e. adjacent pendant methyls are on opposite sides of the chain. The random form is known as atactic polymer.



Blocks of isotactic and syndiotactic segments can co-exist in the same molecule, such products being known as stereo block polymers. Only isotactic polypropylene is of commercial importance. Copolymers with minor amounts of ethylene are also produced in random or block copolymer variants. All commercial polypropylenes contain low concentration of atactic polymer. Densities are in the range 0·90–0·91 g/cm^3.

COPOLYMERS

The structure becomes more complicated, because each monomer used can combine in a regular or random way. By way of example only, it is useful to look at a polypropylene copolymer containing a small amount of ethylene—analytical techniques have shown that, besides the branching groups shown previously, molecules of the ethylene homologue polymers contain some of the same functional groups as discussed above for polyethylene.

CRYSTALLINE NATURE OF POLYMERS

Before dealing with additives, it is important to note that all polyolefins are crystalline polymers, the crystalline content being governed by the regularity of the polymer chain, with branching reducing crystallinity in polyethylene from a maximum level in excess of 80 % in high density polyethylene. In polypropylene, also, crystallinity is a function of the regularity of the molecular structure, with tactility being the dominant factor. Irrespective of the regularity of structure, all polyolefins contain appreciable proportions of non-crystallised amorphous material, which is of particular importance in regard to incorporated additives. Crystalline areas do not allow any significant amounts of foreign structures to be accommodated and the additives are therefore concentrated in the amorphous parts of the materials.

ADDITIVES INCORPORATED INTO POLYOLEFINS

As the additives used in all polyolefins are virtually the same, it is possible to deal with them without making special reference to specific polymers.

(*a*) *Antioxidants* (*stabilisers*) (*A/O*)

There is no shortage of antioxidants for polyolefins and a substantial amount of work has been carried out to prove their suitability for food packaging applications. Besides toxicological assessment of the antioxidants, specific migration testing of the A/O into food simulants, and global (overall) migration testing of polyolefins into which A/O has been incorporated have been carried out.

Antioxidants are usually soluble in organic solvents, whereas the polymers are not, and are normally of low molecular weight. By way of example, a few of the commercially available antioxidants used in polyolefins today, which are approved for food contact packaging, are given below. It is stressed that these are examples and that the approvals and limits of use vary from country to country.

Butylated Hydroxy Toluene (BHT)

Tetrakis Methylene 3-(3′5′-di-tert-butyl-4′-Hydroxy Phenyl) Propionate Methane

N-Octadecyl-3(3′5′-di-tert-Butyl-4′-Hydroxy Phenyl) Propionate

1,3,5,-Trimethyl 2,4,6-tris(3,5-di-tert-Butyl-4-Hydroxybenzyl) Benzene

Tetrakis-(2,4-di-tert-butyl-phenyl)-4′,4′-di-phenylene-diophosphonite

All European food contact regulations and recommendations give a limit to the amount of antioxidant that can be incorporated into the polyolefin. There is some variation from country to country, but amounts of the order of 0·1–0·2 % are normally permissible, which is considerably higher than conventionally used in LDPE (0·02 %) or HDPE (up to 0·1 % usually).

It is possible to incorporate the A/O either during the polymerisation stage (i.e. in the reactor), or during a secondary compounding process. The A/O is present to prevent oxidation of the polymer during all conversion processes, but it is necessary also to prevent any possible degradation of the polymer during storage, especially under hot conditions.

From the food packaging aspect, the important issues are the toxicity and the amount of the A/O which can migrate into the foodstuff, and this is determined with the use of food simulants (e.g. olive oil for fatty foodstuffs), using gas chromatographic analytical methods. It should be realised that some of the A/O's used to stabilise polyolefins are already used to stabilise foodstuffs, and it is important in such cases to ensure that any additional A/O migrating from the polyolefins into the foodstuff does not result in the total A/O content exceeding the legal limit in the foodstuff (a very rare occurrence).

The solubility of most A/O's in common organic solvents means that their extraction from stabilised polyolefin polymers is relatively easy, and quantitative analysis by gas–liquid chromatography, mass spectrometry, colorimetric or polar graphic techniques can be carried out.

(*b*) *Slip agents*

In many polyolefins film (especially LDPE) food packaging applications, it is often necessary to incorporate slip additives, which exude to the film surface and reduce the coefficient of friction (COF). Without such additives, film plies would be very difficult to separate.

Frequently, such modified film is referred to as high/medium/low slip and as slip is the reciprocal of COF, it follows that: high slip film has a low COF and low slip film has high COF.

Most slip agents are fatty amides of the general formula $R.CONH_2$ where R is the parent acid radical. The most important are oleamide and erucamide. Although stearamide has the same general formula, its use is more for its anti-blocking properties.

Both oleamide and erucamide contain a central double bond and the molecules exist in the *cis* form. The latter, however, has increased carbon chain length due to its greater molecular size.

The stearamide has a different molecular structure and, as it is fully saturated, the existence of *cis/trans* isomers is not possible. During and after processing (e.g. film extrusion) the slip agent exudes to the surface, oleamide more rapidly than erucamide. For any given concentration, the former could be expected to give a larger reduction in COF. However, this does not always occur because of the lower melting point of the oleamide, as well as its inferior heat resistance. More can be 'lost' during processing.

Approvals for the use of these fatty amides in food contact packaging applications appear in most European legislations, recommendations and Codes of Practice, the maximum permitted level generally being in the order of 0·2% in the polymer.

Quantitative analysis of these slip agents in polymer and film can be carried out by gas chromatographic techniques.

(*c*) *Anti-block agents*

The use of stearamide was mentioned in the previous section, but today, particularly in the LDPE film area, this fatty amide is being used less, because of difficulties it causes in downstream operations—for example, printing and sealing.

Calcined diatomaceous earths have been the main anti-block agents used for many years and have universal approval for food contact usage. In 1979, it became apparent that there was some evidence of handling hazard in that, when calcined, 30–40% of cristobalite was produced. This form of crystalline silica gel can cause silicosis, if a sufficient dose is inhaled, and replacement anti-block agents were sought and found—amorphous silicas—which are some sixty times safer to handle on production plant. Today, these inorganic anti-block additives are widely used and have the same universal food contact approvals as the calcined diatomaceous earths. Hence, change was really brought about to ensure safety for workers on production plants and not for food contact reasons.

(*d*) *Other possible additives*

In some polyolefin food contact applications, the incorporation of uv stabilisers, cling agents, anti-static agents and colorants may be desirable.

Generally the use of uv stabilisers is more in the area of indirect food contacts—for example, shrink film—not considered in this paper. Cling agents, such as glycerol mono-oleate or stearate, can be incorporated into LDPE resins when a degree of surface tackiness in the film is necessary. Several of the cling agents for LDPE are already used in food manufacture and there are no problems with food contact approvals.

Cling LDPE film is used for wrapping foodstuffs on expanded polystyrene, polystyrene and board trays and, in some countries in Europe, as a stretch wrap (rotary method) film.

Anti-static (A/S) additives are incorporated in many food contact polyolefin films and containers in the UK. Continental Europe does not use A/S agents to the same extent. A typical A/S agent is polyoxyethylene (8–9) stearate, which is approved by F&DA (USA) and the BPF/BIBRA Code of Practice.

A few coloured polyolefins are used in food contact applications, by far the most common being white. Titanium dioxide (TiO_2) of high purity is used to colour polyolefins white, and in most cases the whiteness/opacity can be achieved with about 2% of TiO—widely approved for food contact usage.

Some concern has been expressed in the past regarding coloured polyolefin containers used in contact with liquid foodstuffs—for example, home brewing kits. The best advice here is to check with the manufacturer that food-approved pigments have been used.

WHAT CAN OCCUR DURING CONVERSION/FABRICATION PROCESSES

Most people who have worked with polyolefins know of the characteristic wax-like odour which is especially detectable when the product is hot. During any film extrusion, blow or injection moulding, forming, sealing or any process where the thermoplastic is heated, the odour is noticeable, especially to persons new to that environment. The basic rule is not to overheat the polyolefin and to work in the temperature range indicated by the polymer supplier.

Copolymers, such as EVA, EEA and EBA, have a slightly different odour, commonly described as 'bad apples'.

In almost all cases, the odour originating from the polyolefins does not affect the packaged foodstuffs, but it must be mentioned that, in some European countries, it is the legal responsibility of the food packer to test and give a guarantee that the foodstuff is not 'contaminated'. With foodstuffs that are particularly susceptible to odour pick-up, it is especially important to carry out packaging and storage tests.

From the additives considered previously, there are no decomposition products that would affect the food contact status of the plastics, provided 'good manufacturing practice' is exercised at all stages, especially the avoidance of overheating.

Occasionally, a taint/odour problem can occur if the fatty amide slip additive is

stored badly or kept for a protracted period. The storage conditions for fatty amides are available from the manufacturers and if these are adhered to and stock is rotated properly so that the storage period is less than a year, then, to the author's knowledge, there are no problems.

Information on the storage of polyolefin resins, both modified and unmodified, and how this affects the suitability of the plastics for food contact usage, suggests that these are not problem areas. Certainly, there have been a few reported cases of polymer being stored in silos/bags in countries with hot climates for long periods and which developed somewhat stronger odours. However, the documentation is not very conclusive, and as storage periods usually do not exceed 6 months, this is virtually a non-problem. More frequent, but still quite rare, are reported cases where a polyolefin film or moulding, after prolonged storage, develops an odour which could contaminate a foodstuff. Generally, investigation of such complaints has indicated that any odour build-up resulted from poor ventilation in the storage area, and then was usually associated more with print on the plastics item rather than a degradation of the plastics.

In film, it is conceivable that fatty amides migrating to the surface can cause a slight odour problem, but again evidence that this occurs is inconclusive.

REGULATIONS IN EUROPE

Polyolefins and the additives incorporated (discussed previously) meet the requirements of existing legislation, recommendations and codes of practice in Europe. Additionally, they meet the requirements of F&D (USA).

An accompanying paper in this issue, by Dr John Bell, deals with the control of plastics food packaging materials, especially with regard to the UK. Accordingly, in this paper, only brief references will be made, together with a few comments about the systems adopted in Europe.

The United Kingdom

For many years, plastics manufacturers have relied upon the BIBRA/BPF Code of Practice, which, although not perfect in the eyes of many, has stood the test of time well.

Legally, there is UK Statutory Instrument 1978, No. 1927, which came into force on the 26th of November, 1979, which emphasises 'good manufacturing practice' and the avoidance of any danger to human health or a deterioration in the organoleptic characteristics of the food packaged. This regulation refers to the finished packaging material and the manufacturer of this is responsible for compliance. The polymer manufacturer can assist the fabricator/convertor in complying by using approved additives, but cannot guarantee that the final product is in accord with the regulation.

France

There is specific legislation for all plastics materials intended for use in contact with foodstuffs. In French legislation there is a list of permitted ingredients (incomplete in some respects) and maximum levels of usage. Migration testing of the finished article is not legally necessary, but the law states that there should be no transfer from packaging to food.

Italy

Specific legislation published in the *Gazetta Ufficiale* gives a permitted list of ingredients and migration limits to be applied to the finished article. An overall (global) migration limit into food simulants is given.

West Germany

Recommendations (not legislation) from the Bundesgesundheitsamt (BGA) for each type of polymer, with a permitted list of ingredients and the maximum level to be used, exist.

There are no tests given for the finished product.

Belgium

Specific legislation (not unlike that from Italy) for all plastics materials intended for use in contact with foodstuffs. The law is published in *Moniteur Belge* and, strictly speaking, applies to the finished product, with overall (global) migration limits quoted.

Holland

Specific legislation enacted in October, 1980, which, although basically similar to Belgian law, is rather easier to follow, in that there are individual sections for each polymer type which include a permitted list of ingredients. This again applies to the finished product with respect to the overall (global) migration figures quoted.

Sweden, Norway, Finland and Denmark

These countries rely upon BGA (West Germany) and F&DA (USA) approvals mainly, but can be expected to fall in line with any EEC legislation on food contact materials, possibly adding some special clauses themselves.

USA—F&DA

For completeness, this is added here. The F&DA regulation is 21 CFR 177, 1520 'Olefin Polymers', and no testing of the finished product is required. Copolymers are dealt with slightly differently in other regulations, in that the finished product has to pass certain tests.

The EEC

The UK is part of the EEC, in spite of the stretch of water which tends to isolate it from Continental Europe. To have one law only in Europe for plastics food contact materials would make life easier for all concerned. For over 10 years now the EEC has strived to achieve this and, in fact, the enabling directive was passed two years ago with the promise that specific directives would follow.

Unfortunately, in spite of determined opposition by the UK overall (global) migration into food simulants was pushed as the means of control, with a test method of doubtful value, and maximum limits of 60 ppm on 10 mg/dm^2 quoted. Considerable work by polymer manufacturers managed to get the test methods up to standard whereby inter- and intra-laboratory agreement was obtained, but the argument about the inter-changeability of the maximum limits continues. However, more important was the support finally given by German delegates at the EEC to the UK's standpoint that global migration alone was not an effective means of control.

The position today is unclear regarding EEC legislation on plastics in foodstuff contact applications, but it appears that unanimous support will not be forthcoming until:

(i) There is a classification of foodstuffs.
(ii) A positive list of polymers and ingredients is approved.
(iii) Global migration testing is put into perspective.

We can expect a classification of foodstuffs in the not too distant future, and the SCF of the EEC is dealing with polymers at the present time for inclusion on a positive list. When additives will be considered is anyone's guess.

In the author's opinion, global migration into food simulants should be a back-up test and should never be allowed to become the most important criterion in any legislation enacted by the EEC in future.

In the author's view three final points on legislation are worth making:

(i) West Germany and Holland have laws which can be followed best by polymer manufacturers and fabricators/convertors.
(ii) Do we need further legislation in this area? Already the plastics industry has shown itself to be conscious of its responsibilities to ensure the safety of the general public.
(iii) If glass and paper/board were developed today, would they be accepted as food contact packaging materials?

CONCLUSIONS

Polyolefins are not really new food packaging materials; they have been used for some 30 years, and have been most critically examined in respect of toxicology and migration into foodstuffs and food simulants.

The fact that they are approved in virtually every country in the world is surely indicative of their *safety*.

ACKNOWLEDGEMENT

The author would like to acknowledge help given by colleagues in BP Chemicals (Suisse) SA and BP Chemicals (UK) in the preparation of this paper, and to thank BP Chemicals for permission to publish it.

Food Chemistry 8 (1982) 121–127

THE SELECTION OF PLASTICS FILMS FOR FOOD PACKAGING

C. R. OSWIN

11, Minterne Road, Christchurch, Dorset BH23 3LD, Great Britain

(Received: 8 September, 1981)

ABSTRACT

The packaging of perishable foodstuffs is a form of transfer engineering provided to collate contents and prolong their shelf-life. For many commercial purposes a life of a few weeks or months is adequate, and flexible wrappings meet this need. They impede oxygen and water vapour from the contained micro-climate and so retard the activity of microbes and of reactions which spoil flavour. The highest barrier properties are not always needed—they can sometimes be harmful by promoting anaerobic conditions; so informed selection is necessary to secure optimum value. Barrier properties can be produced over a range of about four decades. Maximum impedance is provided when steric hindrance in the polymer prevents the chain-folding and the spherulitic crystallisation characteristic of simple hydrocarbons, but this makes fabrication more difficult. Minimal barriers use atactic lyophilic polymers with high proportions of plasticiser, or hydrophilic polymers. Further extension of the range can be produced, if necessary, by perforation, or by combination of dissimilar polymers (by coating, co-extrusion or lamination).

INTRODUCTION

This paper describes the variety of wrappings available commercially, showing how each meets a need in the wrapping of food for market.

Packaging is a form of transfer engineering designed to collate contents and prolong their shelf-life in a hostile environment. Elaborate packing, such as sterilisation in a hermetically sealed container, can secure a life of several years, but this is of strategic interest only since growing seasons are less than six months apart. For many commercial purposes a 'life' of 3–9 Ms (1–3 months) is adequate, and often this can be reached by wrapping in a plastics film. Such films provide little

Food Chemistry 0308-8146/82/0008-0121/$02·75

protection against mechanical hazards, but they exclude most small, visible predators. Their main function is to control the internal micro-climate within a range that hinders microbial conquest. They are not hermetic barriers, but are more or less permeable, and their selection should be a numerate operation: this is illustrated by a series of thumbnail sketches below.

The range of barrier properties available for this control can be displayed in a two-dimensional plot of the permeabilities of each film to water vapour and to oxygen (Paine, 1970). It is most informative if the plot is on a logarithmic scale as in Fig. 1. The 'spread' is more open than in a simple arithmetic plot, and changes of unit are easier (Salame, 1970).

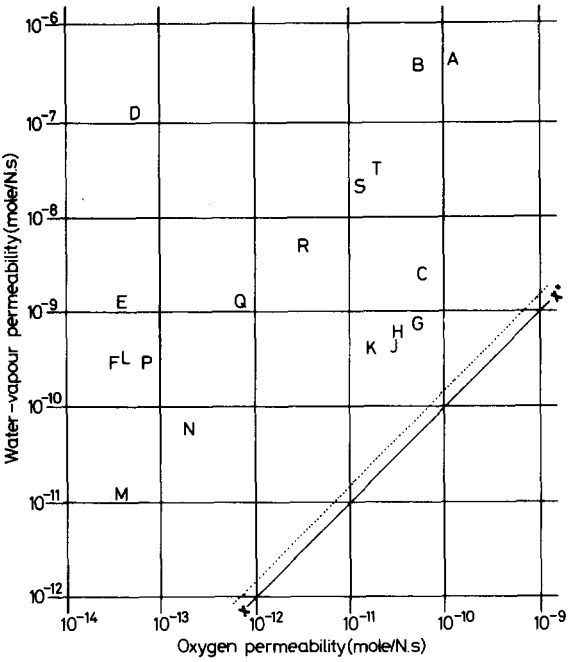


Fig. 1. The permeabilities of plastics films to water vapour and oxygen, plotted in SI units (mole/N.s) on log scales. The regions indicated by lettering are identified in the text.

EXAMPLE 1: BISCUITS

In the simplest case of spoiling, dry biscuits lose their texture and flavour when they have absorbed a certain amount of water vapour from the storage atmosphere. A typical pack might contain 302 g nett, of which 12 g is water. This water is absorbed on starch and has an activity of only about 0·1—in other words, it does not develop the full saturated water vapour pressure (2 kN/m^2 if the storage temperature is

18 °C), but about 200 N/m^2. The water vapour pressure in the storage atmosphere might be 1·3 kN/m^2 (RH = 65 %), so a potential difference of 1·1 kN/m^2 presses water into the biscuits. When the total water in the biscuits increases by another 6 g (0·33 mole), a deterioration might be just noticeable (Burton, 1964) and the value of the pack would fall. The area of film exposed might be 0·05 m^2 and if this film could be chosen to admit no more than 0·01 mole of water per day (86400 s) the 'shelf-life' would be something over 33 days. (It would probably be about 38 days under constant conditions because there is a near-exponential approach to equilibrium (Cairns *et al.*, 1975)). This demands a film having a water vapour permeability no greater than 0·01/(1100 × 0·05 × 86400) mole/N.s, or 2·1 nanomole/N.s. Any of the films below this level in Fig. 1 could be considered, with the cost naturally taken into account. Varying the weight or size of the pack would just shift the cut-off. Storage temperature is particularly important, a rise of 7–11 °C being enough to require halving of the specified permeability. Some measurement of the absorption isotherm is called for, because there is a wide variation in biscuit recipes.

EXAMPLE 2: SNACK FOODS

This is a more demanding case than Example 1, because the snacks are bulkier and usually put up in lighter packs with a higher surface/weight ratio. One way to secure longer life is to lower the starting moisture content, but this introduces a risk (Heiss & Eichner, 1971) of autoxidation of the residual cooking oil and it limits the choice of film to those which also have a low permeability to oxygen. In practice, a water-vapour permeability below 1 nanomole/N.s and an oxygen-permeability below 100 femtomole/N.s are usually required.

EXAMPLE 3: BREAD

A loaf could weigh 800 g, of which about 580 g is dry solid and 220 g water. The water has a high activity (0·95) and in a storage atmosphere of 24 °C (saturation vapour pressure 3 kN/m^2) and 45 % RH there is a potential gradient of 1·5 kN/m^2 pressing water out of the loaf. In the absence of a wrap the loaf would rapidly become dry and lose weight. A film similar to that selected in Example 1 would retain the water effectively—but too well. The relative humidity of the microclimate inside the pack would rise well above 75 %, and mould would grow (Heiss & Eichner, 1971) from any spores that had collected on the surface during cooling. A more permeable film must be used so that the surface of the bread dries to an activity below 0·75. Experience shows that, unlike biscuits, the transient water in the bread can develop an activity gradient, the body of the loaf acting as though it had a limited permeability ('admittance' in electrical circuit reckoning). This is not usually

quoted, but it can easily be estimated by the emballistic equivalent of a Wheatstone bridge: the loaf is wrapped in a film of known permeability, and the water activity at the surface is measured. In practice, various wraps are tried until the absence of a mouldy 'indicator' at the surface shows that the required balance is achieved. A safety margin is allowed to cover temperature changes and fluctuations. A film with permeability of 20–50 nanomole/N.s is usually selected: the loaf dries out eventually, but it is the best that can be done.

EXAMPLE 4: VEGETABLES

Oxygen permeability is involved in packing vegetables, but in the opposite sense to that for snack foods. Cut fresh vegetables are still alive, breathing oxygen from the atmosphere and exhaling carbon dioxide (Platenius, 1946). If this is hindered by a film of low permeability, anaerobic conditions develop and in the moist atmosphere the produce 'rots'. The oxygen demand varies from one vegetable species to another: a permeability exceeding 10 picomole/N.s is acceptable for many. For the most demanding (lettuce, asparagus) the simplest solution is to sacrifice hygiene and leave the end of the bag open; in between, perforation may be enough for some. Excessive water loss produces wilting, but over-retention induces bacterial growth, so a compromise has to be struck. A water vapour permeability of about 15 nanomole/N.s gives about the longest life attainable before one of the competing predators does the damage. When there is likely to be temperature fluctuation, it is probably best to resort to perforation; if the weight of the contents is above 100 g the result is not too serious.

EXAMPLE 5: RED MEAT

All the problems of wrapping in permeable film are presented at once when seeking to specify a film for wrapping freshly cut and 'bloomed' red meat. The colour is at best a transient phenomenon, demanding an adequate supply of oxygen to replace that which is being slowly consumed by harmless, but unsightly reaction in the interior. The highest oxygen permeability attainable is scarcely enough to sustain the colour for more than a day or two, but as the wrap is in close contact, perforation would be ineffective as well as unhygienic. At present an oxygen permeability of about 20 picomole/N.s is the manufacturing limit. It entails a water vapour permeability of 5–25 nanomole/N.s but this achieves a tolerable compromise between drying out and going rotten.

The problems are much less severe if the meat is deep frozen: colour is sacrificed and the need is to prevent dehydration in the freezer. A water vapour permeability of

less than 500 nanomole/N.s is usually specified, so that perforation can be used to counter the harmful effects of condensation when the temperature fluctuates.

The examples above have shown that there is a real need for a variety of films in wrapping—especially as each calculation must be refined by trial and error-correction. It is interesting to see how far the chemical industry has been able to provide the range of properties required. This is shown in Fig. 1, where each capital letter represents an area of availability.

The starting point is paper (A), made from vegetable fibre. Water and oxygen pass fairly freely through the interstices between fibres. Water passes the more freely, the ratio being far greater than Graham's Law would predict, as represented by the dotted line above X–X', the line of equal permeabilities. The permeabilities can be reduced by prolonged 'beating', but this makes manufacture very slow. It produces 'glassine' (B). Greater reduction in permeabilities is obtained by dissolving the fibres chemically (as the xanthate) and re-precipitating them in a continuous sheet. This gives cellulose film, which is still highly permeable to water vapour (D, 120 nanomole/N.s). Paper can be improved by impregnation with wax. Lowest permeabilities are obtained with a crystalline paraffin ($C_{32}H_{66}$) which gives values (C) of 1 nanomole of water and 50 picomoles of oxygen/N.s. Unfortunately, the wax is brittle and easily fractured by creasing, with a fifty-fold increase in permeability. Similar improvements can be achieved with cellulose films by coating them (E) with a lacquer containing wax. One benefit of these coatings is that they enable joins to be made by heat sealing.

These early wrappings have been supplemented by polymers derived from fossil fuels. The polymers are produced by a fairly complicated process, but they are thermoplastic, and their conversion into film-form is simpler. The most common polyolefin—(low density) polyethylene—is at G in Fig. 1, diagonally opposed to D. Water vapour still permeates more easily than oxygen, but the ratio here is only 20:1. If the polyolefin film could be produced as a single crystal it would have a much lower permeability; but it is a pecularity of the paraffins that their crystals grow in the form of folded-chain segments which twin into spherulitic growths. At each meeting of the spheres there is a discontinuity (Way, 1974) which must account for much of the permeation. In compensation, there is much less susceptibility to mechanical damage.

The later polyolefins, high-density polythene (H) and polypropylene (J) are in the same area, but slightly less permeable. Biaxial orientation (K) of polypropylene reduces the permeabilities—perhaps by diminishing the effect of the discontinuities. Coating this film with a soluble vinylidene chloride copolymer takes it back across Fig. 1 to a position (L) near F.

Between D and G there is a useful family of polymers based on halogenated ethylene monomers. Least permeable is the fully fluorinated polymer, polytetrafluoroethylene (PTFE), (M): its outstanding properties seem to come from good alignment of the molecules, held straight by steric hindrance. They pack densely and

there is little scope for transport of water molecules by hydrogen bonding. The polymer has a high melting point and is particularly difficult to process, so the full potential barrier properties are probably never realised. It cannot be heat-sealed easily—in fact, its principal use in packaging is to provide a 'non-stick' surface on the jaws of heat-sealing machines.

Steric rigidity has to be eased if normal extrusion is to be used: this can be done, at the sacrifice of performance, by polymerising a less regular monomer such as chlorotrifluoroethylene (N).

Polyvinylidene chloride is also aligned by steric hindrance, but it is also difficult to process. If copolymerised with other substituted ethylenes, and plasticised with a non-volatile solvent, it can be extruded to give a film (P) with low permeabilities. A 'leaner' copolymer with good properties can be handled as an aqueous emulsion: this is the coating medium used in F and L.

Atactic polymers such as polyvinyl chloride (Q) are not restrained by steric hindrance, and they can be extruded: they decompose if overheated, and a small amount of plasticiser is usually added. More plasticiser is added to produce a softer film (R) which can be stretched in production; it will shrink to a conforming wrap if it is heated gently. The plasticiser increases permeabilities by a factor of 2 or 3; this can be extended by more plasticiser to give a stretchable wrap (S) of high permeability. The highest permeability that can be achieved at present (T) requires the sacrifice of ease in handling and the acceptance of a high proportion of uncombined plasticiser. It is only used when the utmost oxygen permeability is needed.

There are some cases where the permeabilities available in commercial films are still not high enough: in such cases it is necessary to forego hygiene, and perforate the film, or to leave the wrap partly open. This is only feasible when the life is, in any case, short. Alternatively, when less permeation is demanded, a thicker film can sometimes be used, or two consecutive wraps applied; but the effect is no more than the height of a letter in Fig. 1. Bulk overwrapping is a more efficient way of using the extra film. The combination of two or more films with complementary properties sometimes gives useful results not otherwise obtainable; it can be done by coating (F, L), coextrusion, or laminating with an adhesive. In extreme cases, if even this does not yield the low permeability required, a thin layer of aluminium foil can be included in the laminate: the result falls in the bottom left-hand corner of Fig. 1.

When films are laminated, the resulting permeability can be estimated by the addition of reciprocals, in the way common in electrical circuit theory. The analogy is so convenient that it is worth staying in reciprocals, and thinking in terms of resistance rather than of permeability. Thus the water vapour permeability of T, $2{\cdot}5 \times 10^{-8}$ mole/N.s, becomes a resistance of 4×10^{7} N.s/mole. The reciprocal values can be seen simply by inverting Fig. 1.

Many other factors have to be taken into account once the emballistic screening is done: printability, strength, machineability and, above all, cost; but the model is

often illuminating, especially if the concept of a 'capacitor' is introduced to represent the ability of the contents of the pack to absorb water. All the examples have been terrestrial: space-flight conditions are more demanding, but similar reckoning applies (Labuza *et al.*, 1972).

REFERENCES

BURTON, L. V. (1964). In: *Food processing operations*. Vol. 3. (Eds. M. A. Joslyn and J. L. Heid) AVI Publishing Co., Westport, Conn., 473–7.
CAIRNS, J., PAINE, F. A. & OSWIN, C. R. (1975). *Packaging for climatic protection*, Newnes, London.
HEISS, R. & EICHNER, K. (1971). *Food Manufacture* (June), 37–41.
LABUZA, T. P., MIZRAHI, S. & KAREL, M. (1972). *Trans. Am. Soc. Agr. Eng.*, 150–6.
PAINE, F. A. (1970). In: *Food Industries Manual*, (20th edn.), Butterworth, London, 370.
PLATENIUS, L. (1946). *Mod. Packaging*, **20**(2), 106–10.
SALAME, M. (1970). In: *A.M.I. Chem. Eng. Conf. preprints* (*Maths. & Eng. Sci. Div.*), 290.
WAY, G. L. (1974). *J. Mat. Sci.*, **9**, 293–301.

Food Chemistry 8 (1982) 129–145

TRACE CONTAMINATION OF FOODS BY MIGRATION FROM PLASTICS PACKAGING—A REVIEW

M. J. SHEPHERD

Ministry of Agriculture, Fisheries and Food, Food Laboratory, Haldin House, Old Bank of England Court, Queen Street, Norwich NR2 4SX, Great Britain

(Received: 8 September, 1981)

ABSTRACT

Plastic packaging often contains many components in addition to the base polymer. Additives are required both for the manufacturing process to give acceptable results and for the finished product to have the desired characteristics. Furthermore, decomposition products may arise from these additives, while the base polymer itself will contain monomer and oligomers in addition to traces of constituents of the polymerisation mixture (such as catalysts) and any decomposition products arising from these. Printing inks, laminates and their adhesives further complicate the picture. Consequently, the final product can contain a multitude of components at all levels from traces to perhaps 20–30% by weight.

Where these components are of low molecular weight, a potential exists for their migration into packaged foods. It is essential, therefore, that manufacturers and users of plastic packaging intended for food contact be aware of the chemical nature of the range of potential migrants expected from a given polymer/additive/food system and have some understanding of the underlying mechanisms and kinetics of migration.

This paper presents an overview of current knowledge of migration. After an outline of the physical basis of migration, the concept of—and problems inherent in—measuring overall migration are described, followed by a discussion of selected specific migrants.

Although information on contamination from residual monomers forms the bulk of the available literature on specific migrants, migration of other base polymer constituents and of non-volatile plastics additives and their decomposition products can be of importance and is reviewed here.

Food Chemistry 0308-8146/82/0008-0129/$02·75

INTRODUCTION

All food packaging materials undergo a continuous process of evaluation with respect to their possible effects on packaged foodstuffs. This is particularly the case for plastics, both because of their historically recent introduction and also because of their frequently complicated composition.

Many different plastics are used by the packaging industry and although the polymers themselves are generally considered to be inert, all articles of plastics packaging will contain a variety of low molecular weight components which might have the potential to migrate into contacting foods and contaminate them, thus possibly causing tainting or toxicological problems. From a different point of view, migration can also be undesirable because of consequent deleterious changes in the physical properties of the package itself.

The major source of potential migrants is the additives incorporated in all plastics for reasons of manufacture or use. Demanding polymers, such as poly(vinyl chloride) (PVC), may require compounding with a wide range of additives in order to ensure a satisfactory product. Thus a typical PVC bottle intended for use with orange squash or cooking oil may contain 3–6 % by weight of additive. In addition, plastic packaging will contain other components, such as residual monomers, ingredients from the polymerisation reaction and oligomers plus any decomposition products or contaminants of intentional additives. Consequently, these materials may contain a wide range of compounds, some of which might have the potential to migrate into contacting foodstuffs.

This review will present a broad outline of current knowledge of migration science relating to the trace contamination of foods from plastic packaging, with an emphasis on the physical basis of migration. In addition, the measurement of specific migrant species will be discussed, with particular reference to non-volatile plastics additives. However, coverage of the literature has necessarily been highly selective and the balance presented here obviously reflects, to some extent, the interests of the author.

MECHANISMS AND KINETICS OF MIGRATION

The mechanisms of migration have been studied for many years and there is a consensus of opinion concerning the important features of the different processes. The behaviour of specific plastics constituents in given polymer/extractant combinations has sometimes been grouped into three classes (Figge, 1980):

Class I—Non-migration.
Class II—Diffusion controlled.
Class III—Leaching.

Leaching migration is of substantial practical importance and will be dealt with in detail below. Non-migration is not entirely a trivial system, because of surface effects, but in this discussion it will be considered as the limiting case for Class II migration, where movement of material is controlled principally by diffusion and where Fick's laws apply. Diffusion coefficients are inversely proportional to molecular weights in the absence of polarity effects and this is reflected in the consequent levels of migration.

Thus Figge has reported (Figge *et al.*, 1978) that the migration rates, into aqueous extractants, of 2,6-di-*t*-butyl-4-hydroxytoluene (BHT, molecular weight, 220) and 3-(3,5-di-*t*-butyl-4-hydroxyphenyl)-*n*-octadecylpropionate (Irganox 1076, molecular weight, 530) were in the proportion of about 8 to 1. For powdered and pelleted solids the ratio was nearer to 100 to 1, reflecting the different vapour pressures of the two antioxidants which, under these conditions, had not only to migrate to the surface of the container but also to vaporise into the headspace around the product (Luston, 1980).

A number of factors can influence the simple diffusion controlled concept of migration. Additives are generally polar compounds and thus interactions with the polymer can be important, increasing the rate of migration where incompatibility exists (culminating in such effects as 'plate-out' where additive exudes onto the polymer surface) and decreasing it where attractive forces exist. It has been suggested that additives could be added in polymeric form (Scott, 1981; Ayrey & Poller, 1980) or that effects such as charge-transfer complex formation could be utilised in order to reduce their potential for migration (Karcher *et al.*, 1980) and Gilbert has shown that at very low levels of residual vinyl chloride in PVC the monomer may be physically trapped at active sites in the plastic (Morano *et al.*, 1977). Consequently, at some combination of concentration, polarity and molecular weight, additives become effectively unavailable for migration under class II conditions.

EFFECTS OF POLYMER PROPERTIES

A number of approaches to the mathematical modelling of migration have been reported (Crank, 1975; Sanchez *et al.*, 1978; Anon., 1979, 1981; Figge, 1980; Rudolph, 1980) and the derived equations fit some of the experimental data quite well. Interest in this work is considerable and its application to complex foodstuffs has been investigated as part of an extensive study funded by the USA Food and Drug Administration (Anon, 1981). When the restrictions of an infinitely thick plastics and an infinite well-stirred reservoir of extractant are introduced it has been shown that, at constant temperature, the amount of migration depends upon the initial concentration of the migrant and upon the square root of contact time (Fig. 1). Experimentally, 'infinite thickness' is equivalent to about 2 mm (see, for example,

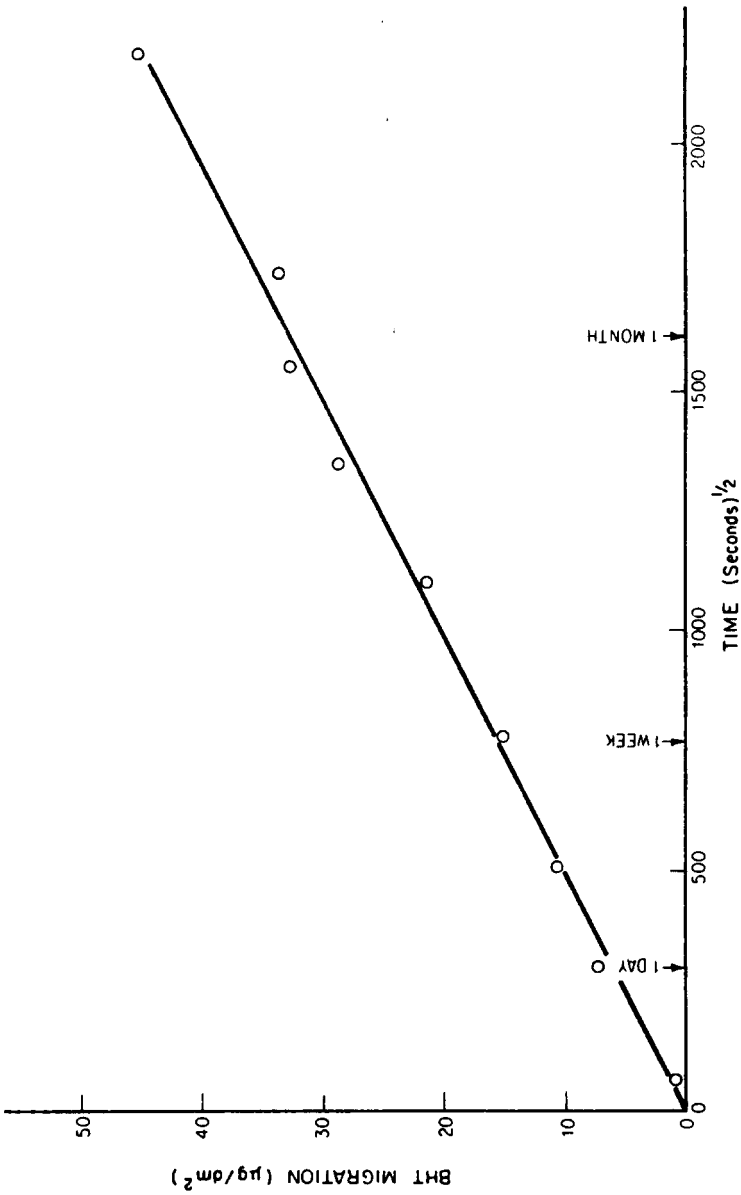


Fig. 1. Migration of BHT from HDPE foil into corn oil at 21 °C, showing 'infinite polymer: infinite extractant' behaviour (Anon., 1981). Note: 100 % loss corresponds to 108 $\mu g/dm^2$.

Fig. 2) and in high density polyethylene (HDPE), these theoretical predictions have been verified (Anon., 1981). The effect of additive 'bloom' (that is, an increase in the surface concentration compared with the bulk concentration) is of practical importance, giving rise to higher than predicted initial rates of migration (Anon., 1979). In addition, the data have been shown to fit an Arrhenius-type relationship with temperature (over the limited range of practical interest) (Luston, 1980; Figge & Klahn, 1980). This relationship will not hold in the region of the glass transition temperature (T_g) of the plastics where the mobility of segments of the macromolecular chains increases markedly for a relatively small increase in temperature and where, in consequence, diffusion coefficients change by orders of magnitude (Luston, 1980; Pfab, 1973). This is significant for highly plasticised PVC where, for example, the T_g of PVC containing 24·5% dioctyl phthalate has been reported as about 11 °C (Pfab, 1973). Although food-grade PVC cling film is plasticised with dioctyl adipate, rather than the phthalate ester, its T_g may well be in the same range.

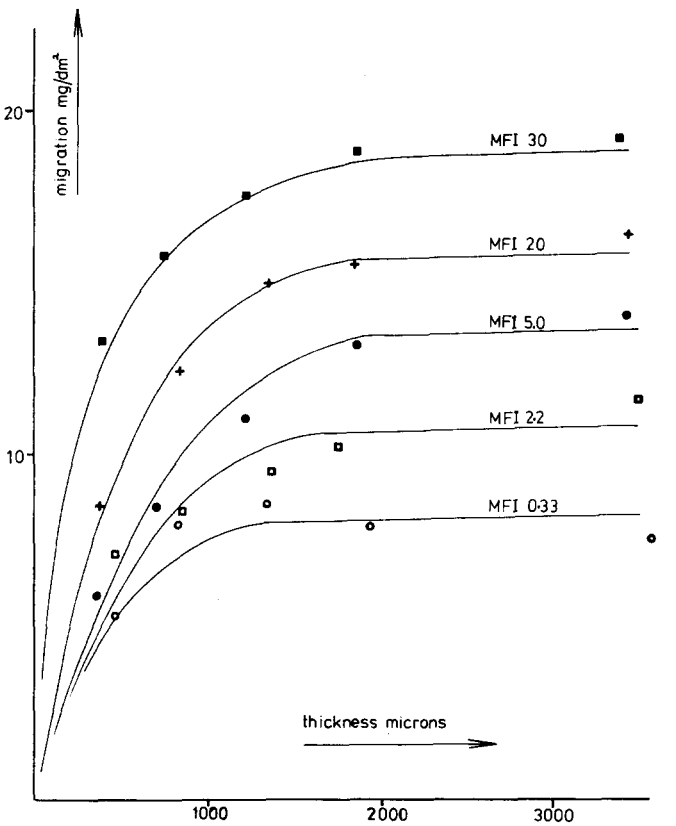


Fig. 2. Overall migration into olive oil—10 days at 40 °C (Ashby & Krul, 1980).

Molecular weight

Crystallinity

Chain branching

Density

Affinity for migrant
(with respect to partition coefficient between polymer and extractant)

Fig. 3. Polymer properties affecting migration.

Other polymer properties, such as chain branching (Anon., 1981), crystallinity (Anon., 1981) and molecular weight (Kampouris, 1975; Adcock, 1980) (Fig. 3) can also have a significant effect on migration rates, as can the overall thermal history of the plastics (Juskevicinte & Shlyapnikov, 1965). The distribution of additives is affected by crystallinity of the polymer as low molecular weight compounds are concentrated in the amorphous phase (Luston, 1980). As expected, an increase of molecular weight, polarity or packing density of the polymer reduces migration of incorporated additives (Luston, 1980). These effects can be explained in terms of the 'hole' or free volume theory of diffusion (Luston, 1980).

One other aspect of polymer molecular weight which can be of importance for migration is the concentration of oligomers in the plastics. The mobility of species of

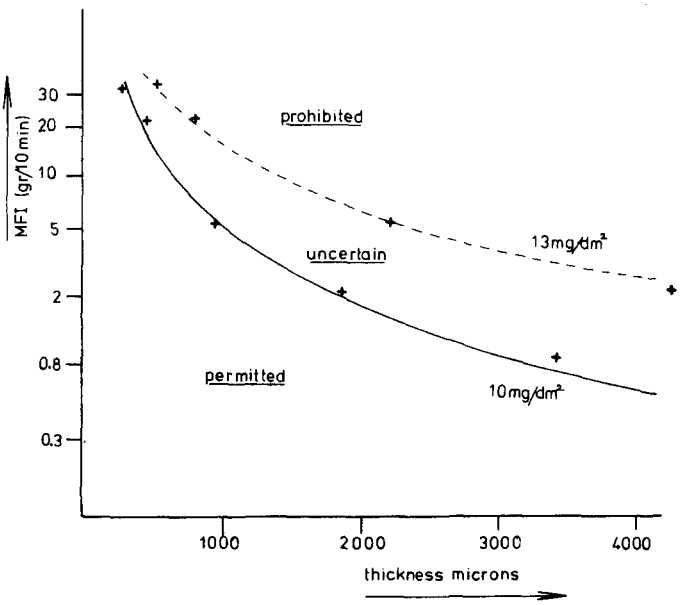


Fig. 4. The boundaries of MFI and thickness for overall migration from additive-free low density polyethylene (Ashby & Krul, 1980).

any given chain length will vary according to the chemical nature of the monomer, as discussed above, but oligomers can represent a significant mass of potential migrants, particularly from polyolefins. Overall migration into olive oil from additive-free low density polyethylene (LDPE) has been correlated (Fig. 2) with polymer melt-flow index (MFI—a measure of the molecular weight of the polymer) (Ashby & Krul, 1980) and, on this basis, these workers are able to predict the acceptability of foils of given MFI/thickness combinations with regard to the proposed EEC regulations on overall migration (Fig. 4).

MIGRATION EXPERIMENTS AND TESTING

Thus an extensive range of variables must be considered in any investigation of migration phenomena. The experimental parameters which must be reported to allow meaningful comparisons of results from different laboratories have been discussed (Katan, 1980) and these are summarised in Fig. 5. It is highly desirable that, where possible, standard conditions of contact are employed, as the literature contains many interesting results which are difficult to relate to other work because of an apparently arbitrary choice of temperatures or times. A wide range of conditions has been used for migration experiments. Extended periods at normal product storage temperatures are often required in order to establish the maximum possible extent of mass transfer and thus accelerated testing is common. Typically, contact periods of 10 days at 40 °C (EEC) or 10 days at 49 °C (FDA) have been recommended for packaging products intended to be stored under ambient conditions (Figge, 1980). Other combinations are used where, for example, products are customarily refrigerated or subjected to higher temperatures during use (Figge, 1980). Migration levels are often reported as either ppm in the extractant or as mg/dm^2 of plastics surface, but the full experimental details are required in order to interpret either satisfactorily.

A. Intensive
- Temperature
- Time
- Specific gravity of plastics
- Specific gravity of extractant

B. Extensive
- Initial mass of migrant in plastics
- Mass of migrant transferred during specified time
- Mass of solvent
- Area of contact
- Geometry of system

Fig. 5. Basic migration variables (Katan, 1980).

EFFECTS OF EXTRACTANT PROPERTIES

Thus far, emphasis has been placed upon the properties of the plastics but in order fully to evaluate the potential for migration of a given polymer/extractant system, the properties of the extractant must also be considered. Obviously, its physical form is important. Migration into a solid extractant will be limited by the formation at the plastics surface of a zone of extractant containing a high level of migrant, thereby reducing the concentration gradient across the plastics which is the driving force for migration. Consequently, in this situation migrant diffusion in the extractant strongly influences the total amount of migration (Anon., 1979).

The chemical nature of the extractant—aqueous (acidic, neutral or basic), alcoholic, fatty or emulsion (oil-in-water or water-in-oil)—is also critical. Migration of BHT into water is restricted because of the very low solubility of the antioxidant (Anon., 1979). In certain circumstances, however, migration can exceed solubility limits. For example, plasticiser transfer from PVC film into stirred water was thought to have been enhanced by co-migration of other ingredients of the film, especially surface-active materials incorporated as anti-fog agents (glycerol mono-oleate or polyethylene oxide) (Anon., 1979).

Of considerable importance is the ability of the extractant to penetrate or 'leach' polymer materials. Some low molecular weight components of food, such as triglycerides or terpenes (derived, for example, from orange juice) are capable of extensive movement *into* plastics. Where this occurs, migration rates can be substantially increased (Class III migration). Resistance of polymers to penetration varies considerably, depending upon both chemical factors and the parameters listed in Fig. 3. Furthermore, for each polymer, penetration by saturated triglycerides (as measured by extraction of additives) is dependent upon the chain length, n, of their acyl groups (Figge, 1980). The highest values of n at which detectable extraction occurred were for polystyrene, 14, and for PVC, 10, while substantial extraction from HDPE was seen at all values of n tested (2–18), (see Fig. 6). For all three polymers, maximum extraction was observed at intermediate acyl chain lengths (3–8) (Figge, 1980).

Significant penetration of polymers is usually accompanied by swelling of the plastics affected. The consequent changes in inter-macromolecular dimensions and free volume lead to particularly rapid migration. Not all polymers swell, but in all cases where a plastics is penetrated by oil, diffusion coefficients in the mixed phase increase by several orders of magnitude. It is important to note that the physical form in which the oil is present is critical to its ability to penetrate (Figge, 1980). Oil-in-water emulsions, such as milk or cream, are not effective penetrants although their lipid content may be considerable (Figge, 1980). Similar considerations can also apply when fats are associated with other components of foods, such as proteins. Since this penetration is also a diffusion-controlled process, mathematical modelling shows that total mass transfer of additive to extractants remains proportional to the square root of contact time (Anon., 1979). Obviously, this

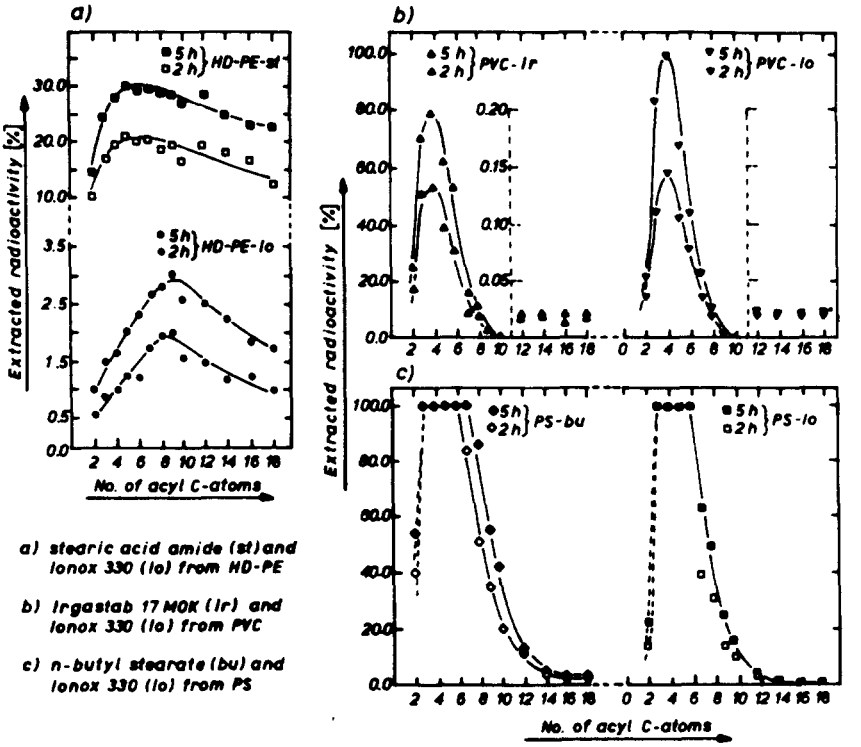


Fig. 6. Dependence of the amounts of additives that migrated from different plastics films into triglycerides on the length of the acyl residues (test conditions: 2 and 5 h at 65 °C, all-sided contact). (Figge, 1980).

mechanism is most significant for additives which are non-migrating under Class II conditions.

It is apparent that migration is likely to be of reduced importance when the potential migrant is in a part of the plastics away from the surface in contact with the extractant. Contamination of foods from adhesives and printing inks is therefore not commonly a problem, because of the thickness of polymer through which components of these materials would have to travel. Nevertheless, care must be taken in their uses, particularly to ensure the complete evaporation of solvents, which can migrate rapidly because of their low molecular weights. In addition, any potential penetration of the product must be carefully considered.

FOODS AND FOOD-SIMULATING SOLVENTS AS EXTRACTANTS

Throughout this discussion of migration mechanisms the term 'extractant' has been used for the medium in contact with the plastics without distinguishing between

foods and food-simulating solvents. Simulants are commonly used because of the extensive difficulties inherent in the analysis of migrants in any but the simplest foods. A true simulant will necessarily fulfil two conditions (Adcock, 1980). It must effect migration from the plastics to the same extent as the food simulated and, in addition, it must allow relatively simple analysis for the migrant of interest. None of the simulants in common use are completely satisfactory in both respects. All simulants are to some extent compromises between these conflicting demands and, in particular, fat simulants, even the triglyceride mixture HB307 (Figge, 1980) still present substantial analytical problems. In this context it should be recognised that vegetable oils cannot truly be regarded as simulants.

The migration of plastics components into actual foods other than vegetable oils has been measured only infrequently (Figge, 1980; Anon., 1980; Haesen *et al.*, 1981). Most of the work that has been done used antioxidants as model migrants and, as discussed below, there are practical difficulties in the analysis of these compounds which, unless recognised by the analyst, can cause problems in any assessment of the results. Furthermore, in the most extensive report in this area, which described the migration of BHT from HDPE into a variety of foods (Anon., 1980), although the analysis was performed using radiotracer techniques, no account appears to have been taken of potential degradation of the antioxidant during preparation of the stabilised plastics foils used.

Nevertheless, in general, where simulants are carefully chosen to represent individual foodstuffs, results obtained by the use of the simulant can be used with some degree of confidence. It is essential, however, that simulants do adequately reflect the chemical and physical properties of the food. Water is an inadequate simulant for milk (Haesen *et al.*, 1981), considerably underestimating the likely migration levels whilst, in contrast, fatty simulants overestimate migration (Figge, 1980). Solvents such as heptane are totally unsuitable as replacements for fatty foods as they cause unrealistically rapid migration (Figge, 1980).

It must be recognised that foods are typically multi-component systems and this may be reflected, for example, in differential penetration into plastics under Class III migration conditions. Work on methanol/tetrahydrofuran mixtures as fat simulants represents an interesting approach to the problem and is potentially of great value (Adcock, 1980).

OVERALL MIGRATION

The problems experienced with fat simulants such as edible oils are clearly highlighted in the procedures suggested for the determination of overall migration values (Rossi, 1977). These are derived directly from the gravimetric mass loss of a plastics article weighed before and after testing and can give no indication of the chemical composition of the migrant. Because analysis for many specific migrants is

uncertain, the overall migration value is intended to give some guide to the potential transfer to foods of such individual species. Evaluation of overall migration into aqueous simulants is relatively straightforward but considerable manipulative skills are required in order to obtain reliable values for simulants of fatty foods where the mass gain, because of absorption of fats by the plastics, will frequently exceed the mass loss due to overall migration (Figge, 1980). It is necessary to re-extract and determine the absorbed lipids, and if this extraction is selective or incomplete, errors will be introduced into the final result for overall migration.

MIGRATION OF MONOMERS

Although the measurement of specific migrants frequently poses formidable methodological problems, these difficulties have been generally overcome in one area of major importance, monomer analysis. The monomers of primary concern to date have been vinyl chloride, vinylidene chloride, acrylonitrile and styrene.

In most cases the preferred method, both for plastics and for foods, is that of headspace gas chromatography (headspace GC) using, where possible, specific detectors either to eliminate interferences or to increase sensitivity, or both (Hachenberg & Schmidt, 1977; Gilbert & Shepherd, 1981). The analysis of styrene in foods requires an alternative approach because of its low volatility compared with the other monomers. Only small amounts of styrene are expected and a pre-concentration step is essential unless a mass spectrometer is available (Gilbert & Shepherd, 1981).

The outstanding feature of monomer analysis in this way is the ease with which direct analysis of foods may be performed. Nevertheless, interfering volatile components can be present in even the simplest food and it is essential to confirm the identity of suspected monomer chromatographic peaks. Ideally, this would be done by single or multiple ion-monitoring using a mass spectrometer. This can also offer a

TABLE 1
DETECTION LIMITS FOR MONOMERS

Monomer	*Method*	*Detection limit* (mg/kg)*
Vinyl chloride	Headspace FID	0·002–0·005
Acrylonitrile	Headspace AFID	0·005
Styrene	Distillation FID	0·005–0·200
	Headspace SIM	0·001–0·015
Vinylidene chloride	Headspace ECD	0·005

* Dependent on type of food.
FID = Flame ionisation detector.
AFID = Alkali flame ionisation detector.
SIM = Single ion monitoring.
ECD = Electron capture detector.

TABLE 2
TYPICAL MONOMER LEVELS IN RETAIL FOODS PACKAGED IN PLASTICS, UK (1978/79)

Monomer	*Food type*	*Typical range*
Vinyl chloride[a]	Orange squash	<0·002–0·005 ppm
Acrylonitrile	Soft margarines	<0·01–0·04 ppm
Vinylidene chloride[b]	Biscuits	<0·005 ppm
Styrene	Cream	0·005–0·04 ppm

[a] Ministry of Agriculture, Fisheries and Food (1978).
[b] Ministry of Agriculture, Fisheries and Food (1980).

means of increasing the analytical sensitivity. A comparison of detection limits now attainable for monomers in foods (Table 1) and the range of concentrations currently expected (Ministry of Agriculture, Fisheries and Food, 1978, 1980) (Table 2) indicates that sensitivity is important.

MIGRATION OF NON-VOLATILE PLASTICS COMPONENTS

Analyses for other specific migrants are less well developed, principally because facile separation methods comparable to headspace GC do not exist. One area which has been much studied is the migration of plasticisers where the high concentrations added to plastics, combined with the relative stability of these compounds, reduce the severity of the analytical problem. A plasticised polymer is in some ways analogous to one swollen with oil and, viewed in this manner, it is not surprising that migration rates are generally increased. Particularly rapid loss occurs when plasticised films are in contact with fatty foods or simulants. The relationship between migration and percent incorporation into a polymer of a typical plasticiser is shown in Fig. 7 (Figge, 1980). Migration of other additives present is also enhanced, as expected.

One general feature of the analysis of specific migrants, sometimes overlooked, is that additives are frequently degraded during packaging fabrication and use. Other migrating species (secondary migrants) may be derived from a single additive and apart from the further reduced concentrations of the compounds to be measured, it will often be necessary for the analyst to distinguish between closely related degradation products. Figure 8 gives some indication of the potential complexity of such products (Lichtenthaler & Ranfelt, 1978). In addition, many additives are relatively unstable compounds and the possibility of continued chemical reaction after migration cannot be ignored. This is of special relevance to the trace analysis of antioxidants where considerable care may be required in order to obtain valid results (Wheeler, 1968). For example, the peroxide content of natural oils must be very carefully controlled where these are used as extractants.

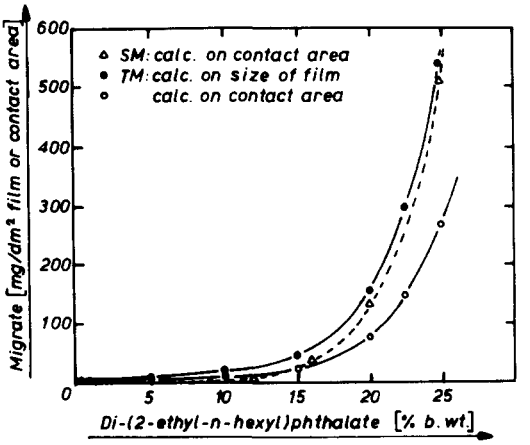


Fig. 7. Influence of plasticiser content of PVC test films on the specific migration (SM) of the additive under one-sided and on the total migration (TM) under all-sided contact between the test specimens and test fat HB 307. (Test conditions: 10 days at 40 °C). (Figge, 1980).

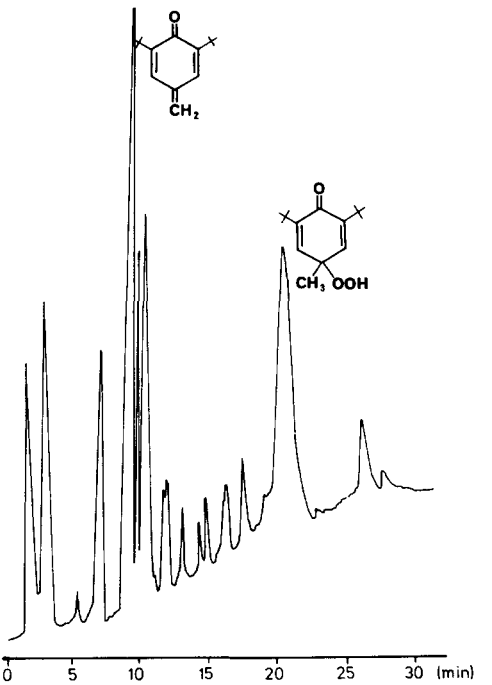


Fig. 8. Photochemical transformation products of BHT in polyethylene (HPLC-uv detection). (Lichtenthaler & Ranfelt, 1978).

There have been many reports describing the products obtained from individual additives subjected to degradation in bulk or solution by heat or uv radiation (Schroeder, 1973; Pospisil, 1979; Guyot & Michel, 1980), but comparable results for additives incorporated into plastics under realistic conditions of use are less common. One type of additive which has been studied in some detail is the organotin stabilisers for PVC (Figge & Zeman, 1973; Figge & Findeiss, 1975; Figge *et al.*, 1979; Ayrey & Poller, 1980). These do not migrate to any appreciable extent, except under leaching conditions, and consequently where this does not occur contamination of contacting foods is due mainly to the additive initially present on the surface of the plastics. Prior cleaning of the surface reduces transfer of the additive considerably (Downes & Gilbert, 1975).

The identities of any transformation products and the rates at which they are formed obviously depend upon the particular stabiliser involved. It has been shown by X-ray photoelectron spectroscopy that di-*n*-octyltin bis (2-ethylhexylthioglycollate) was completely decomposed at the surface of a stabilised PVC film (Figge & Zeman, 1973). The tin–sulphur bond no longer existed and thiols, sulphides, sulphoxides, sulphones and sulphonic acids (some of which were bound to the polymer) were all detected (Figge & Zeman, 1973). It is difficult to distinguish between migration of intact stabiliser or of a combination of cleavage products, ethylhexylthioglycollate and di-*n*-octyltin dichloride (or oxide) and this question remains, to some extent, unresolved, but it is probable that these secondary migrants predominate (Figge *et al.*, 1979).

Much of this work was done using radio-labelled tin stabilisers and in principle this is the favoured approach for all additives. The fate of epoxidised triglyceride stabilisers for PVC has also been studied in this manner (Shepherd & Gilbert, 1981). Glyceryl tri [1-^{14}C] epoxyoleate was incorporated into rigid PVC foils which were heated to simulate packaging fabrication conditions. Progressive loss of intact stabiliser was found with increased heating times (Fig. 9) and complex mixtures of chlorohydrins and oligomeric material were isolated from the plastics while 20–30 % of the additive remained unaccounted for. Except in the case of penetrating extractants, migration of stabiliser or of chlorohydrins was not detectable. However, small amounts of both had migrated into groundnut oil after 10 days' contact at 40 °C (Shepherd & Gilbert, 1981).

Even if radio-tracer experiments are not designed to characterise individual transformation products, judicious choice of the label ensures that total migration due to a tested additive can be assessed with confidence. All other methods are open to the objection that important secondary migrants might have escaped detection. In many instances radiochemical methods will not be feasible and the analyst must give due consideration to this problem. Much remains to be done in terms of characterising secondary migrants derived from plastics additives, principally because of the difficulties inherent in this area of analysis.

Only a very broad outline of current knowledge concerning the migration into

foods of low molecular weight components of plastics packaging has been presented here. Further information on some aspects of migration can be found in two recent books. Crompton (Crompton, 1979) gives details of a large number of analytical methods for the determination of additives, principally in simulants, while Crosby (Crosby, 1981) discusses some of the points mentioned here and includes other material not covered in this review, such as toxicology.

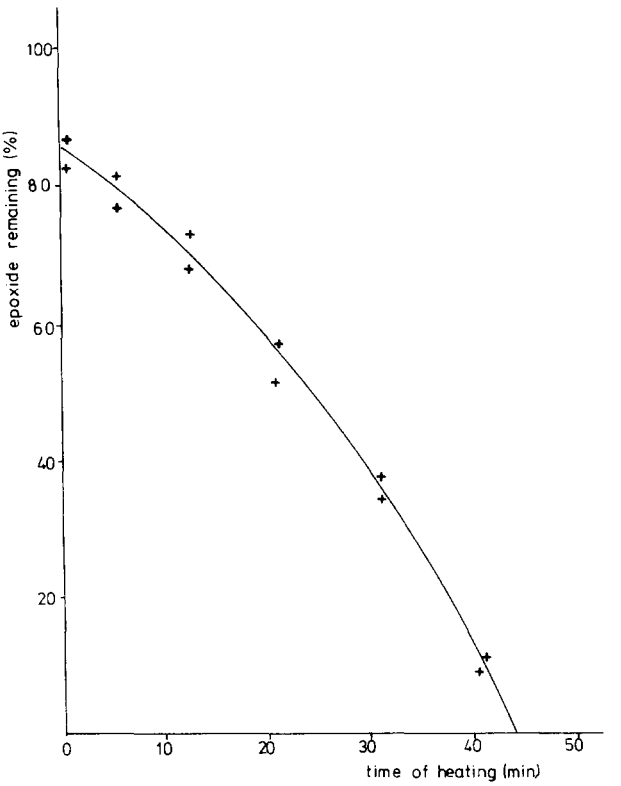


Fig. 9. Progressive loss of epoxide stabiliser through heating PVC sheet (Shepherd & Gilbert, 1981).

In conclusion, it must be emphasised that despite the complexity of the topic, substantial advances have been made over the past ten years with consequent benefit to the consumer. This progress can be expected to continue, particularly directed towards developing a better understanding of the theoretical basis of migration, with the consequence that the packaging fabricator will be able to tailor his product in order to reduce still further the level of trace contamination of foods from plastics.

ACKNOWLEDGEMENTS

I wish to thank Dr J. Gilbert for his helpful advice during the preparation of this paper and for his critical appraisal of the manuscript and Professor A. W. Birley for discussions on polymer crystallinity.

I am also grateful to the following for permission to reproduce figures: Fig. 1, Arthur D. Little, Inc. and FDA; Figs 3 and 4, R. Ashby; Figs 6 and 7, Dr K. Figge and Pergamon Press; Fig. 8, Dr R. Lichtenthaler and Elsevier Scientific Pub. Co.; Fig. 9, Pergamon Press.

REFERENCES

ADCOCK, L. H. (1980). *Proc. Third Internat. Symp. Migration*, Unilever, Hamburg.

ANON. (1979). *A Study of Indirect Food Additive Migration.* Arthur D. Little Inc., Second Annual Technical Progress Report, FDA contract 223-77-2360, USA Food and Drug Administration, Washington.

ANON. (1981). *Migration of BHT Antioxidant from High Density Polyethylene.* Arthur D. Little Inc., Summary Report, FDA contract 223-77-2360 USA Food and Drug Administration, Washington.

ASHBY, R. & KRUL, N. (1980). *Proc. Third Internat. Symp. Migration*, Unilever, Hamburg, p. 122.

AYREY, G. & POLLER, R. C. (1980). *Developments in polymer stabilisation*, Vol. 2. (Scott, G. (Ed.)), Applied Science Publishers Ltd, London, p. 1.

CRANK, J. (1975). *The mathematics of diffusion* (2nd ed.), Clarendon Press, Oxford.

CROMPTON, T. R. (1979). *Additive migration from plastics into foods*, Pergamon Press, Oxford.

CROSBY, N. T. (1981). *Food packaging materials: Aspects of analysis and migration of contaminants*, Applied Science Publishers Ltd, London.

DOWNES, T. W. & GILBERT, S. G. (1975). *Dtsch. Lebensm.-Rundsch.*, **71**, 422.

FIGGE, K. (1980). *Progr. Polym. Sci.*, **6**, 187.

FIGGE, K. & FINDEISS, W. (1975). *Angew. Makromol. Chem.*, **47**, 141.

FIGGE, K. & KLAHN, J. (1980). *Proc. Third Internat. Symp. Migration*, Unilever, Hamburg, p. 81.

FIGGE, K., KOCH, J. & FREYTAG, W. (1978). *Food Cosmet. Toxicol.*, **16**, 135.

FIGGE, K. & ZEMAN, A. (1973). *Kunststoffe*, **63**, 543.

FIGGE, K., FREYTAG, W. & BIEBER, W.-D. (1979). *Dtsch. Lebensm.-Rundsch.*, **75**, 333.

GILBERT, J. & SHEPHERD, M. J. (1981). *J. Assoc. Publ. Anal.*, **19**, 39.

GUYOT, A. & MICHEL, A. (1980). *Developments in polymer stabilisation*, Vol. 2, (Scott, G. (Ed.)), Applied Science Publishers Ltd., London, p. 89.

HACHENBERG, H. & SCHMIDT, A. P. (1977). *Gas chromatographic headspace analysis*, Heyden, London.

HAESEN, G., LE GOFF, B. & GLAUDE, Ph. (1981). *Mikrochimica Acta*, **1**, 25.

JUSKEVICINTE, S. & SHLYAPNIKOV, Yu. A. (1965). *Vysokomolekul. Soedin.*, **7**, 2094.

KAMPOURIS, E. M. (1975). *Rev. Gen. Caout. Plast.*, **52**, 289.

KARCHER, W., HAESEN, G., LE GOFF, B., SCHWARZE, A. & FIGGE, K. (1980). *Proc. Third Internat. Symp. Migration*, Hamburg, p. 48.

KATAN, L. L. (1980). *Proc. Third Internat. Symp. Migration*, Unilever, Hamburg, p. 9.

LICHTENTHALER, R. G. & RANFELT, F. (1978). *J. Chromatogr.*, **149**, 553.

LUSTON, L. (1980). *Developments in polymer stabilisation*, Vol. 2, (Scott, G. (Ed.)), Applied Science Publishers Ltd, London, p. 185.

MINISTRY OF AGRICULTURE, FISHERIES AND FOOD (1978). *The Second Report of the Steering Group on Food Surveillance. The Working Party on Vinyl Chloride*, Food Surveillance Paper No. 2, HMSO, London.

MINISTRY OF AGRICULTURE, FISHERIES AND FOOD (1980). *The Third Report of the Steering Group on Food Surveillance. The Working Party on Vinylidene Chloride*, Food Surveillance Paper No. 3, HMSO, London.

MORANO, J. R., GIACIN, J. R. & GILBERT, S. G. (1977). *J. Fd Sci.*, **42**, 230.

PFAB, W. (1973). *Dtsch. Lebensm.-Rundsch.*, **69**, 151.
POSPISIL, J. (1979). *Developments in polymer stabilisation*, Vol. 1, (Scott, G. (Ed.)), Applied Science Publishers Ltd, London, p. 1.
ROSSI, L. (1977). *J. Assoc. Offic. Anal. Chem.*, **60**, 1282.
RUDOLPH, F. B. (1980). *Proc. Third Internat. Symp. Migration*, Unilever, Hamburg, p. 70.
SANCHEZ, J. C., CHANG, S. S., MCCRACKIN, F. L. & SMITH, L. E. (1978). NBSIR, 78–1499, USA Food and Drug Administration, Washington.
SCHROEDER, E. (1973). *Pure Appl. Chem.*, **36**, 233.
SCOTT, G. (1981). *Developments in polymer stabilisation*. Vol. 4, (Scott, G. (Ed.)), Applied Science Publishers Ltd., London, p. 181.
SHEPHERD, M. J. & GILBERT, J. (1981). *Eur. Polymer J.*, **17**, 285.
WHEELER, D. A. (1968). *Talanta*, **15**, 1315.

Food Chemistry 8 (1982) 147–155

NEW MATERIALS FOR FOOD PACKAGING—THE FUTURE

J. H. BRISTON

30 Ranmore Rd., Cheam, Surrey SM2 7LT, Great Britain

(Received: 8 September, 1981)

ABSTRACT

It is unlikely that there will be many new materials used in future food packaging, but there will be new combinations of materials and new uses to which they are put. Changes in relative costs may reverse certain trends.

Developments in the following areas are covered: metals, such as tin-free steel and the combination of aluminium and plastics; paperboard, such as tubs and ovenable board; glass, such as 'Plastishield', and plastics, themselves, dealing with developments in processing, such as stretch blow moulding and solid phase pressure forming.

Developments in the newer materials such as poly(ethylene phthalate), the nitriles, poly(vinyl alcohol) and ethylene/vinyl alcohol copolymer and polycarbonate, are also considered.

INTRODUCTION

It is an intriguing commentary on the way times have changed in the past twenty or thirty years, that a symposium covering the chemistry of food packaging materials should be devoted almost entirely to plastics, but it is interesting to note that many of the developments in other materials are being made in combination with plastics. This paper is unable to do more than point to the the various areas of development.

METALS

Containers based on tin-coated steel (tinplate) have been used for food packaging since the early 19th century and they still hold an appreciable share of the market, particularly for processed foods. Because of the high cost of tin, much research has

Food Chemistry 0308-8146/82/0008-0147/$02·75

been carried out over the years to reduce the coating of tin. A great deal of success has been achieved in this area, but there is a practical limit in terms of the performance required. The emphasis shifted, therefore, to a search for alternative steel coating materials. The most successful development to date is the duplex chromium/chrome oxide treated low-carbon steel known as tin-free steel or TFS. Figure 1 shows a cross-section of a typical TFS sample compared with a typical tinplate sample.

The surface of TFS is more acceptable to protective lacquers and printing inks than is tinplate and higher lacquer stoving temperatures can be used because of the absence of the low melting point tin layer (232 °C). Shorter stoving times are possible, therefore.

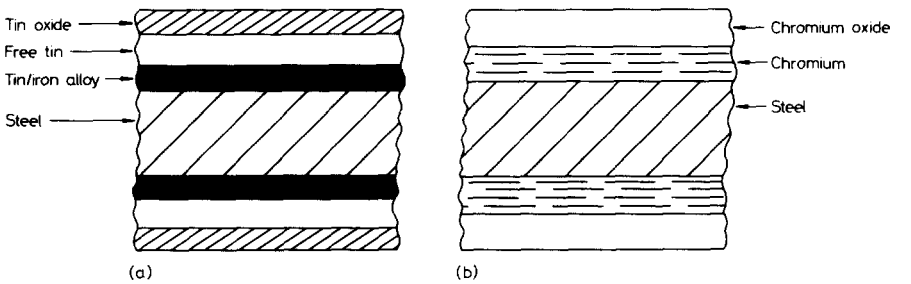


Fig. 1. Diagrammatic sections of (a) tinplate and (b) tin-free steel (TFS).

However, TFS lacks a protective sacrificial tin layer and so is less resistant to corrosion than is tinplate, and both surfaces must be protected by a lacquer system. A further disadvantage of TFS is that containers made from it cannot be soldered with traditional tin or lead solders. This is overcome by the use of organic adhesives or by welding. One process (the Miraseam®) uses a polyamide adhesive and this has been successful in the manufacture of cans for carbonated beverages. Welding processes may be thermal (as in the Conoweld® process), or based on an alternating electric current with a continuous copper wire electrode. The latter system is being developed in Europe for the production of food and beverage containers—for tinplate, as well as TFS.

In the UK, TFS is used for ends of certain tinplate cans, including petfoods, beer and rhubarb, as well as for some crown type closures for beer bottles. The use of TFS in the UK will certainly expand if American experience is any guide. In the USA, TFS is being used for two-piece cans such as the Drawn and Wall Ironed can.

Turning now to aluminium, perhaps the most significant development has been the retort pouch, formed from a laminate of aluminium foil and plastics such as polyester, modified high density polyethylene or propylene/ethylene copolymers. Such pouches are designed to be filled with a food product and fully heat processed. The food can then be stored for up to two years or more at ambient temperatures. There has been a certain amount of misunderstanding attached to the retort pouch

because of its initial over-ambitious billing as the 'flexible can'. Admittedly, it has some advantages over the can inasmuch as its geometry results in more rapid (and more even) heat penetration, shorter processing cycles and improved product quality in comparison with the cylindrical can (or glass jar).

It is not so robust as the tinplate or TFS can, however, and filling speeds at present are well below those achieved on modern can filling lines. Normal pouch filling speeds at present are 50–100 packs a minute, with 250 as a top speed, whereas a modern can filling line runs at about 1000 cans a minute. It is usual to overwrap a retort pouch with a polyester/polyethylene laminate or place it in a carton.

Future growth for the retort pouch should be examined under two headings—retail and catering. In the retail sector the growth will be governed by the convenience offered to the retailer and the ultimate consumer. Benefits to the retailer are related to the pressures on space, present in any retail outlet, whether it be corner store or supermarket. The fact that retort pouches can be stored at ambient temperatures relieves the pressure on cabinet space and removes the cost of back-up deep freeze. The pouch also takes up less space than cans and bottles in the ambient storage areas. Benefits to the consumer include ease of preparation, particularly for recipe meals, such as Chicken Supreme or Paella. The pouch can be re-heated quickly by the boil-in-the-bag technique and the meal is then ready to eat, saving time, labour and energy by the user.

In the catering sector, a key factor is the shortage of skilled labour, so that the convenience factors are extremely attractive to schools, canteens, hospitals and hotels. In addition, many catering establishments have a limited frozen storage capacity so that the retort pouch offers advantages over frozen foods.

A development of the flexible pouch is the semi-rigid tray. This is based on a heavier than normal gauge material and is cold formed. Conventional pouch material is used for lidding the tray. Material costs are higher but, for many products, such as sausages and fish fillets, it is possible to use less material because the tray can be tailor-made to fit the product. Because of its more robust construction, the tray can be marketed without the protective wrap or carton.

One other combination pack should be briefly mentioned. This consists of a seamless body tube, extruded from polypropylene. The body is then overwrapped with a multi-layer pre-printed web incorporating aluminium foil, and a pre-moulded base and top are sealed on. The filled container is able to withstand processing temperatures and pressures and has already been used for packaging meat products such as frankfurters.

PAPERBOARD

Although paper and board still have the largest single share of the packaging market, they have lost ground over the past few years, mainly to plastics. However,

there are signs that in some areas there may be a swing back because of the rapid price rises in certain plastics. Thus, paperboard formed tubs are capturing markets in ice cream and margarine. In Germany, for example, 250 g and 500 g margarine packs are commonly made from paperboard.

Another development area for paperboard is the so-called 'ovenable' board. This is used for the packaging of convenience foods that are eventually heated, in the tray, by either conventional of microwave ovens. A common type of ovenable board is a bleached sulphate board extrusion coated with poly(ethylene terephthalate). Containers are made either by existing carton technology, to produce folded cartons, or by press forming, to give trays. The bulk of ovenable board sales are for containers to be used with microwave ovens. In the USA, some 12% of homes possess a microwave oven and sales of ovenable board are already appreciable. Growth in the UK and in Europe generally is slow but could be appreciable by the mid-1980s. In addition, of course, there is scope for growth in the catering sector.

GLASS

One of the most successful lines of development in glass bottle manufacture is that of weight reduction. Glass is intrinsically a heavy material and this is a disadvantage in packaging applications. The success of the glass industry's efforts to overcome this disadvantage is well illustrated by the reduction in weight of the 1-pint milk bottle, from $19\frac{1}{4}$ oz in the 1930s to the present 9·3 oz.

One interesting development that utilises plastics is the 'Plastishield' bottle. This consists of a low weight bottle, fitted with a pre-printed sleeve of expanded polystyrene. The expanded polystyrene protects the surface of the bottle during filling and processing and also reduces noise in the bottling hall. Finally, the protective shield greatly reduces damage to surfaces such as kitchen or bathroom ceramic tiles.

PLASTICS

As far as plastics are concerned, a great deal of information about five materials that have served the cause of food packaging well during the past two or three decades has been given in previous papers, i.e. PVC, polystyrene, low density polyethylene, high density polyethylene and polypropylene. Although the subject of this paper is new materials and the future, it must be emphasised that these five materials (and their variants) are going to be around for a long while yet.

Apart from developments in the materials themselves, there have been developments in conversion processes that have given a new lease of life to the older established polymers.

One of these processes is stretch blow moulding or biaxial blow moulding. This is a process whereby bottles are produced having an appreciable orientation in both the longitudinal and the transverse directions. All blow moulding processes produce orientation in the transverse direction but none or little in the longitudinal direction. Biaxial orientation improves tensile and impact strength, while in transparent resins there is a reduction in haze. Other advantages include reduced creep and improved gas and water vapour barrier properties. Container weight may also be reduced. The basic principle is that either a length of plastics tube or an injection moulded pre-form is mechanically stretched in the longitudinal direction. During this stretching process the temperature is maintained within a narrow range which is specific for each polymer. The stretched tube or pre-form is then blown to the final desired shape.

Of the five polymers mentioned, two in particular (namely PVC and polypropylene), have been blown into bottles using the stretch blow moulding process and have been found to have improved properties. Oriented PVC bottles, for example, have a high resistance to breakage at minimum unit weight, high resistance to pressure and excellent optical properties, such as brilliance, absence of streakiness and high transparency. The greater strength imparted by orientation enables lower weight bottles to be made, yet with satisfactory impact strength. Examples quoted in the literature include 0·33-litre bottles to hold carbonated drinks (6 vols carbonation) where a 25 g biaxially oriented bottle is claimed to be equivalent to a 40 g bottle produced by normal extrusion blow moulding. One of the additives normally found in PVC is an impact modifier, to increase impact strength. Orientation is said to allow a great reduction to be made in the impact modifier content. In fact, a 30 % higher impact strength has been claimed, with a 20 % reduction in bottle weight and with no impact modifiers added. As well as reducing cost, this has the advantage that there is one less additive to worry about! Orientation of polypropylene also improves the physical properties. Thus, oriented polypropylene bottles have a higher impact strength, better low temperature resistance and higher water vapour barrier properties.

New resin formulations, made exclusively for orientation, have also been introduced, based on random copolymers as opposed to the more normal block copolymers. Display clarity of oriented polypropylene bottles is said to be comparable to that of PVC and far superior to that of high density polyethylene. Clarity is also improved by the use of nucleating agents.

Another process that increases the scope of polypropylene is solid phase pressure forming. In this process, polypropylene sheet is heated to a temperature below its melting point and then formed into shape by pressure. The sheet is heated by infra-red heaters as in normal thermoforming processes but, as the actual forming takes place below the crystalline melting point, the heat input must be carefully controlled so that the sheet is uniformly heated to about 155 °C. The heated sheet is first stretched into the mould cavity by a heated plug. At the lowest point, cold air is

forced into the plug chamber under high pressure, pushing the hot sheet against the cooled inner wall of the mould and thus finishing the forming operation at a high speed. This process improves the strength of containers made from polypropylene sheet, as well as their clarity. There is another important consequence from the food packaging point of view: because of the lower temperature involved, the containers so formed are less likely to suffer from residual odour and taint.

Finally, there is the process of coextrusion. This can benefit the older established materials as well as the newer but more expensive ones. As the previous papers have already pointed out, the established polymers each have some very good properties, but they also have some deficiencies. These can best be overcome, not by looking for some super plastics, but by combining two or more plastics to give the desired combination of properties. Film lamination has been carried out for very many years, but multi-layer bottles were not normally available until the technique of coextrusion was developed. Coextrusion consists in coupling two or more extruders to one die, thus giving a multi-layer tube which is then blow moulded to give a multi-layer bottle. Coextrusion provides the facility for producing individual plies of very low gauge and it is, therefore, possible to produce bottles where a thin layer of a relatively expensive barrier material is sandwiched between inner and outer layers of low cost materials.

Let us now look at some other polymers that may become more important in the future. One of the most important of the newer plastics now being used in food packaging is poly(ethylene terephthalate)—commonly known as PETP or PET. This material is already widely used for the packaging of carbonated soft drinks, particularly in the larger sizes. Other polymers of interest in the field of food packaging are the nitriles, such as poly(acrylonitrile) and copolymers of acrylonitrile, poly(vinyl alcohol) and copolymers of ethylene and vinyl alcohol (EVAL).

Polycarbonate, too, is worth a mention. Although it has been available for a long time, it has not been used very much in food packaging. It now looks, judging by developments in the USA and West Germany, as if it may play an increasing rôle in the packaging of milk, and I will return to this topic later.

POLY(ETHYLENE TEREPHTHALATE)

PETP was developed first as a textile fibre (Terylene—trade mark of Imperial Chemical Industries Ltd.). Later, it was developed as a film and has been widely used in packaging boil-in-the-bag foods. Only fairly recently (since the advent of stretch blow moulding) has it found application as a bottle blowing material. Oriented PETP has a high tensile strength and is well able to resist the pressures generated by normal carbonated soft drinks (50–60 psi). Gas barrier properties are superior to those of PVC and the polyolefins but are not as good as poly(acrylonitrile). It is also a good barrier to water vapour. The softening point is high, the material melting

over the range 243°–270°C. It has a high thermal and thermo-oxidative stability, is resistant to many chemicals and has good sparkle and optical clarity. Clarity is affected by the catalyst system used in the polymerisation process. Catalyst systems are usually based on antimony, germanium or a mixture of antimony/germanium containing compounds. All produce very clear bottles, but germanium also adds an extra sparkle to the bottle.

Although gas barrier properties are good, there are areas where improvements in carbon dioxide retention and resistance to oxygen ingress would open up large areas of business, such as beer bottling. It is known that PETP bottles of 1-litre or above can retain carbon dioxide to an acceptable extent (not more than 15% lost over about 4 months), but as bottle size is reduced, the increase in the surface area/volume ratio makes this limit difficult to achieve.

In addition, beer is affected by the presence of small quantities of oxygen. The ingress of oxygen in the present small PETP bottles is sufficient to make them unacceptable for beer bottling. Work is currently proceeding on modifications to the PETP polymer to give it inherently better barrier properties, and on the production of multi-layered bottles. One possibility is PETP/acrylonitrile, while dip coating, using PVDC latices, is also being evaluated.

Although PETP is an inert polymer, degradation of the molten polymer does occur and leads to the formation of acetaldehyde. Although not toxic in the amounts likely to be found in PETP bottles, it can, nevertheless, taint the taste of certain critical drinks. Acetaldehyde, of course, has a characteristically pungent and penetrating odour and is used as a flavour and fragrance ingredient. It can be found in beverages, ice cream and chewing gum and also occurs naturally in citrus fruits, grapes and grape products, apples and many other fruits and berries. In the packaging of beverages, therefore, any additional acetaldehyde coming from the package may affect the product's taste and consumer acceptance. Colas are particularly susceptible to the presence of even very low levels of acetaldehyde. Much work has been, and is being carried out, therefore, to reduce acetaldehyde levels in the finished PETP bottle. Residence time of the molten polymer in the extruder or injection moulding machine is one factor, the generation of acetaldehyde being almost directly proportional to melt residence time. Another factor is temperature, but the picture here is not so simple. Processing temperatures are limited at the lower end in terms of bottle clarity and at the upper end by the generation of acetaldehyde. Taking into account other restraints, the ideal processing temperature range is said to be between 277° and 282°C.

NITRILES

The homopolymer, poly(acrylonitrile) (PAN), also has a long history as a textile fibre, but has only recently been used for bottles. It has good gas barrier properties but is only a fair barrier to water vapour. It is hard and has good clarity. Its impact

strength and creep resistance are borderline for bottle uses but the impact strength is improved by stretching and heat setting.

A number of copolymers based on acrylonitrile or methacrylonitrile have been developed, giving improved properties. One fairly complex material is Barex (trade name for a material made by the Vistron Division of Standard Oil of Ohio). It is made by copolymerising a 75:25 mixture of acrylonitrile and methyl acrylate in the presence of a small amount of a butadiene/acrylonitrile rubber.

It has good clarity, excellent gas barrier properties and a high resistance to creep. In addition, it has good impact strength and is insoluble in a wide range of organic solvents. In film form, it has been laminated to low density polyethylene and then thermoformed into containers for cheese and meat.

It has also been used in the Rigello Pack, which is a novel container, formed from four component parts. The bottom is a cylinder with a hemispherical base moulded from Barex, which acts as a liquid and gas barrier. Mechanical strength is enhanced by a sleeve, wound from three layers of paper. The outer layer carries the design and may be coated with polyethylene or lined with aluminium foil. A conical top, also of Barex, is welded to the base component. Finally, the cone has an orifice into which is fitted a cap, injection moulded from a medium density polyethylene, coated inside with PVC to reduce gas transmission.

Rigello pack bottles have been used for the packing of beer in Sweden for over four years and have recently made their appearance as beer bottles in the UK.

In the USA, nitrile polymers and copolymers have been under attack because of carcinogenicity (determined on test animals at certain intake levels). Acrylonitrile polymers were banned from use in beverage packaging in the USA but a recent appeal has led to the question being re-opened by the F. and DA. One exception to the original ban was the use of PAN where it was prevented from contacting the beverage by a functional barrier that would prevent migration of any acrylonitrile into the beverage.

POLY(VINYL ALCOHOL) AND ETHYLENE/VINYL ALCOHOL COPOLYMER (EVAL)

Poly(vinyl alcohol) is a very good barrier to gases and to many organic solvents but is hydrophilic and is used for bottle blowing only in the form of a coextrusion with other polymers. The poly(vinyl alcohol) is made the centre ply between layers of high density polyethylene or polypropylene. Such bottles have very high resistance to the passage of oxygen and to organic solvents, coupled with the rigidity of the polyolefins mentioned.

EVAL also possesses extremely high gas and solvent barrier properties. Coextruded bottles made from EVAL and either high density polyethylene or polypropylene have been shown to be excellent barriers to oxygen when used to store orange juice, thus preserving the vitamin C content.

POLYCARBONATE

This material has a high impact strength, a high softening point and excellent clarity. Unfortunately, it is an expensive polymer and is based on expensive starting materials. Understandably, it has found few outlets in the one-trip container market. Its physical properties do make it an excellent material for multi-trip use, however, and great interest is being shown in its use for milk bottles, both in the USA and in Germany.

In the USA, for example, a dairy that uses 1-gallon polycarbonate bottles claims journeys of up to 100. Multi-trip bottles can pose problems in the area of possible contamination through alternate use by the consumer. In Germany, the returned polycarbonate bottles are inspected visually and then by an automatic measuring device that takes an air sample from every bottle. The air is analysed by gas chromatography to detect aromatic hydrocarbons and any contaminated containers are automatically destroyed.

Food Chemistry **8** (1982) 157 168

SOME ASPECTS OF THE CONTROL OF PLASTICS FOOD PACKAGING IN THE UK

J. R. BELL

Ministry of Agriculture, Fisheries and Food, Food Science Division, Great Westminster House, Horseferry Road, London SW1P 2AE, Great Britain

(Received: 8 September, 1981)

ABSTRACT

One of the principal reasons for the use of food packaging is to prevent the undue contamination of food. However, the package can itself give rise to contamination by acting as a source of chemical substances which may migrate into the food. The number of potential contaminants from this source is very large and any controls have to be formulated with this in mind. Legal restraints which are currently in force in the UK concerning contaminants which may enter food from plastics packaging are, with one exception, of a general nature. However, this is not to say that no regard is paid to the nature and potential health implications of specific migrants; in fact, several investigatory programmes in this area have already been conducted and others are currently in progress. The results of such studies are always carefully assessed to ascertain whether or not the need for specific legislative controls is indicated.

This paper considers in some detail the approach to gathering information and assessing potential problems associated with the use of plastics for food packaging in the UK. In particular, the influence that this has had so far on the need—or otherwise—for formal controls is discussed and the effects that UK membership of the EEC has had on this area is outlined. A brief comment on emerging trends is made.

INTRODUCTION

One of the principal reasons for the use of food packaging is the prevention of the microbial contamination of food. However, whilst serving this purpose, the packaging can itself become a source of contamination through the migration of chemical substances from the package into the contained food. The extent and nature of this migration depends on many factors which include the type of food, the

Food Chemistry 0308-8146/82/0008-0157/$02·75

type of packaging, the time of contact, the storage temperature and, probably also, the degree of penetration of the food into the packaging material. The number of contaminants which could arise in this way is very large, extending perhaps to several thousands. It is important, though, to keep this source of potential chemical contaminants in perspective by realising that there are many other routes by which undesirable substances can enter food; for instance, from the environment or through the use of materials such as pesticides and herbicides. In this paper, however, attention is focused on the possible hazard to health arising from just one source of food contamination—that of plastics for food packaging.

ASSESSMENT OF HEALTH HAZARD

Toxicity of packaging materials

Before it is possible to make any assessment of potential health hazards arising from the use of food packaging it is necessary to be able to identify the substances which have migrated from the packaging into the food. In the ideal situation, information would be available on the migration of a component of packaging for all types of packaging containing that component into all the various foods contained in these packagings. However, this is not a perfect world and such data are not only rarely available but also expensive to obtain. Analysis of a food for a migrant present in only trace amounts is often very difficult, as foods themselves are usually complex mixtures of chemicals. In the case of plastics the task can, however, be simplified to a degree by dividing the components of a package into categories about which some conclusions can be drawn concerning migration ability. The principal categories are as follows.

(a) High molecular weight polymers (which are unlikely to migrate).
(b) Oligomers (which may migrate).
(c) Monomers (which are very likely to migrate owing to their relatively small size and often high volatility and which may well be present in significant amounts).
(d) Polymerisation ingredients, such as initiators and chain transfer agents (which are likely to decompose in the course of performing their function and so their decomposition products are most likely to be the migrating species. However, the quantities may well be extremely small).
(e) Additives, such as plasticisers, colourants, lubricants (which may migrate in significant amounts).
(f) Additives, such as antioxidants and stabilisers (which undergo some change in the lifetime of the plastics and which may migrate in significant amounts both in the changed and unchanged form).

Experience has shown that there is unlikely to be an acute risk to health from the contamination of food by migrants from packaging. However, it is possible that long-

term exposure to small amounts of a chemical might give rise to a toxic effect in man at some point during a normal lifespan. Such risks are very difficult to evaluate, but feeding studies conducted on laboratory animals, together with epidemiological studies on populations known to have been exposed to the chemical, can provide an estimate of the highest intake level of the migrant tolerable to man.

Per capita daily intake of a migrant

Having identified the chemical nature of the migrant and obtained some measure of its likely toxicity to man, the next step in a hazard assessment of the packaging material is to obtain an estimate of the per capita daily intake of the substance through the diet. The particular chemical may be present in a range of different packaging materials and each of these may be in contact with a number of different types of food. In addition, information on the level of migrant in each of the types of food may not be available. Therefore, it is often difficult to make an exact calculation of the quantity of the chemical ingested by the average man per day. To overcome this problem, approximation techniques are often used. These may involve the following:

(a) The use of per capita daily consumption figures for the proportion of each major food that is packaged in material likely to contain the migrant, together with figures for the maximum observed level of the migrant in each food.
(b) The use of per capita daily consumption figures for the proportion of each major food that is packaged in material likely to contain the migrant, together with migration figures based on the assumption that all the migrant in each pack actually migrates.
(c) The use of a per capita daily consumption figure for all packaged food, together with the migration figure for the food into which migration is greatest.

The first approach is probably the most exact—and the third the crudest but simplest. Other techniques are also available, but, in general, they have no definite advantages over those described.

An estimate of the per capita weekly consumption of most of the foods consumed in the UK can be obtained by reference to the National Food Survey (Ministry of Agriculture, Fisheries and Food, 1981). However, the determination of the level of a migrant in a packaged food is, as mentioned earlier, often a very difficult matter. In the case of plastics and certain other materials it is common practice, especially in some European countries, to use solvents, often called food simulants, for migration testing. The common solvents, which have been chosen on the basis that they may be expected to resemble—but be generally more aggressive than—groups of foods, are water, dilute acetic acid, aqueous ethanol and olive oil. These are supposed to simulate the action of neutral or alkaline aqueous foods, acid foods,

alcoholic foods and fatty foods, respectively. Of course, the amount of migrant that will appear in the solvent is dependent on, amongst other things, the time and temperature of contact and these are usually chosen to resemble the intended conditions of use of the material. Where there is any doubt, the most stringent conditions are advocated. In the case of fatty foods, 10 days at 40 °C using olive oil has been suggested as the most exacting test. The level of migration determined by using solvents is normally expressed in mg/dm^2 of packaging surface and this has to be related to the amount likely to migrate into food expressed as milligrammes of migrant per kilogramme of food. The best approach is obviously to determine the area of package in contact with the food, but this may be difficult. An alternative is to assume that the total area of package contacts the food (which may be fully justified in certain cases anyway, such as when a product like yogurt is packaged in a plastic tub) or that 1 kg of food is packaged in general in $6\,dm^2$ of package (often used, but strictly only valid if the food is of unit density and packaged as a cube). One kilogramme of food per day is sometimes used as a crude approximation of the total per capita consumption of all packaged food.

Despite the apparent analytical attraction of using solvents, this approach to migration assessment has proved to be too inaccurate to serve as more than a rough guide to the situation with respect to food. Thus, in the UK, stringent attempts are always made to develop suitable techniques for measuring the relevant contaminant levels in the foods themselves wherever possible.

FOOD CONTAMINATION FROM PLASTICS PACKAGING IN THE UK

Surveillance

In 1971 the Steering Group on Food Surveillance, an inter-departmental committee, was set up at the request of the Minister of Agriculture, Fisheries and Food. Its function is to review the position of chemical contaminants in food, assess the need for analytical surveys of food and, when necessary, to arrange for such surveys to be performed. Reports from the Steering Group are submitted to various Expert Committees including the Food Additives and Contaminants Committee (FACC) and the Department of Health and Social Security Committees on Toxicity and Carcinogenicity of Chemicals in Food, Consumer Products and the Environment.

All of the Expert Committees are composed of members who are independent of the Civil Service. The FACC advises various Ministers on matters relating to food contaminants, food additives and similar substances which are, or may be, present in food or used in its preparation. The Committees of the Department of Health and Social Security advise, on request, a number of committees such as the FACC, as well as Ministers, on any hazard to health, including carcinogenic risk, which might result from the presence or use of additives, contaminants or pollutants in food or the environment.

Contaminant problems which are considered to require a detailed examination are referred by the Steering Group to Working Parties. Their task is to collect data from all the relevant sources and report these to the Steering Group. A number of such Working Parties have been set up but the following are particularly relevant to plastics packaging matters:

Working Party on Vinyl Chloride.
Working Party on Vinylidene Chloride.
Working Party on Acrylonitrile and Methacrylonitrile.
Working Party on Styrene.
Working Party on Migration from Plastics.

The membership of Working Parties is established having regard to the topic under consideration and the nature of the data to be collected and assessed. In the case of the Working Parties listed above the membership was drawn mainly from the UK food and food packaging industries. The first Working Party to be set up to investigate an issue associated with the use of plastics packaging was that on vinyl chloride (VC).

Working Party on Vinyl Chloride

In 1973 evidence came to light that showed that certain foods packaged in vinyl chloride polymers (PVC) and copolymers could become contaminated with significant amounts of VC. In view of the doubts which were being raised at that time by toxicologists about the hazards to health caused by exposure to this monomer, it was considered prudent to investigate the extent to which food in the UK might be contaminated with this substance. The Working Party on Vinyl Chloride was therefore set up in late 1973 in order to obtain the following information:

(1) The food uses of vinyl chloride polymers and copolymers.
(2) The levels of vinyl chloride in food.
(3) The factors affecting the vinyl chloride content of packaged food, in particular the effect of the vinyl chloride content of PVC.
(4) The total intake of vinyl chloride from foods packaged in PVC.

The principal food uses of PVC were identified as being bottles, rigid film for formed and shaped containers and flexible film for wrapping. A list of the foods most commonly packaged in PVC was also compiled. When this part of the investigation was complete, members of the Working Party turned their attention to the problem of measuring the levels of VC in VC polymers and copolymers and in retail foods. Suitable methods of analysis were identified and over a period of four years repeated surveys were conducted of the VC levels present in retail foods and their packaging. This enabled the significant reduction in monomer content of food contact VC polymers and copolymers, which was achieved by the industry under the co-ordination and encouragement of the Working Party, to be monitored and

recorded. An indication of the success of this exercise can be obtained from the fact that, whereas in a survey conducted by the Working Party in 1974 only 20 % of PVC bottles analysed contained less than 10 mg/kg VC, by February, 1977 100 % of the bottles examined contained less than 1 mg/kg VC. This, together with similar improvements in PVC used in other food applications, resulted in a marked decrease in the maximum likely intake of VC per person in the UK from 1·3 to 0·1 μg/day. As a result, amongst other things, of this decrease in exposure, the FACC, to whom the findings were submitted, felt that no legislative action in the UK need be recommended. A report by the Working Party containing its findings and recording the successful efforts made by industry in reducing the level of vinyl chloride in PVC and the contained food was published (Ministry of Agriculture, Fisheries and Food, 1978).

Since this time two further investigations have been successfully completed—one covering the presence in food of the monomer vinylidene chloride (VDC) and the other, acrylonitrile and methacrylonitrile. A very similar approach to that used for VC was followed in both subsequent investigations.

Working Party on Vinylidene Chloride

In the case of vinylidene chloride (VDC) it was discovered that the usage of this monomer in food packaging materials was considerably more diverse than that of VC, but that the amounts used per annum were smaller. This diverse usage, coupled with the low level of residual monomer present in most materials investigated (because VDC is mainly used as a comonomer in thin coatings), meant that only a crude calculation of the likely per capita intake could be attempted. This estimate was based on the assumption that all the VDC present in the materials was available for migration into the food. Obviously, the figure obtained in this way was a gross overestimate, but, even so, at 1 μg/day, was considered by the Working Party to be minute. The Committee on Carcinogenicity considered that, given the low level of migration into foods, VDC from this source did not pose a significant risk to health. Taking this into account, together with the information gathered by the Working Party, the FACC felt that, as in the case of VC, no legislative action in the UK need be recommended. A report detailing the findings of the investigation and including the remarks of the various Expert Committees was also published (Ministry of Agriculture, Fisheries and Food, 1980).

Working Party on Acrylonitrile and Methacrylonitrile

This Working Party has recently completed its work and the findings are now being considered by the Expert Committees. A report of these findings is expected to be published later this year.

Working Party on Styrene

The investigation into the usage of styrene in food packaging and its presence in food was begun in 1979, but still has some way to go before it will have generated

sufficient data to enable a per capita intake figure to be calculated. However, a large body of information has already been collected, so the picture on exposure to this monomer via the food supply in the UK is now beginning to take shape.

Working Party on Migration from Plastics

Unlike the Working Parties on monomers, which were set up to investigate specific contaminant problems in the UK, the Working Party on Migration from Plastics was created in response to a proposal of the EEC Commission in May, 1978 to establish a directive to control migration from food contact plastics (see later). It was decided that data on the levels of migration of constituents of the plastics used for food contact in the UK, when tested according to the provisions laid down in the EEC proposal, should be collected and assessed. A large quantity of such data was generated through an exercise involving collaborative testing of selected plastics by members of the Working Party, and this has proved extremely useful in negotiations on the Directive which have taken place in Brussels since 1978.

FORMAL CONTROLS ON PLASTICS FOOD PACKAGING IN THE UK

At the present time there are two main legislative controls in the UK on the contamination of food arising from the use of packaging materials. These are the Food and Drugs Act 1955 which applies to England and Wales (Anon., 1955) (Scotland and Northern Ireland have their own similar legislation) and The Materials and Articles in Contact with Food Regulations, 1978; as amended (Anon., 1978, 1980) (which do not apply to Northern Ireland).

Food and Drugs Act, 1955

The Food and Drugs Act, 1955 is designed to control food as sold or intended for sale and, since the contamination of food is included under this control, the Act also covers indirectly the use of materials and articles made of plastics where they give rise to contamination. The Sections of the Act which are of interest in this respect are:

Section 1 which makes it an offence to add anything to food so as to render it injurious to the health of the consumer.

Section 2 which ensures that the food must be of the nature, quality and substance demanded by the purchaser.

Section 8 which renders it an offence to sell unsound food.

Section 4 which empowers Ministers (in this case the Minister of Agriculture,

Fisheries and Food; the Secretary of State for Wales and the Secretary of State for Health and Social Security) to make regulations as they see fit to reinforce the general provisions of the Act in specific areas, with the important proviso that they should have regard to restricting the addition of non-nutritive substances to food as far as is practicable.

A number of specific regulations have been made under the Act and several of these contain provisions relating to contaminants, such as lead, which could arise from the use of packaging. One set of regulations that impinges directly on the use of plastics is The Preservatives in Food Regulations, 1979, as amended, which contain a provision allowing the presence in food of a specified level of formaldehyde arising from the use of certain plastics food containers or utensils.

The Materials and Articles in Contact with Food Regulations, 1978, as amended

In addition to indirect controls on plastics packaging, there are also in operation within the UK the Materials and Articles in Contact with Food Regulations 1978, as amended, (MA Regulations) which control materials and articles directly. These Regulations, which fulfil obligations arising from a directive agreed in Brussels in 1976, control, amongst other things, migration into food from materials and articles and the labelling of all such materials and articles where they are used or are intended to be used for food contact. In respect of migration, Regulation 4(2) is worth noting in more detail. It states that:

> 'Materials and articles to which this regulation applies shall be manufactured in accordance with good manufacturing practice; that is to say, in such a way that under normal or foreseeable conditions of use they do not transfer their constituents to foods with which they are, or are likely to be, in contact, in quantities which could: (i) endanger human health, or (ii) bring about a deterioration in the organoleptic characteristics of such food or an unacceptable change in its nature, substance or quality'.

The MA Regulations and the general provisions of the Food and Drugs Act are therefore similar in intent, although it is important to be aware that they are complementary and both can apply to a particular situation. An amendment to the original MA Regulations of 1978, which came into force on 1 January, 1981, is important to note. This amendment controls directly, for the first time in Great Britain, the level of a monomer allowable in plastics food contact materials. In this case the control applies to vinyl chloride present in vinyl chloride polymers and copolymers and requires that the quantity of VC present shall not exceed 1 milligramme per kilogramme of material or article. This amendment again arises from an EEC commitment, this time made in 1978. The mandatory EEC method of analysis for determining compliance is also referred to in the amended Regulations.

EEC ACTION ON FOOD CONTAMINATION ARISING FROM THE USE OF PLASTICS PACKAGING

The Community has been active in the food contact materials field since long before the UK joined in 1973. However, to date, only two Council Directives have been accepted by the Member States, one leading to the UK Materials and Articles in Contact with Food Regulations, 1978, and the other controlling VC levels in certain plastics and in foods (part of which has been implemented in an amendment to the aforementioned Regulations). A number of proposals for further EEC action are under discussion at the present time. Briefly these are as follows.

Plastics

A draft directive was submitted to the Council of Ministers on 30, May 1978 (Commission of the European Communities, 1978*a*). The aim of this directive is to limit total (or overall) migration from food contact plastics into foods or certain specified solvents (olive oil, 3 % acetic acid, water and 15 % alcohol are examples) to 60 mg/kg of food, or solvent, or, in certain cases, to 10 mg/dm^2 of material. In addition, the proposal specifies those materials which are to be tested and gives a definition of plastics. As is common practice, this draft directive was first passed to a Working Party of representatives of the Member States for detailed consideration. In November, 1980 it was forwarded to the Committee of Permanent Representatives (who have ambassadorial status) and they, in turn, referred it to the *ad hoc* Working Party of Agricultural Counsellors and Attachés for further detailed discussions. At the present time the proposal is residing with this latter group, but it looks as if little or no progress is being made as there is a fundamental difference of opinion between the Member States on the need for such a directive.

In addition to the directive controlling overall migration, two further complementary directives on plastics have been suggested by the Commission. The first is intended to contain instructions on which solvents are to be used in place of which foods, where the tests cannot, for analytical reasons, be made with the foods themselves, and, in addition, to indicate how the results should be treated in cases where the solvent is believed to be more aggressive than the food towards the plastic. The second is intended to give details of the methods of analysis to be used with each solvent. Neither of these two proposals for directives has as yet been submitted to the Council of Ministers.

Monomers

The Commission has expressed the intention to eventually submit proposals for directives to control all food contact plastics by one or more positive lists of permitted ingredients. Although such a system is unlikely to become a reality in the near future because of the volume of work involved in its preparation, the Commission has already started along the road by beginning a toxicological

evaluation of the monomers used for the manufacture of food contact plastics in Europe. This work is being carried out by the EEC Scientific Committee for Food (SCF) who are a group of internationally recognised scientific experts appointed by, and reporting to, the Commission. It does not appear, though, that any further monomers will be singled out for special consideration and control, in the way that vinyl chloride was in the past.

The data gathering exercises conducted in the UK through the medium of the various Working Parties of the Steering Group on Food Surveillance have proved very valuable, both from the point of view of influencing the EEC action on contamination from plastics food packaging mentioned above, and of preparing for such action. The information collected by the Working Party on Vinyl Chloride formed a significant part of the data made available by the UK to the SCF during its consideration of the health implications of this monomer in food. The Working Party, by acting as a forum for the encouragement and co-ordination of the efforts of the UK plastics industry in reducing the VC levels in packaging (and consequently in food), also proved to be extremely useful in preparing the UK generally for the introduction of the EEC-agreed controls. Certain data generated by the Working Parties on Vinylidene Chloride and on Acrylonitrile and Methacrylonitrile were also made available to the SCF during its preliminary examination of these monomers in 1978. The report of the former Working Party was sent to the EEC Commission immediately it was published last year, so that it could be considered by the SCF during its latest exercise on monomers. The report of the Working Party on Acrylonitrile and Methacrylonitrile will similarly be made available as soon as it is published, as eventually will a report on styrene. The Working Party on Migration from Plastics, by acting as a source of information on the way plastics used for food packaging in the UK might be expected to fare under a proposed Directive on overall migration, has provided, as mentioned earlier, an important background for discussions taking place in Brussels.

FUTURE TRENDS IN PLASTICS PACKAGING CONTROL

Based on developments in packaging control in Europe in the last 10 years, it is possible to identify three trends for the future.

The first is that future legislation is likely to be formulated on an EEC basis and should, in theory, be uniform throughout the Member States. Inevitably, controls of this nature will be lengthy in their inception, but should result in existing barriers to trade being progressively dismantled. In existing proposals a system is envisaged whereby amendments to agreed legislation could be made reasonably rapidly so as not to stifle unduly any new packaging developments.

The second trend is that legislation will be increasingly specialised in nature. Ten years ago the EEC Commission favoured only a few directives in this field, each

encompassing a large group of controls such as positive lists, overall migration limits, specific migration limits and detailed testing procedures for all eventualities. Thus it was envisaged at that time that only one directive would be required to encompass all plastics controls. More recently, this approach has been superseded by one of dealing with the more narrowly defined aspects such as overall migration, positive lists, etc., in separate directives. A possibility for the future is that this trend could be progressed further still, so that, in the case of plastics, controls might be recommended for each plastics type separately. The main attraction to the Commission of this specialised approach is that it might result in a more rapid agreement between the Member States on each set of controls.

The third trend is that increasingly detailed information will be required before chemicals used to make packaging for food are permitted within a legal framework. The establishment of permitted lists of substances and the addition of new substances to existing lists is likely to put an increasing burden on the producers and users of such substances to provide detailed supporting data. This information will most likely take the form of technological data relevant to the polymer use in food packaging together with the results of toxicological testing. An indication of the form that demands within the EEC for the latter type of data may take can be obtained by consulting the guidelines issued by the Scientific Committee for Food in its report published in 1978. (Commission of the European Communities, 1978*b*).

CONCLUSIONS

In the UK, control of food contamination arising from the migration of chemical components of plastics packaging is currently exercised in two ways. The first is through formal legal restraints, mostly of a general nature, and the second is through a monitoring programme incorporating a framework within which the relevant sectors of the packaging industry can be encouraged, where necessary, to improve voluntarily their plastics materials and so reduce the possibilities for unacceptable migration. In the future it appears increasingly likely that formal controls in this area will be Europe—rather than nationally—orientated and will result from agreement amongst the Member States of the EEC.

REFERENCES

ANON. (1955). *Food and Drugs Act, 1955*, 4 & 5. Eliz. 2c16, HMSO, London.

ANON. (1978). *The Materials and Articles in Contact with Food Regulations, 1978*, HMSO, London.

ANON. (1980). *The Materials and Articles in Contact with Food (Amendment) Regulations, 1980*, HMSO, London.

COMMISSION OF THE EUROPEAN COMMUNITIES (1978*a*). Proposal for a Specific Council Directive on the Overall Migration Limit for the Constituents of Plastics Materials and Articles Intended to Come into Contact with Foodstuffs, *Off. J. of the Eur. Communities*, **C141**, 4.

COMMISSION OF THE EUROPEAN COMMUNITIES (1978*b*). *Reports of the Scientific Committee for Food*, Fifth Series. Office for Official Publications of the European Communities, Luxembourg.

MINISTRY OF AGRICULTURE, FISHERIES AND FOOD (1978). *Survey of Vinyl Chloride Content of Polyvinyl Chloride for Food Contact and of Foods*, HMSO, London.

MINISTRY OF AGRICULTURE, FISHERIES AND FOOD (1980). *Survey of Vinylidene Chloride Levels in Food Contact Materials and in Foods*, HMSO, London.

MINISTRY OF AGRICULTURE, FISHERIES AND FOOD (1981). *Household Food Consumption and Expenditure, 1979*, HMSO, London.

Food Chemistry 8 (1982) 169–177

OXALATE CONTENT OF SOME LEAFY VEGETABLES AND DRY LEGUMES CONSUMED WIDELY IN EGYPT

S. S. GAD, M. ESMAT EL-ZALAKI, M. S. MOHAMED & S. ZEINAB MOHASSEB

Food Science and Technology Division,
College of Agriculture, Alexandria University, Egypt

(Received: 17 June, 1980)

ABSTRACT

Oxalic acid and total oxalate contents were determined in four leafy vegetables and six dry legumes consumed widely in Egypt. The four vegetables, normally eaten raw, can be arranged according to their oxalate content, in descending order, as follows: green onion (leaves), green onion (bulbs), leek, radish (leaves), radish (roots) and roquette. Oxalic acid represented 76–86% of the total oxalates.

Dry faba beans had the highest oxalic acid content, followed, in descending order, by fenugreek, lentils, peas, chickpeas and lupin. The ratio of oxalic acid to total oxalates differs according to the total oxalate content of the legume variety. Decortication, steeping, sprouting, stewing, boiling and baking reduced the oxalate content of seeds in different ways. Sprouting and steeping, followed by decortication, resulted in the most pronounced decrease in oxalates, especially in faba beans and lupin.

INTRODUCTION

Oxalic acid is found in the free state and, more commonly, in the form of salts in vegetables (Wilson & Wilson, 1961). The greater part of oxalic acid in plants is present in the form of soluble oxalates. Ageing, as well as over-ripening of vegetables, is accompanied by an increase in the proportion of calcium oxalate, as reported by Cuthbertson (1968). The oxalate content of some Nigerian leafy vegetables was determined by Oke (1968). He found that oxalate levels were high and, to improve the quality of these dietary vegetables, attention should be concentrated on breeding strains low in oxalate. Gemuses & Herrmann (1972) found that the oxalic acid content of fruits and vegetables ranged from 0 to 10 mg/100 g fresh weight with the exception of those vegetables known to be rich in oxalic acid such as beetroot, rhubarb, dill and beans.

Food Chemistry 0308-8146/82/0008-0169/$02·75

In Egypt, numerous studies have been made on the gross composition of vegetables. Information concerning the effect of processing on the oxalate content of vegetables and legumes is lacking. Accordingly, the present work was carried out with the aim of determining the oxalic acid and total oxalate content of some leafy vegetables and dry legumes consumed widely in Egypt. The effect of decortication, steeping, sprouting, boiling, stewing and baking of dry legumes on the oxalate content is considered.

MATERIALS AND METHODS

Legume seeds

Different kinds of legume were obtained from the Plant Breeding Department, Ministry of Agriculture, Giza, Egypt. The dry seeds investigated were:

1. Broad bean: *Vicia faba* L. Varieties Giza 1, Giza 2 and Rebaya 40.
2. Chickpea: *Cicer arietinum* L. Varieties Giza 1 and Ala 2.
3. Fenugreek: *Trigonella foenum-graecum* L. Varieties Giza 2 and Giza 30.
4. Lentil: *Lens esculenta*. Varieties Giza 9 and Pakistany.
5. Lupin: *Lupinus termis*. Varieties Giza 1 and Giza 2.
6. Pea: *Pisum sativum*. Variety Little Marvel.

Leafy vegetables

Four kinds of leafy vegetable, commonly consumed raw in Egypt in large amounts, were obtained from a local market in Alexandria. The four vegetables were roquette (*Eruka sativa*), radish (*Raphanus sativus*), Egyptian leek (*Allium kurrat*) and green onion (*Allium cepa*). The vegetables were washed, dried in an oven at 55 °C to a constant weight and ground to pass a 30 mesh sieve.

Decortication

The dry seeds were decorticated mechanically. The steeped and sprouted seeds were decorticated manually.

Baked broad beans (medamis)

The baked broad bean is a popular food in Egypt, known locally as medamis. In the present study canned baked broad beans were prepared. The dry seeds were cleaned, washed and steeped in water for 6 h. No. 1 cans (211 × 409) were filled with beans (150 g) and boiled water (200 ml), exhausted, steamed and heat processed at 115·5 °C for $4\frac{1}{2}$ h, a period long enough to soften and cook the beans.

Stewed broad bean paste (bisara)

Stewed broad bean paste is used in the preparation of a delicious and popular food, known in Egypt as bisara. Dry, decorticated broad beans were cooked in

sufficient water in a 1:4 proportion. The cooking period differed according to the variety and the beans were cooked until disintegrated into a paste upon mixing. The beans were cooked for $1\frac{1}{2}$ h in the case of the Giza 1 variety and for $2\frac{1}{4}$ h in the case of both the Giza 2 and Rebaya 40 varieties. The cooked paste was strained, then allowed to cool and set.

Sprouted broad beans

Sprouting of broad bean seeds was carried out by first steeping them in cold tap water for 24 h. The seeds were then spread between moistened cloth sheets for 3 days.

Cooked sprouted broad beans

The sprouted beans were cooked with water in a normal proportion (1:2) for 45 min in the case of the Giza 2 variety and for 30 min in the case of the Rebaya 40 and Giza 1 varieties.

Whole cooked lentils

The seeds were cooked in water (1:3) for 2 h in the case of the Giza 9 variety and for $1\frac{1}{2}$ h in the case of the Pakistany variety. The seeds disintegrated upon mixing.

Decorticated cooked lentils

Dry, decorticated lentils were cooked in water (1:4) for $2\frac{1}{2}$ h in the case of the Giza 9 variety and for 2 h in the case of the Pakistany variety. The paste was strained and allowed to cool and set.

Steeped lupin seeds

The dry seeds were steeped overnight in water, boiled in water for 10 min and soaked in sufficient water to cover them for 4 days. The water was changed twice daily throughout the soaking period.

Sprouted fenugreek

The dry seeds were steeped in cold tap water for 24 h, then spread between moistened cloth sheets for 3 days.

Boiled fenugreek

The dry seeds were boiled in water (1:3) for 20 min, drained and cooled.

Sample preparation

Dry seeds were cleaned, ground and passed through a 30 mesh sieve. The processed products were dried in an air-oven at 55 °C to a constant weight, ground and passed through a 30 mesh sieve.

Moisture

The moisture content was determined by using an air-oven at 135 °C according to the methods of the AOAC (1970).

Oxalic acid and total oxalate

A volumetric method using potassium permanganate, as described by Abaza *et al.* (1968), was followed.

RESULTS AND DISCUSSION

Leafy vegetables

The oxalic acid and total oxalate contents varied from one kind of vegetable to another and the distribution in the same plant was also uneven, as shown in Table 1.

TABLE 1

OXALIC ACID AND TOTAL OXALATE CONTENTS OF SOME RAW EDIBLE VEGETABLES

Raw edible vegetables	*Moisture content* (%)	*Oxalic acid* % *on a dry weight basis*	*Total oxalate*	*Oxalic acid as % of total oxalate*
Roquette	92·7	0·320	0·369	86·7
Radish, leaves	91·6	0·442	0·576	76·7
Radish, roots	94·7	0·358	0·448	79·9
Leek	89·6	0·552	0·613	90·5
Green onion, leaves	87·5	0·669	0·879	76·1
Green onion, bulbs	91·4	0·624	0·748	83·4

The leaves were usually much richer in oxalates than the other parts. The vegetables being investigated can be arranged according to their oxalic acid and total oxalate content in descending order as follows: green onion leaves, green onion bulbs, leek, radish leaves, radish roots and roquette. These vegetables are eaten raw in Egypt and, from the results shown in Table 1, it is interesting to note that the oxalic acid in green vegetables represented 76–90 % of the total oxalates while 10–24 % appeared as insoluble oxalates. Cuthbertson (1968) stated that the greater part of the oxalic acid content in plants was present in the form of soluble oxalates and only 0–20 % appeared as calcium oxalate. Ageing, as well as over-ripening, of vegetables was accompanied by an increase in the proportion of calcium oxalate and the presence of crystals of this salt inside the cells was a sign of over-ripening in harvested products.

Dry legume seeds

The results shown in Table 2 indicate that faba beans exhibited the highest oxalic acid content (0·561 %), followed in descending order by fenugreek (0·554 %), lentils

TABLE 2
OXALIC ACID AND TOTAL OXALATE CONTENTS OF SOME WHOLE DRY LEGUME SEEDS

Legume seed varieties	*Moisture content* (%)	*Oxalic acid* % *on a dry weight basis*	*Total oxalate*
Broad beans:			
Giza 1	9·4	0·545	0·921
Giza 2	10·3	0·576	0·969
Rebaya 40	10·0	0·561	0·849
Chickpea:			
Giza 1	9·2	0·155	0·220
Ala 2	9·2	0·207	0·233
Fenugreek:			
Giza 1	9·6	0·675	0·909
Giza 30	10·4	0·433	0·734
Lentils			
Giza 9	9·9	0·416	0·538
Pakistany	9·7	0·375	0·480
Lupin:			
Giza 1	8·8	0·180	0·271
Giza 2	8·9	0·168	0·310
Peas:			
Little Marvel	10·2	0·275	0·667

(0·396 %), peas (0·275 %), chickpeas (0·181 %) and lupin (0·174 %). With regard to total oxalate content, the legumes can be arranged in descending order as follows: broad beans (0·913 %), fenugreek (0·821 %), peas (0·667 %), lentils (0·509 %), lupin (0·291 %) and chickpea (0·227 %). Therefore, the oxalic acid content, as a percentage of total oxalates, differs and the sequence of seeds, based on this ratio, is chickpea (79·9 %), lentils (77·7 %), fenugreek (67·4 %), broad beans (61·4 %), lupin (59·8 %) and peas (41·2 %).

Broad beans

The results shown in Table 3 indicate that decortication reduced the oxalic acid content of beans by 30·8–38·4 %. This reduction was more pronounced in the Giza 2 variety, as shown in the Table, which indicates that the hulls of this variety contained higher amounts of oxalic acid than the other two varieties. Decortication also lowered the total oxalates in the Giza 1, Giza 2 and Rebaya 40 varieties by 50·7, 40·2 and 31·7 %, respectively, indicating that the insoluble oxalates were high in bean hulls, especially in Giza 2, followed by Giza 1, then Rebaya 40. Accordingly, the ratio of oxalic acid to total oxalates differed between varieties, as shown in Table 3.

Cooking of the sprouted beans resulted in the highest reduction in oxalate content, followed by stewing, baking and sprouting of beans. The reductions in the

TABLE 3

THE EFFECT OF PROCESSING AND COOKING ON OXALIC ACID AND TOTAL OXALATE CONTENTS OF BROAD BEANS

Broad bean varieties	*Moisture content* (%)	*Oxalic acid* % *on a dry weight basis* (*Loss* %)	*Total oxalate* (*Loss* %)
Giza 1:			
Whole beans	9·4	0·545	0·921
Decorticated beans	9·5	0·377 (30·8)	0·454 (50·7)
Baked beans (medamis)	75·0	0·282 (30·8)	0·496 (46·2)
Stewed bean paste (bisara)	65·7	0·214 (43·3)	0·302 (33·5)
Sprouted beans (nabet)	55·0	0·495 (9·2)	0·774 (16·0)
Cooked sprouted beans	70·4	0·307 (43·7)	0·446 (51·5)
Giza 2:			
Whole beans	10·3	0·576	0·969
Decorticated beans	10·7	0·355 (38·4)	0·579 (40·2)
Baked beans (medamis)	78·0	0·380 (34·1)	0·393 (59·4)
Stewed bean paste (bisara)	68·0	0·178 (49·9)	0·344 (40·5)
Sprouted beans (nabet)	67·3	0·507 (12·0)	0·824 (15·0)
Cooked sprouted beans	71·0	0·264 (54·2)	0·364 (62·4)
Rebaya 40:			
Whole beans	10·0	0·561	0·849
Decorticated beans	10·4	0·380 (32·2)	0·580 (31·7)
Baked beans (medamis)	80·0	0·316 (43·6)	0·445 (37·1)
Stewed bean paste (bisara)	67·4	0·189 (50·3)	0·378 (34·8)
Sprouted beans (nabet)	61·4	0·501 (10·6)	0·749 (11·8)
Cooked sprouted beans	70·0	0·252 (55·1)	0·378 (55·5)

TABLE 4

THE EFFECT OF DECORTICATION AND COOKING ON OXALIC ACID AND TOTAL OXALATE CONTENTS OF LENTIL SEEDS

Lentil varieties	*Moisture content* (%)	*Oxalic acid* % *on a dry weight basis*	(*Loss* %)	*Total oxalate*	(*Loss* %)	*Oxalic acid as % of total oxalate*
Giza 9:						
Whole seeds	9·9	0·416		0·538		77·3
Decorticated seeds	10·6	0·399	4·1	0·460	14·5	86·7
Whole cooked seeds	68·1	0·314	24·5	0·364	32·3	86·3
Decorticated cooked seeds	70·0	0·206	48·4	0·325	29·3	63·4
Pakistany:						
Whole seeds	9·7	0·375		0·480		78·1
Decorticated seeds	9·9	0·295	21·3	0·336	30·0	87·8
Whole cooked seeds	68·0	0·264	29·6	0·310	35·4	85·1
Decorticated cooked seeds	70·4	0·158	46·4	0·245	27·1	64·4

oxalic acid and total oxalate contents in the cooked sprouted beans are, respectively, 43·7 %–55·1 % and 51·5 %–55·5 %; in the stewed beans, 43·3 %–50·3 % and 33·5 %–40·5 %; in the baked beans, 30·8 %–43·6 % and 37·1 %–46·2 % and, in the sprouted beans, 9·2 %–12·0 % and 11·8 %–16·0 %, as shown in Table 3 for each bean variety. The present study indicates that the reduction in oxalic acid and total oxalate during processing depends on the bean variety, the original concentration in the dry seed and the processing method.

Lentils

The results presented in Table 4 show that decortication of dry lentils reduced the oxalic acid and total oxalate content of the Giza 9 and Pakistany varieties by 4·1 % and 21·3 % and 14·5 % and 30·0 %, respectively. This indicates that the hulls of the Pakistany variety had a higher oxalate content than the local variety, Giza 9. Accordingly, cooking of decorticated lentils resulted in a more pronounced decrease (46·4 %–48·4 %) in the oxalic acid content than cooking of whole seeds (24·5 %–29·6 %). In whole cooked seeds, although Pakistany lentils were cooked for $1\frac{1}{2}$ h and the Giza 9 variety for 2 h, the oxalate loss was higher in the former variety.

Lupin

Decortication of dry lupin seeds diminished their oxalic acid and total oxalate content by 7·7 % and 10·4 %–12·6 %, respectively, as shown in Table 5. This indicates that the Giza 1 and Giza 2 varieties do not differ greatly in their oxalate content. Steeping of lupin seeds greatly reduced the oxalic acid and total oxalates by 43·3 %–46·4 % and 46·5 %–53·2 %, respectively, this reduction being more pronounced (51·1 %–52·4 % and 51·3 %–62·7 %, respectively) upon decortication of the steeped seeds. It is quite well known that steeping of lupin seeds is an important step in improving the taste of seeds consumed widely in Egypt as snacks. As noted by

TABLE 5

THE EFFECT OF DECORTICATION AND WATER STEEPING ON OXALIC ACID AND TOTAL OXALATE CONTENTS OF LUPIN SEEDS

Lupin varieties	*Moisture content* (%)	*Oxalic acid* % *on a dry weight basis*	(*Loss* %)	*Total oxalate*	(*Loss* %)	*Oxalic acid as % of total oxalate*
Giza 1:						
Whole seeds	8·8	0·180		0·271		66·4
Decorticated seeds	8·0	0·166	(7·7)	0·243	(10·4)	68·3
Whole steeped seeds	69·8	0·102	(43·3)	0·127	(53·2)	80·3
Decorticated steeped seeds	67·2	0·088	(51·1)	0·101	(62·7)	87·1
Giza 2:						
Whole seeds	8·9	0·168		0·310		54·2
Decorticated seeds	8·8	0·155	(7·7)	0·271	(12·6)	57·2
Whole steeped seeds	71·9	0·090	(46·4)	0·166	(46·5)	54·3
Decorticated steeped seeds	68·7	0·080	(52·4)	0·151	(51·3)	58·2

Gad *et al.* (1982), decortication of steeped lupin greatly decreased the phytic acid content of the seeds and decortication must be recommended before consumption, especially for children, who consume steeped lupin seeds widely and are therefore in real need of mineral availability.

Fenugreek

Boiling of fenugreek reduced the oxalic acid and total oxalates of seeds by 22·4 %–31·9 % and 29·4 %–35·2 %, respectively, while sprouting diminished the two components by 12·0 %–15 % and 13·5 %–17·1 %, respectively, as shown in Table 6. The loss of oxalates is mainly in the form of oxalic acid. Boiled fenugreek as a hot beverage is used to some extent in Egypt nowadays, while sprouted fenugreek is used widely as a snack, together with sprouted lupin seeds. As detected in the present study and demonstrated by Gad *et al.* (1982), sprouting of fenugreek markedly decreases the phytic acid and oxalate content, which adds to the high nutritive value of deflavoured fenugreek (Kamer *et al.*, 1980*a*) as a good source of easily digested proteins (Kamer *et al.*, 1980*b*) rich in amino acids; it is recommended for use to supplement foods.

TABLE 6

THE EFFECT OF SPROUTING AND BOILING ON OXALIC ACID AND TOTAL OXALATE CONTENTS OF FENUGREEK SEEDS

Fenugreek varieties	*Moisture content* (%)	*Oxalic acid* % *on a dry weight basis*	(*Loss* %)	*Total oxalate* % *on a dry weight basis*	(*Loss* %)	*Oxalic acid as % of total oxalate*
Giza 2:						
Dry seeds	9·6	0·675		0·909		74·2
Sprouted seeds	76·4	0·571	(15·0)	0·777	(17·1)	73·4
Boiled seeds	74·5	0·478	(31·9)	0·607	(35·2)	78·7
Giza 30:						
Dry seeds	10·4	0·433		0·734		58·9
Sprouted seeds	76·6	0·381	(12·0)	0·635	(13·5)	60·0
Boiled seeds	74·6	0·336	(22·4)	0·518	(29·4)	64·9

REFERENCES

ABAZA, R. H., BLAKE, J. T. & FISHER, E. J. (1968). Oxalate determination: Analytical problems encountered with certain plant species. *J.A.O.A.C.*, **51**, 89–95.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS (AOAC) (1970). *Official methods of analysis.* Washington, DC, USA.

CUTHBERTSON, D. (1968). *Natural Antinutritive Substances in Foodstuffs and Forages.* S. Karger, Basel and New York.

GAD, S. S., MOHAMED, M. S., EL-ZALAKI, M. ESMAT & MOHASSEB, S. ZEINAB (1982). Effect of processing on phosphorus and phytic acid content of some Egyptian varieties of legumes. *Fd Chem.*, **8**, 11–19.

GEMUSES, U. O. D. & HERRMANN, K. (1972). Oxalic acid content of fruits and vegetables. *Zeitschrift für Lebensmitteluntersuchung und Forschung*, **148**, 206–10.

KAMER, A. AYATE, EL-ZALAKI, M. ESMAT, ABO-BAKR, M. TAISEER & ZOUEIL, M. B. (1980*a*). Deflavouring of fenugreek powder as a cereal supplement. *Alex. J. Agric. Res.* (In press).

KAMER, A. AYATE, ABO-BAKR, M. TAISEER, EL-ZALAKI, M. ESMAT & ZOUEIL, M. B. (1980*b*). Evaluation of the proteins of deflavoured fenugreek powder. *Alex. J. Agric. Res.* (In press).

OKE, O. L. (1968). Composition of some Nigerian leafy vegetables. *J. Amer. Diet. Ass.*, **53**, 130–2.

WILSON, B. J. & WILSON, C. H. (1961). Oxalate formation in mouldy foodstuffs as a possible factor in livestock toxic disease. *Amer. J. Vet. Res.*, **22**, 961–9.

Food Chemistry 8 (1982) 179–186

CARBOHYDRATE CONSTITUENTS OF SOME NIGERIAN MAIZE VARIETIES GROWN AT THREE SOIL NITROGEN LEVELS

OYEBIODUN G. LONGE*, B. L. FETUGA* & M. E. AKEN'OVA†

* *Division of Nutritional Biochemistry, Department of Animal Science.*
† *Department of Agronomy.*
University of Ibadan, Ibadan, Nigeria

(Amended manuscript received: 3 August, 1981)

ABSTRACT

Of eight varieties of maize analysed, average values for constituent carbohydrates in g/100 g dry matter were: soluble sugars, 1·61; starch, 62·04; water-soluble polysaccharides, 2·60; alkali-soluble polysaccharides, 6·85 and cellulose, 1·08. Lignin varied from 1·18 to 1·86%. Significant varietal differences ($P < 0·05$) were found for the carbohydrate fractions, although differences within varieties due to nitrogen fertilisation were not significant. Soluble sugars and starch, which contribute appreciably to the energy value of the maize, ranged between 56·68% for WCUI and 67·12% for Lag ABCD. Predicted digestible energy (DE) and metabolisable energy (ME) values for pigs were in the range 4005–4073 kcal/kg and 3881–3947 kcal/kg, respectively whilst metabolisable energy for poultry varied between 3692 and 4007 kcal/kg.

INTRODUCTION

Maize has long been recognised as a good source of utilisable carbohydrates in human diets and is also an important energy contributing ingredient in diets for pigs and poultry.

A considerable amount of information is available on the chemical composition of maize (Montgomery & Smith, 1956; Seckinger *et al.*, 1960; Bond & Glass, 1963; Boundy *et al.*, 1967). It has also been established that this composition can be influenced genetically or by variations in soil nutrient levels (Cameron, 1947; Sauberlich *et al.*, 1953; Aiguiree *et al.*, 1953; Bressani & Mertz, 1958; Bressani *et al.*, 1962; Boundy *et al.*, 1967). The free sugars and cell wall carbohydrates of maize have also been characterised and changes which occur in them during storage and

Food Chemistry 0308-8146/82/0008-0179/$02·75

germination have been studied by Montgomery & Smith (1956), Taufel *et al.* (1960), Peat *et al.* (1956) and Bond & Glass (1963).

The present study was initiated to provide information on the possible genetic variations in the carbohydrate constituents of eight varieties of maize which have been recommended for southwestern Nigeria. It also had, as a secondary objective, the investigation of the possible relationship of carbohydrate content to soil nitrogen fertility levels. It is hoped that established variations could be of value in future efforts aimed at selecting or breeding for varieties of high utilisable carbohydrate content.

MATERIALS AND METHODS

Field experiment

Eight maize varieties were planted in April, 1978 at the University of Ibadan Teaching and Research Farm and fertiliser was applied at the rate of 0, 72 and 144 kg N/ha. The fertiliser was applied as urea in two equal split doses, two weeks after planting and at tasselling. Triple superphosphate was applied to supply 13 kg P/ha along with 25 kg K/ha, applied as murate of potash.

Seed production was by hand pollination within each variety using random samples of pollen from a number of plants, and pollinated plants were bagged for effective isolation. Plant population for each variety was maintained at forty plants and the planting distance was one plant at every 30 cm in rows 90 cm apart.

Ears of maize were harvested and dried immediately to 7–10% moisture and stored in dry air conditions until used.

Analytical methods

Ground samples of maize for each variety and nitrogen level were analysed for dry matter, ether extract and nitrogen by the official methods of analysis (AOAC, 1970). Free sugars, starch, water-soluble and water-insoluble non-cellulosic polysaccharides, cellulose and lignin were estimated following the methods outlined by Southgate (1969*a*, *b*) while sugars were identified on Whatman No. 1 chromatography paper using ethylacetate–pyridine–water (8:2:1, v/v) as solvent. Oligosaccharide (raffinose) was identified and quantified by the method of Dubois *et al.* (1956). Cell wall constituents were also determined by the detergent fibre procedure of Van Soest & McQueen (1973) but modified as recommended by Terry & Outen (1973).

Carbohydrate values obtained were subjected to analysis of variance (Steel & Torrie, 1960).

Prediction of energy values

Energy values of the maize varieties for pigs and poultry were predicted using the equations of Morgan *et al.* (1975*a*) and Sibbald *et al.* (1963), respectively.

RESULTS AND DISCUSSION

Sugar constituents of the ethanol-soluble fraction (free sugars) and starch are shown in Table 1. No significant differences ($P > 0.05$) were observed for the component sugars as a result of nitrogen fertilisation within any variety. Only the mean values for each variety are therefore presented. Significant differences ($P < 0.05$), however, existed among varieties for the different carbohydrate fractions.

TABLE 1
FREE SUGARS AND STARCH OF MAIZE VARIETIES (% DRY MATTER BASIS)

Variety	*Sucrose*	*Fructose*	*Glucose*	*Raffinose*	*Total free sugars*	*Starch*
Western Yellow	0·3	0·0	0·3	0·2	0·8	62·0
WC UI	0·8	0·3	0·4	0·1	1·6	56·0
Local	0·8	0·3	0·3	0·1	1·5	61·7
TZPB	1·0	0·2	0·5	0·1	1·9	63·5
TZB	1·0	0·3	0·4	0·1	1·8	62·2
096EP6	0·9	0·3	0·5	0·1	1·8	64·8
Lag ABCD	1·0	0·2	0·5	0·1	1·9	65·2
NCD	0·9	0·2	0·4	0·1	1·7	61·1
Mean	0·9 ± 0·24	0·2 ± 0·09	0·4 ± 0·08	0·1 ± 0·01	1·6 ± 0·37	62·04 ± 2·8

Sugars identified in the ethanol-soluble fraction include sucrose, glucose, fructose and raffinose. These sugars have been previously isolated from maize by Bond & Glass (1963). The sugar pattern in all varieties investigated followed the same trend. Sucrose was present in the highest concentration, ranging from 0·31 % in Western Yellow to 1·03 % in TZB and Lag ABCD. Glucose and fructose concentrations were generally low but glucose was consistently present in higher concentration. Percentage raffinose in the samples was variable but extremely low.

Starch—the major component in the endosperm—varied between 56 % for WCUI and 65 % for 096EP6 and Lag ABCD. These varieties therefore have available carbohydrates (sugar + starch) as high as 67 %. Such varieties could be particularly important as raw materials for industrial manufacture of starch or dextrin and are to be preferred as major energy sources in compounding diets for pigs and poultry, as well as food for humans, since these are likely to be more digestible by these species and will therefore provide more available energy. Further genetic improvements may be possible with these varieties, if selection is for high starch and sugar content.

Sugars identified in the water-soluble fraction include glucose, xylose and arabinose, although the last two sugars were quantified as pentoses (Table 2). For the water-insoluble non-cellulosic fraction, arabinose, xylose, glucose, galactose and glucuronic acid were identified. The pentoses were present in the highest concentration. TZPB and 096EP6 had pentose values of 5·2 % and 5·8 %, respectively, whilst Western Yellow had the lowest value at 4 %. The pentosan

TABLE 2
UNAVAILABLE CARBOHYDRATE FRACTIONS OF MAIZE VARIETIES (% DRY MATTER BASIS)

Variety	*Non-cellulosic polysaccharides*						*Cellulose*	*Lignin*
	Water-soluble		*Water-insoluble*					
	Glucose	*Pentose (Xylose + Arabinose)*	*Glucose*	*Galactose*	*Pentose (Xylose + Arabinose)*	*Uronic acid*		
Western Yellow	2·0	0·6	1·4	0·6	4·0	0·8	1·2	1·2
WC UI	1·8	0·7	0·9	0·5	4·2	0·6	1·2	1·7
Local	1·9	0·6	1·7	0·6	4·9	1·0	1·2	1·3
TZPB	2·2	0·6	2·4	0·5	5·1	1·2	0·9	1·6
TZB	1·8	0·6	0·8	0·4	4·9	0·7	1·0	1·5
096EP6	1·9	0·6	1·1	0·5	5·8	0·6	1·2	1·6
Lag ABCD	2·3	0·6	2·0	0·5	4·9	0·8	1·0	1·7
NCD	2·2	0·6	2·6	0·5	4·0	0·6	0·9	1·9
Mean	2·0 ± 0·19	0·6 ± 0·04	1·6 ± 0·68	0·5 ± 0·08	4·7 ± 0·62	0·8 ± 0·22	1·1 ± 0·12	1·6 ± 0·23

fractions of cereals have been assigned significant rôles in the quality of cereal grains. Elder *et al.* (1953) and Karim & Rooney (1972) have been able to relate the pentosan content of sorghum to variation in proportion of pericarp and germ to the endosperm. Kernel hardness was also positively related to pentosan content by these authors.

Cellulose and lignin values are also presented in Table 2, while cell wall constituents, measured by the acid detergent fibre method, are presented in Table 3. Hemi-cellulose, largely as pentoses, represents a major proportion of the cell wall fraction.

TABLE 3
CELL WALL CONSTITUENTS, ETHER EXTRACT AND CRUDE PROTEIN OF MAIZE VARIETIES (% DRY MATTER BASIS)

Variety	*NDF*	*ADF*	*Lignin*	*Ether extract*	*Crude protein*
Western Yellow	12·6	1·2	1·0	5·6	12·6
WC UI	11·3	1·9	1·4	5·4	13·0
Local	12·4	1·8	0·7	4·5	12·1
TZPB	12·8	1·6	0·2	4·6	11·9
TZB	12·4	1·4	0·8	3·4	11·7
096EP6	11·9	1·5	1·2	3·6	12·1
Lag ABCD	10·1	1·7	1·3	4·4	12·2
NCD	11·8	2·0	1·4	4·5	13·2
Mean	11·9 ± 0·88	1·7 ± 0·29	1·0 ± 0·42	4·5 ± 0·74	12·4 ± 0·54

An assessment, such as that carried out in this work, is of nutritional and agronomic importance since it provides information which could aid in the continuous improvement of the varieties studied, particularly in relation to increasing the starch or pentosan contents.

Energy value

Since the chemical composition of a feedstuff is the major determinant of its energy value to an animal (Morgan *et al.*, 1975*b*), data obtained in the present study can be safely used to ascribe to the different varieties of maize their utilisable energy values. Similar techniques have been used for pigs and poultry by Schneider *et al.* (1952); Carpenter & Clegg (1956); Sibbald *et al.* (1963) and Drennan & Maguire (1970). The digestible energy (DE) and metabolisable energy (ME) values obtained for the maize varieties by the prediction method are presented in Table 4.

The prediction equation is useful in assessing the energy value of the ingredients as it takes into account the variability which might exist among samples of the same ingredients. It does not, however, take into consideration factors other than composition which may affect the energy value, such as the nature of other dietary components or breed and age of the animal. Predicted energy values may therefore be considered to be of limited accuracy but they do serve as a rough guide,

particularly in the computation of balanced least cost rations. Calculated mean values obtained from the present chemical analysis of maize, however, compare favourably with some reported values. The mean values of 4036 kcal/kg DM, DE and 3907 kcal/kg DM, ME for pigs are in close agreement with the 4000, 4050 and 4020 kcal/kg DM of DE and the 3890, 3810 and 3940 kcal/kg DM of ME reported from animal feeding experiments by Diggs *et al.* (1965); NRC (1969) and Morgan *et*

TABLE 4
PREDICTED ENERGY VALUES OF MAIZE VARIETIES (kcal/kg DRY MATTER BASIS)

Variety	*Pig*		*Poultry*
	DE	*ME*	*ME*
Western Yellow	4073	3947	3947
WC UI	4011	3887	3692
Local	4018	3893	3829
TZPB	4044	3919	3919
TZP	4061	3904	3757
096EP6	4052	3927	3880
Lag ABCD	4030	3905	4007
NCD	4005	3881	3819
Mean	4036 ± 24	3907 ± 22	3856 ± 103

al. (1975*b*). A slightly higher DE value of 4170 kcal/kg DM has been reported by Takahasi *et al.* (1968) whilst Robinson *et al.* (1965) obtained a value of 3940 kcal/kg DM of digestible energy which is slightly lower than that obtained by prediction in this paper. For poultry, different ME values have been reported, ranging from 3170 kcal/kg (Squibb, 1971) and 3430 kcal/kg (Hill *et al.*, 1960) to 3810 kcal/kg (Gipp *et al.*, 1968); 3840 kcal/kg (Sibbald *et al.*, 1960); 3940 kcal/kg (Hill *et al.*, 1960) and 4010 kcal/kg (Shimada & Cline, 1974). A mean ME value of 3856 kcal/kg DM was obtained for the maize varieties. Variations in chemical composition of different samples of maize used by the various authors mentioned above are probably responsible for the differences in the values reported.

REFERENCES

AIGUIREE, F., BRESSANI, R. & SCRIMSHAW, N. S. (1953). The nutritive value of Central American corns. III. Tryptophan, niacin, thianine and riboflavin content of twenty-three varieties in Guatemala. *Food Res.*, **18**, 273–9.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS (1970). *Official methods of analysis of the AOAC.* (11th edn), Washington, DC.

BOND, A. B. & GLASS, R. L. (1963). The sugars of germinating corn (*Zea mays*). *Cereal Chem.*, **40**, 459–66.

BOUNDY, J. A., WOYCHIK, J. H., DIMLER, R. J. & WALL, J. S. (1967). Protein composition of dent waxy and high amylose corn. *Cereal Chem.*, **44**, 160–9.

BRESSANI, R. & MERTZ, E. T. (1958). Studies of corn proteins. IV. Protein and amino acid content of different corn varieties. *Cereal Chem.*, **35**, 227–35.

BRESSANI, R., ELIAS, L. G., SCRIMSHAW, N. S. & GUZMAN, M. A. (1962). Nutritive value of Central American Corns. VI. Varietal and environmental influence of the nitrogen, essential amino acid and fat content of ten varieties. *Cereal Chem.*, **39**, 59–67.

CAMERON, J. W. (1947). Chemico–genetic bases of the reserve carbohydrates in maize endosperm. *Genetics*, **32**, 459–85.

CARPENTER, K. J. & CLEGG, K. M. (1956). The metabolisable energy of poultry feeding stuffs in relation to their chemical composition. *J. Sci. Fd Agric.*, **7**, 45–51.

DIGGS, B. G., BECKER, D. E., JENSEN, A. H. & NORTON, A. W. (1965). Energy values of various feeds for the young pig. *J. Anim. Sci.*, **24**, 555–8.

DRENNAN, P. & MAGUIRE, M. F. (1970). Prediction of the digestible and metabolisable energy content of pig diets from their fibre content. *Irish J. Agric. Res.*, **9**, 197–202.

DUBOIS, M., GILLES, K. A., HAMILTON, J. K., REBERS, P. A. & SMITH, F. (1956). A colorimetric method for the determination of sugars and related substances. *Anal. Chem.*, **28**, 350–6.

ELDER, A. H., LUBISICH, T. M. & MECHAM, D. K. (1953). Studies on the relation of the pentosans extracted by mild acid treatments to milling properties of Pacific Northwest wheat varieties. *Cereal Chem.*, **30**, 103–14.

GIPP, W. F., CLINE, T. R. & ROGLER, J. C. (1968). The metabolizable energy of Opaque-2 and floury-2 corn for the chick. *Poult. Sci.*, **57**, 2019–21.

HILL, F. W., ANDERSON, D. L., RENNER, R. & CAREW, JR, L. B. (1960). Studies of the metabolizable energy of grain products for the chickens. *Poult. Sci.*, **39**, 573–9.

KARIM, A. & ROONEY, L. W. (1972). Pentosans in sorghum grain. *J. Fd Sci.*, **37**, 365–8.

MONTGOMERY, R. & SMITH, F. (1956). A review of carbohydrates of wheat and other cereal grains. *J. Agric. Fd Chem.*, **4**, 716–20.

MORGAN, D. J., COLE, D. J. A. & LEWIS, D. (1975*a*). Energy values in pig nutrition. I. The relationship between digestible nutrient values of a range of feedstuffs. *J. Agric. Sci. Camb.*, **84**, 7–17.

MORGAN, D. J., COLE, D. J. A. & LEWIS, D. (1975*b*). Energy values in pig nutrition. II. The prediction of energy values from dietary chemical analysis. *J. Agric. Sci. Camb.*, **84**, 19–27.

NATIONAL RESEARCH COUNCIL (1969). *Joint U.S.–Canadian Tables of Feed Composition.* Publ. 1232. Washington, DC.

PEAT, S., WHELAN, W. J. & TURVEY, J. R. (1956). The soluble polyglucose of sweet corn (*Zea mays*). *J. Chem. Soc.*, 2317–22.

ROBINSON, D. W., PRESCOTT, J. H. D. & LEWIS, D. (1965). The protein and energy nutrition of the bacon pig. IV. Digestible energy values of cereals in pig diets. *J. Agric. Sci. Camb.*, **64**, 59–65.

SAUBERLICH, H. E., CHANG, W. & SALMON, W. D. (1953). The amino acid and protein content of corn as related to variety and nitrogen fertilization. *J. Nutr.*, **51**, 241–50.

SCHNEIDER, E. D., EARLY, E. B. & DETURK, E. E. (1952). Nitrogen fractions of the component parts of the corn kernel as affected by selection and soil nitrogen. *Agron. J.*, **44**, 161–9.

SECKINGER, H. L., WOLF, M. J. & MACMASTERS, M. (1960). Hemicelluloses of the cementing layer and of some cell walls of the corn kernel. *Cereal Chem.*, **37**, 121–8.

SHIMADA, A. & CLINE, T. R. (1974). A comparison of *in vivo* and *in vitro* metabolizable energy for the chick, rat and pig. *J. Anim. Sci.*, **38**, 1257–60.

SIBBALD, R., SUMMERS, J. D. & SLINGER, S. J. (1960). Factors affecting the metabolizable energy content of poultry feeds. *Poult. Sci.*, **39**, 544–56.

SIBBALD, I. R., CZARNOCKI, J., SLINGER, S. J. & ASHTON, G. L. (1963). The prediction of the metabolizable energy content of poultry feeding stuffs from a knowledge of their chemical composition. *Poult. Sci.*, **42**, 486–92.

SOUTHGATE, D. A. T. (1969*a*). Determination of carbohydrates in foods. I. Available carbohydrate. *J. Sci. Fd Agric.*, **20**, 326–30.

SOUTHGATE, D. A. T. (1969*b*). Determination of carbohydrates in foods. II. Unavailable carbohydrates. *J. Sci. Fd Agric.*, **20**, 331–5.

SQUIBB, R. L. (1971). Estimating the metabolizable energy of foodstuffs with an avian model. *J. Nutr.*, **101**, 1211–16.

STEEL, R. G. D. & TORRIE, J. H. (1960). *Principles and procedures of statistics.* McGraw-Hill Book Co. Inc., London.

TAKAHASI, S., FURUYA, S., JITSUKAWA, Y. & MORIMOTO, H. (1968). Studies on the nutritive evaluation of feedstuffs for pigs. I. Grains and potatoes. 2. Fishmeals and oil meals. *Bulletin of the National Institute of Animal Industries, Japan*, **17**, 1–7, 9–14.

TAKAHASI, S., MORI, A., JITSUKAWA, Y., HIMENO, K. & MORIMOTO, H. (1968). Studies on the nutritive evaluation of feedstuffs for pigs. 3. Bran and others. *Bulletin of the National Institute of Animal Industries, Japan*, **17**, 15–20.

TAUFEL, K., STEINBACH, K. J. & HARTMAN, B. (1960). The lower saccharides of corn and their behaviour during storage and germination. *Nahrung*, **4**, 452–65.

TERRY, R. A. & OUTEN, G. E. (1973). The determination of cell wall constituents in barley and maize. *Chem. Ind.*, (23), 1116–17.

VAN SOEST, P. & MCQUEEN, R. W. (1973). The chemistry and estimation of fibre. *Proc. Nutr. Soc.*, **32**, 123–30.

Food Chemistry 8 (1982) 187–201

WINGED BEAN LIPOXYGENASE—PART 1: ISOLATION AND PURIFICATION

TRUONG VAN DEN†, LEONCIO C. RAYMUNDO & EVELYN MAE T. MENDOZA‡

University of the Philippines at Los Banos (UPLB), Laguna, Philippines 3720

(Received: 8 October, 1981)

ABSTRACT

Lipoxygenase was isolated and purified from dried winged bean seeds by ammonium sulphate fractionation, gel filtration, DEAE–Sephadex ion exchange chromatography and hydroxyapatite chromatography. Two major isoenzymes, FI and FII, were separated by ion exchange chromatography and were further purified by elution through a hydroxyapatite column. These resulted in a 105- and 171-fold purification and 7% and 9% recovery for FI and FII, respectively. FI and FII had similar Rf values of 0·25 on polyacrylamide gel electrophoresis. A minor band of Rf 0·01 was detected in ammonium sulphate fractions but was not further enriched and purified in succeeding steps.

INTRODUCTION

Legumes play an increasing rôle in meeting the protein needs in areas of food scarcity and widespread malnutrition. However, of the approximately 13,000 species of legumes identified, only about twenty are of relative economic importance as food items. This limitation is due to the lack of intensified research on other, lesser known, tropical legumes whose full potentials have not been exploited. One of these little known legumes is the winged bean, *Psophocarpus tetragonolobus* (L.) DC.

A recent publication of the National Academy of Sciences (USA) focused attention on winged bean as a promising legume due to its high protein and oil contents (National Academy of Sciences, 1975) and its relative ease of growth with high yield in the tropics where the soybean cannot grow well. The mature seeds contain about 34% protein and 17% oil, which is comparable to soybean. The seed is a good source of protein whose nutritive value is similar to that of soybean in terms

† Present address: National Institutes of Biotechnology and Applied Microbiology (BIOTECH), University of the Philippines at Los Baños College, Laguna, Philippines 3720.
‡ To whom correspondence should be addressed.

Food Chemistry 0308-8146/82/0008-0187/$02·75

of amino acid composition. The fatty acid composition of the winged bean oil appears to be quite similar to that of peanut, with linoleic acid accounting for about 27% (Garcia *et al.*, 1979; Ekpenyong & Borchers, 1980; Truong, 1980). This fatty acid is particularly susceptible to oxidation which is responsible for the development of a beany and rancid off-flavour. This could be the possible cause of the strong beany flavour of winged bean seed products—e.g. winged bean milk, curd, tempeh and miso (Wong, 1978)—which are usually produced from soybean. The beany and off-flavour in soybean and other legume products has been studied and reported as the problem limiting their utilisation (Wolf, 1975). Several methods have been developed to overcome this technical problem in legume processing, among which the inactivation of a key enzyme oxidising polyunsaturated fatty acid—lipoxygenase—is the most common. Lipoxygenase (linoleate:oxygen oxidoreductase EC1.13.11.12) catalyses the oxidation of *cis*, *cis*-1,4-pentadiene systems in unsaturated fatty acid to conjugated *cis*, *trans*-hydroperoxides in the presence of molecular oxygen. The enzyme is well distributed in plants such as cowpea, pea, peanut, potato, wheat, corn, rice, tomato, etc. (Gardner & Weisleder, 1970; Sanders *et al.*, 1975; Bonnet & Crouzet, 1977; Sekiya *et al.*, 1977; Yoon & Klein, 1979; Truong *et al.*, 1979; Yamamoto *et al.*, 1980). A survey of the lipoxygenase activity in various winged bean lines indicated that the mature seeds possess this enzyme in a range comparable with that of soybean (Truong, 1980).

The purpose of the investigation reported in this paper is to purify lipoxygenase from winged bean, a step necessary to the understanding of the mechanism of formation of the astringent smell and strong beany off-flavour of winged bean seeds, the latter being evident after disrupting the endosperm. The results of the study will hopefully provide a sound basis for further research on the improvement of the flavour pattern of winged bean products in order to increase their acceptability to humans, especially in the tropics and subtropics where malnutrition is prevalent.

MATERIAL AND METHODS

Material

Dried seeds of the winged bean line, Batangas medium, the most widely grown variety in the Philippines, were used in this work. The seeds were ground in a Wiley mill to pass through a 40 mesh screen. Defatted flour was prepared from winged bean meal by extracting with acetone three times (1:10 meal to solvent) at 4°C.

All reagents were of analytical grade except ammonium sulphate which was of enzyme grade. Linoleic acid (99% pure), Tween 20, Soybean lipoxygenase Type II, Sephadex G-150, DEAE–Sephadex-A-50, CM–Sephadex A-50, Blue dextran 2000 and bovine serum albumin were purchased from the Sigma Chemical Company. Acrylamide, *N,N'* methylene-bis-acrylamide, *N,N,N',N'*-tetramethylethylenediamine (TEMED), thioglycolic acid and bromphenol blue were obtained from

BioRad. Hydroxyapatite was prepared according to the method of Tiselius as described by Bernardi (1971). Deionised distilled water was used to prepare all solutions.

Preparation of crude extract and ammonium sulphate fractionation

Twenty grammes of acetone powder, prepared from winged bean meal, were stirred with 400 ml of 0·1M sodium phosphate buffer (pH 6·5 at 4°C) for 1 h. The slurry was forced through four layers of cheesecloth and centrifuged at 12,000 × *g* for 20 min. The supernatant was treated with ammonium sulphate. The precipitate at 30–50% ammonium sulphate saturation was collected, dissolved in a minimal amount of 0·05M phosphate buffer (pH 7·0) and dialysed against two 500-ml portions of the same buffer at 4°C.

Sephadex G-150 gel filtration

The dialysate (6 to 8 ml containing about 860 mg of protein) was applied to a Sephadex G-150 column (2·5 × 70 cm) and eluted with 0·05M phosphate buffer (pH 7·0) at a flow rate of about 17 ml/h. Fractions of 3 ml were collected with the aid of a fraction collector. All chromatography runs were carried out in a chromatography refrigerator at 4°C.

Alternate fractions were assayed for peroxidase, catalase and lipoxygenase activity. The lipoxygenase active fractions were pooled and concentrated by ultrafiltration.

DEAE–Sephadex A-50 ion-exchange chromatography

The concentrated Sephadex G-150 pooled fraction was dialysed overnight against three 500-ml portions of 0·01M sodium phosphate buffer (pH 7·0). A small amount of precipitate was removed by centrifugation and discarded. The supernatant was applied to a DEAE–Sephadex A-50 column (1·2 × 20 cm) previously equilibrated with the same buffer. The bed was eluted stepwise with 0·01M phosphate buffers containing 0·025M, 0·03M, 0·04M and 0·1M NaCl. Elutions were achieved by gravity flow at a rate of 32 ml/h and fractions of 3 ml were collected. Lipoxygenase active fractions corresponding to the three enzyme active protein peaks were pooled separately, concentrated and dialysed against 0·01M phosphate buffer (pH 7·0). The two major fractions (PI and PII) were applied separately to the hydroxyapatite columns. PI and PII were eluted with 0·15M and 0·10M phosphate buffer, respectively. The active fractions (FI and FII) were pooled and stored at 4°C.

Disc gel electrophoresis

Acrylamide gel electrophoresis was used to study the protein pattern, to check the purity of the enzyme preparation and to observe the isoenzyme pattern of the samples. The procedure was performed as described by Davis (1964) using 7·5% polyacrylamide gel (pH 9·3) with 1% soluble starch for lipoxygenase activity

staining (Guss *et al.*, 1967). Electrophoresis proper was carried out with a Buchler electrophoresis apparatus at 3 mA per tube for 2 h at 4 °C. Protein was stained by immersing the gels in 1 % amido black 10B. Destaining was performed in a Bio-Rad diffusion destainer. Gels were scanned at 600 nm using a Gilford Spectrophotometer Model 250 with a gel scanner attachment.

To detect lipoxygenase activity, the gels were immersed in 0·1 M phosphate buffer (pH 5·8), containing $1{\cdot}6 \times 10^{-3}$ M linoleic acid and 0·05 % Tween 20, for 30–45 min at room temperature. The gels were then rinsed with distilled water and the brown coloured bands were developed by starch–iodine staining (Guss *et al.*, 1967).

Half gel technique

Electrophoresis was carried out according to the procedure described above in 12·5 × 0·6 cm inside diameter gel tubes. After removal from the gel tube, the gel was sliced longitudinally with a razor blade. Half was stained using the staining procedure described above and the other half was sliced into 0·5 cm segments using a lateral gel slicer. These segments were separately ground into fine pieces, using a glass rod, and suspended in 0·5 ml of 0·05 M phosphate buffer (pH 7·0). The supernatants were assayed for lipoxygenase activity.

Enzyme assay

Lipoxygenase assay: Lipoxygenase activity was determined spectrophotometrically by measuring the increase in absorbance due to the formation of conjugated diene hydroperoxide at 234 nm (Ben-Aziz *et al.*, 1970). The assay was performed at 30 °C with a Gilford Model 1250 recording spectrophotometer.

Linoleic acid substrate was prepared by the method of Rackis *et al.* (1972). The solution had a concentration of $16{\cdot}1 \times 10^{-3}$ M linoleic acid and 0·75 % (v/v) Tween 20. Each reaction mixture contained 0·3 ml of substrate solution, 0·02 ml of diluted enzyme preparation and 2·7 ml of 0·1 M sodium phosphate buffer (pH 5·8). One unit of enzyme activity is defined as the amount of enzyme which produces a change of one unit of absorbance at 234 nm/min.

An initial lag period was observed, especially with newly prepared substrate. The initial velocity of the reaction was taken in the linear part of the enzyme reaction progress curve.

Peroxidase assay: Peroxidase activity was determined by the decomposition of hydrogen peroxide with *o*-dianisidine as the hydrogen donor, as described in the Worthington Enzyme Manual (1972) with some modifications. The winged bean peroxidase was assayed at pH 5·0 which was found to be the optimal pH of the enzyme in the preliminary experiments.

One unit of peroxidase activity was that amount of enzyme decomposing 1 μM of peroxide a minute at 25 °C.

Catalase assay: Catalase activity was determined by following the disappearance of peroxide spectrophotometrically at 240 nm, as described in the Worthington

Enzyme Manual (1972). Winged bean catalase was assayed at its optimal pH which was a plateau in the pH range at 6–9 observed in the preliminary experiment. The concentration of H_2O_2 in the reaction mixture was found to be optimal at 0·12% H_2O_2.

One unit of catalase activity was that amount of enzyme decomposing 1 μM of peroxide a minute at 25°C.

The specific activity of lipoxygenase, peroxidase and catalase was defined as the enzyme unit activity per milligramme of protein.

RESULTS AND DISCUSSION

Lipoxygenase extraction

The activity of winged bean lipoxygenase obtained with different buffers in the pH range 4·5 to 7·5 is relatively high. The highest specific activity was observed at pH 5·0 with 53 units per milligramme of protein. However, to obtain a high specific activity with minimal reduction in the total activity, 0·1M phosphate buffer at pH 6·5 was selected for winged bean lipoxygenase extraction. Increasing the molarity of the extracting buffers to values higher than 0·1M did not significantly increase the total activity, the specific activity and the protein content of the crude extract. An extraction time of between 30 min and 1 h was sufficient and the dark colour of the seed coat had no effect on the activity of the enzyme.

Ammonium sulphate fractionation

The purification of lipoxygenase isoenzymes from winged bean seeds was accomplished by conventional procedures for protein purification—$(NH_4)_2SO_4$ fractionation, gel filtration on Sephadex G-150, DEAE–Sephadex ion exchange and hydroxyapatite chromatography. Mean values for lipoxygenase activity during purification are given in Table 1.

Lipoxygenase active proteins were precipitated from 30% $(NH_4)_2SO_4$ supernatant at 50% saturation, resulting in a 1·5-fold purification with 90% recovery (Table 1). This fraction accounted for about 59% of the original proteins. To purify soybean lipoxygenase, Christopher *et al.* (1970) reported a 5·6-fold degree of purification of the 30–50% $(NH_4)_2SO_4$ precipitate but with a much lower percentage of recovery (34·6%). With a 40–60% $(NH_4)_2SO_4$ cut, Stevens *et al.* (1970) obtained 95% recovery of the original activity and 1·3-fold purification.

In a separate experiment catalase was localised mainly in the 0–30% and partly in the 30–40% $(NH_4)_2SO_4$ precipitate (Table 2). On the other hand, about 55% and 58% of peroxidase activity was recovered in the 40–50% and 50–60% $(NH_4)_2SO_4$ precipitates, respectively. A substantial amount of peroxidase and catalase, however, was still present in the ammonium sulphate fraction, 30–50% precipitate, where lipoxygenase was localised. This experiment was conducted to determine if

TABLE 1
SUMMARY OF THE PURIFICATION OF WINGED BEAN LIPOXYGENASE

Fraction	*Volume (ml)*	*Total activity (Units)*	*Total protein (mg)*	*Specific activity (Units per milligramme of protein)*	*Degree of purification*	*Per cent recovery*
Crude extract	100	36341	1379	26·35	1·0	100
30% $(NH_4)_2SO_4$ Supernatant	107	32706	1290	25·35	0.96	90
30–50% $(NH_4)_2SO_4$ Precipitate	7·5	40488	852	47·52	1·80	111·4
Sephadex G-150 Pooled fractions	52	26746	50·87	525	19·9	73·6
DEAE–Sephadex pooled fractions						
Peak I (PI)	41	3149	3·61	872	33·0	9·6
Peak II (PII)	60	4268	3·97	1075	40·8	11·7
Hydroxyapatite						
Fraction I (FI)	21	2440	0·885	2757	104·6	6·7
Fraction II (FII)	43	3214	0·712	4514	171·3	8·8

Values are means for at least two replications.

TABLE 2

ACTIVITY OF LIPOXYGENASE, CATALASE AND PEROXIDASE IN DIFFERENT AMMONIUM SULPHATE FRACTIONS

Enzyme	*Crude extract*	*Ammonium sulphate fractions*					
		30% supt	*30% ppte*	*30–40% ppte*	*40–50% ppte*	*50–60% ppte*	*60% supt*
Lipoxygenase							
Total units	41000	36600	368	11948	33280	280	260
Total protein (mg)	1354	1096	50	201	609	79	260
Specific activity	30·2	33·4	7·4	59·4	54·6	3·7	1·0
Per cent recovery	—	89	0·9	29	81	0·7	0·6
Purification	1·0	1·1	—	2	1·8	—	—
Catalase							
Total units	7654	5153	4584	2353	0	0	0
Specific activity	5·6	4·7	91·6	11·7	—	—	—
Per cent recovery	100	67	60	31	—	—	—
Purification	1·0	0·83	16·3	2·0	—	—	—
Peroxidase							
Total units	19·4	14·8	0·4	1·1	10·8	11·4	0
Specific activity	0·014	0·013	0·008	0·005	0·017	0·144	—
Per cent recovery	—	76·2	2·0	5·6	55·6	58·7	—
Purification	1·0	0·93	0·57	0·36	1·21	10·3	—

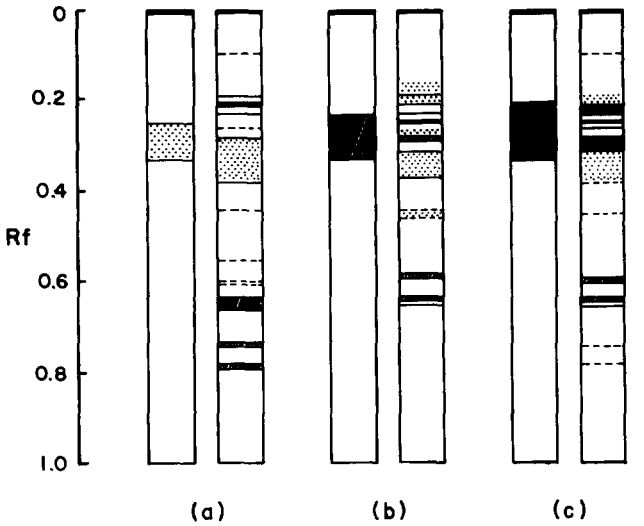


Fig. 1. Disc gel electrophoresis patterns of ammonium sulphate fractions. (a) 30–40 %. (b) 40–50 %. (c) 30–50 %. Lipoxygenase activity and proteins, left and right gels, respectively, were stained as described under the section in the text headed 'Materials and Methods'.

catalase or peroxidase would co-purify with lipoxygenase. It is established that, as well as lipoxygenase, haem-containing proteins such as catalase and peroxidase are powerful lipid peroxidisers due to their ability to accelerate autoxidation (Eriksson & Svensson, 1970). These enzymes have been reported to contaminate lipoxygenase isolated from pea and soybean (Eriksson & Svensson, 1970; Johns *et al.*, 1973). However, further steps in the purification of lipoxygenase from winged bean seeds were able to separate catalase and peroxidase from lipoxygenase, as shown below.

Disc gel electrophoresis of ammonium sulphate fractions on polyacrylamide gel at pH 9·3 revealed the presence of isoenzymes (Fig. 1). All the gels with 30–40 %, 40–50 % and 30–50 % previously dialysed ammonium sulphate fractions, upon specific activity staining, exhibited one non-migrating band of Rf 0·01 and a broad band of Rf 0·20–0·33 of lipoxygenase activity. The broad band corresponded to the major protein and, as shown below, consisted of two isoenzymes that can be separated by ion exchange chromatography. The half gel slicing technique confirmed the positive lipoxygenase reaction of the broad band and the band at Rf 0·01.

Eskin & Henderson (1974*a*, *b*) also reported a different isoenzyme pattern of the faba bean lipoxygenase in the crude extract and $(NH_4)_2SO_4$ precipitates using electrophoretic conditions similar to those followed in our study. They obtained a major lipoxygenase band at Rf 0·22–0·25 and 0·27 for the 40 % and 50 % $(NH_4)_2SO_4$ precipitates, respectively. Guss *et al.* (1967) obtained one major band of lipoxygenase activity at Rf 0·33 in a commercially purified soybean preparation and in mungbean crude extracts. Kalac (1973) reported that both commercial and

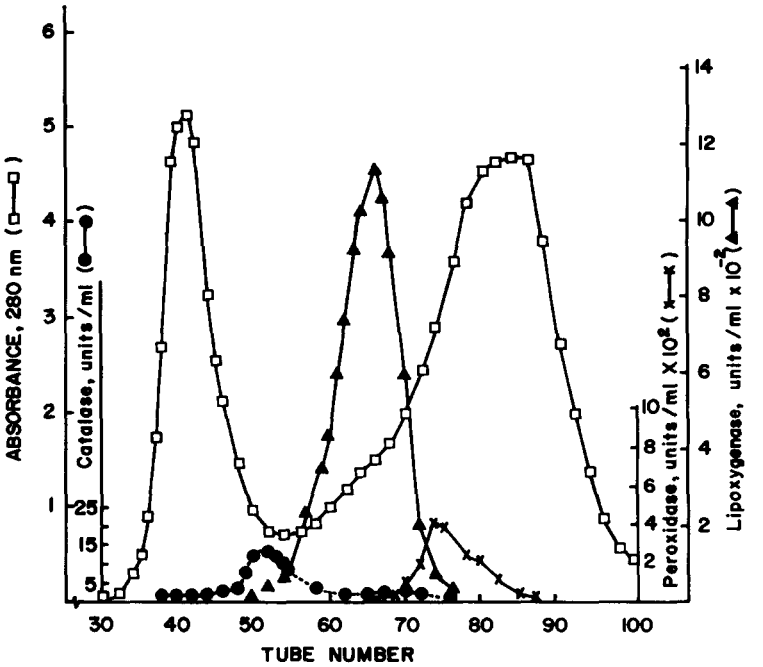


Fig. 2. Separation of winged bean lipoxygenase on Sephadex G-150 column (2·5 × 70 cm). Fractions of 3 ml were collected at the rate of 17 ml/h using 0·50M sodium phosphate buffer (pH 7·0).

purified soybean lipoxygenase preparations contain a main band with Rf 0·22 and a smaller fraction of Rf 0·33.

Gel filtration

Subjecting the dialysed 30–50% $(NH_4)_2SO_4$ precipitate to a Sephadex G-150 column resulted in the recovery of 19,152 units—or 50·6% of the total lipoxygenase activity (Table 2)—with specific activity of 455 units per milligramme of protein, which is 20·8 times that of the crude extract. The pooled lipoxygenase active fraction was free from any detectable peroxidase activity but was contaminated with negligible catalase activity of 75·6 units, corresponding to only 1% of the total activity in the crude extract. Eriksson & Svensson (1970) reported that most of the pea lipoxygenase was separated from the peroxidase and catalase originally present by passing the dialysed 25–50% $(NH_4)_2SO_4$ precipitate through a Sephadex G-150 column. However, the complete removal of these haem-containing enzymes was achieved after only two steps of ion exchange chromatography. Haydar *et al.* (1975), however, claimed that a single DEAE-cellulose chromatography was sufficient to free the purified lipoxygenase from any detectable catalase and peroxidase activities.

A typical elution pattern from the Sephadex G-150 column (2·5 × 70 cm) is shown in Fig. 2. This purification step was highly reproducible and resulted in two major

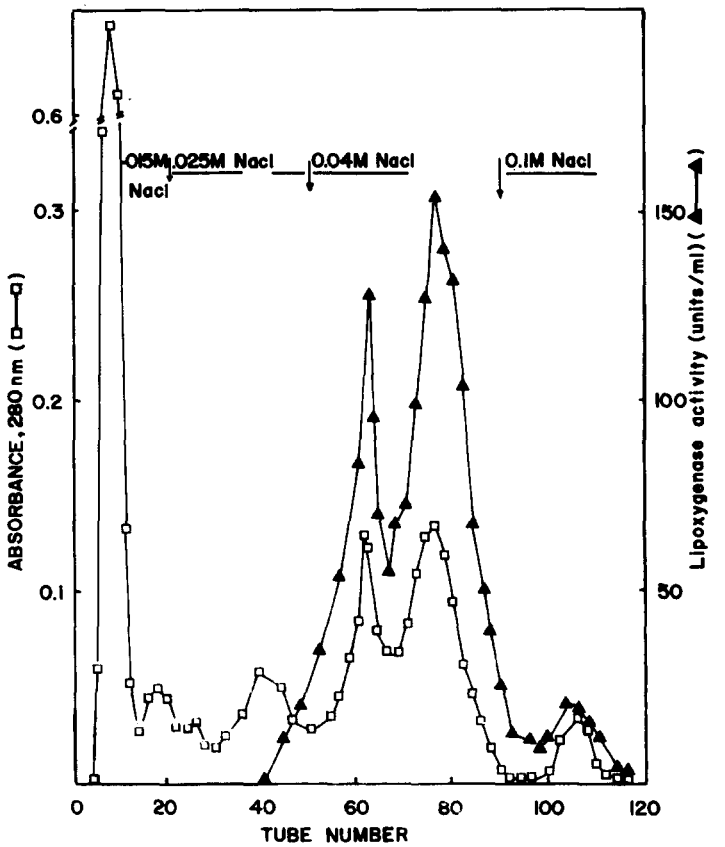


Fig. 3. Stepwise elution of winged bean lipoxygenase isoenzymes on DEAE–Sephadex-50 column (1·2 × 20 cm). Fractions of 3 ml were collected at the rate of 12 ml/h using 0·01 M sodium phosphate buffer (pH 7·0) containing different NaCl concentrations.

protein peaks. Increasing the length of the column had no effect on the separation. Lipoxygenase activity was concentrated in the shoulder between the two major protein peaks, indicating a molecular weight of 78,000 daltons (Truong *et al.*, 1982). Sanders *et al.* (1975) reported a similar elution pattern of peanut lipoxygenase from a Sephadex G-150 column.

Pooled lipoxygenase-active fractions from the Sephadex G-150 column were concentrated by ultrafiltration and dialysed against 0·01 M phosphate buffer (pH 7·0) before applying to the ion-exchange column. A loss of about 30 % of the enzyme activity occurred during this concentration step. Concentration by $(NH_4)_2SO_4$ precipitation and dialysis also resulted in a similar loss, as indicated in a preliminary experiment.

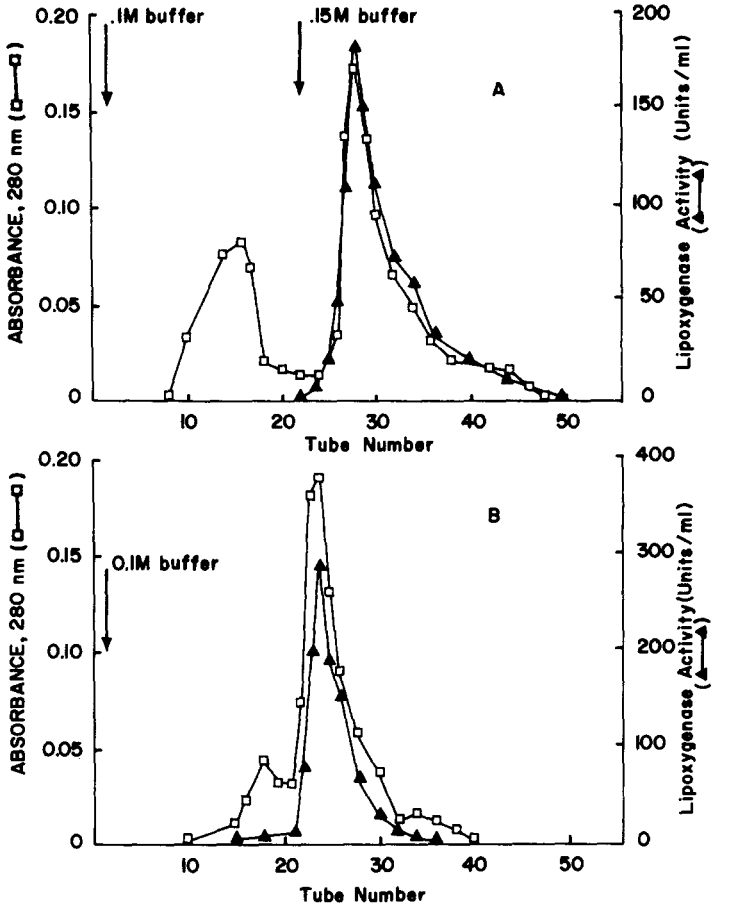


Fig. 4. Hydroxyapatite column chromatography of DEAE–Sephadex A-50 pooled fractions, PI (A) and PII (B). Column: 1·2 × 15 cm. Fractions of 3 ml were collected using sodium phosphate buffer (pH 7·0) at the rate of 12 ml/h.

DEAE–Sephadex ion exchange chromatography

Ion-exchange chromatography was performed on a DEAE–Sephadex A-50 column (1·2 × 23 cm). The column was equilibrated with 0·01 M sodium phosphate buffer (pH 7·0) containing 0·015 M NaCl and was eluted stepwise with a higher NaCl concentration. The two lipoxygenase active protein peaks corresponding to two distinct protein peaks were separated with 0·04 M NaCl buffer and designated PI and PII in the order of elution (Fig. 3). They consisted of heterogeneous proteins as shown on polyacrylamide gels (Fig. 4). A small protein peak with lipoxygenase activity, designated PIII, was eluted with 0·1 M NaCl (Fig. 3). However, due to its small amount, PIII was not subjected either to further purification or to characterisation. Chromatography of the partially purified lipoxygenase fraction

from Sephadex gel filtration on a larger DEAE–Sephadex A50 column (2·5 × 30 cm) with a linear gradient of 0–0·2M revealed only two close lipoxygenase peaks of PI and PII. Re-chromatography of the pooled PI and PII on a smaller DEAE–Sephadex column (1·0 × 20 cm) eluted with 0·04M NaCl gave a better resolution of PI and PII. However, this step resulted in a severe loss of enzyme activity and interfering proteins were still present, as shown by polyacrylamide gel electrophoresis. Eriksson & Svensson (1970) reported similar results in an attempt to purify pea lipoxygenase using two step ion-exchange chromatography.

Ion-exchange chromatography using CM–Sephadex A-50 revealed only one lipoxygenase active protein peak. The pooled fraction had a lower specific activity than that obtained with DEAE ion-exchange chromatography.

The degree of purification and per cent recoveries of PI and PII were comparable with those obtained in the purification of pea and soybean lipoxygenase (Eriksson & Svensson, 1970; Klein, 1976; Yoon & Klein, 1979). There were differences in the number of isoenzymes of pea and soybean reported in the literature based on the elution pattern of ion exchange chromatography (Christopher *et al.*, 1970, 1972; Haydar *et al.*, 1975; Klein, 1976; Yoon & Klein, 1979; Stevens *et al.*, 1970). This was probably due to varietal differences and variations in chromatographic conditions and techniques.

Hydroxyapatite column chromatography

After concentration by ultrafiltration and dialysis against 0·01M phosphate buffer at pH 7·0, PI and PII were applied separately on hydroxyapatite columns (1·2 × 15 cm) which had been previously equilibrated with the same buffer.

The typical elution patterns of the PI and PII from hydroxyapatite columns are shown in Fig. 5. With PI. the lipoxygenase inactive proteins were eluted in the first peak when 0·1M sodium phosphate buffer was used as the eluant. The lipoxygenase activity was concentrated in the major protein peak which was eluted with 0·15M sodium phosphate buffer. This pooled lipoxygenase-active fraction (FI) had a specific activity of 2757 units per milligramme of protein (Table 2). An enrichment of 105-fold and 6·7% recovery were achieved compared with the crude extract.

Interfering proteins in PII were also separated from lipoxygenase active proteins on a hydroxyapatite column (Fig. 5). The elution revealed one major protein peak where lipoxygenase is found, and two minor peaks, all eluted with 0·1M sodium phosphate buffer (pH 7·0). The pooled lipoxygenase active fractions (FII) exhibited a specific activity of 4514 units per milligramme of protein—a 171-fold purification compared with the crude extract.

The FI and FII preparations were stable for a month at 4°C and, unlike the DEAE–Sephadex pooled fractions, no adherence of the enzyme on the wall of the container or on the surface of the micropipette tip was observed. Lyophilisation greatly denatured both enzyme preparations. FI and FII so obtained can thus be used for enzyme characterisation.

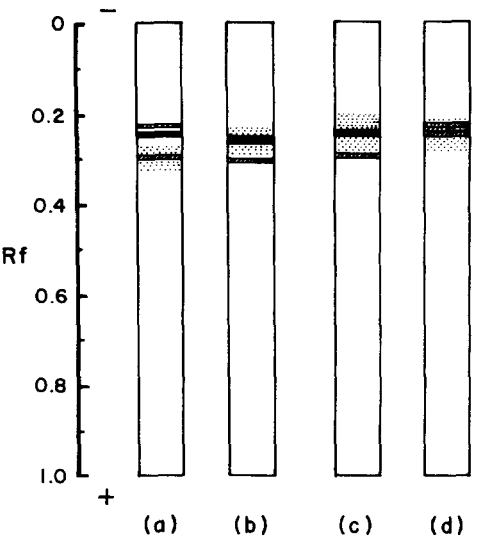


Fig. 5. Disc gel electrophoresis protein patterns of winged bean lipoxygenase isoenzymes. (a) PI. (b) PII. (c) FI. (d) FII.

FI and FII on polyacrylamide gels exhibited a major band at Rf 0·25 and minor bands of Rf 0·28 and 0·21 for FI and FII, respectively (Fig. 4). It was also observed that, coincident with the major protein bands at Rf 0·25 for the two fractions (Fig. 4), there was a broad diffused protein band, Rf 0·21–0·28, which may arise by partial transformation of the protein structure during the absorption and desorption step in hydroxyapatite chromatography. Diel & Stan (1978) also observed the diffused protein band on soybean lipoxygenase-2 in the disc polyacrylamide gel electrophoresis and attributed this to the partial transformation of the tertiary or the quaternary protein structure of lipoxygenase-2. However, Christopher *et al.* (1970) did not observe this phenomenon with the soybean lipoxygenase-2 eluted from the hydroxyapatite column, the final step in their purification procedure. Their lipoxygenase-2 preparation had a 50-fold purification and a 0·2% recovery compared with the crude extract and showed homogeneous protein by disc gel electrophoresis.

The above results indicate the presence of two major isoenzymes and one minor isoenzyme of lipoxygenase in winged bean seeds. Detailed characterisation of the two major isoenzymes, designated FI and FII, is presented in the next paper of this series (Truong *et al.*, 1982).

REFERENCES

BEN-AZIZ, A., GROSSMAN, S., ASCARELLI, I. & BUDOWSKI, P. (1970). Linoleate oxidation induced by lipoxygenase and haem protein: A direct spectrophotometric assay. *Anal. Biochem.*, **34**, 88–100.

BERNARDI, G. (1971). *Chromatography of proteins in hydroxyapatite.* In: *Methods of enzymology*, Vol. 22. (Jakoby, W. B. (Ed.)), New York, Academic Press, pp. 325–39.

BONNET, J. L. & CROUZET, J. (1977). Lipoxygenase from tomato fruits: Partial purification and study of some properties. *J. Food Sci.*, **42**, 625–8.

CHRISTOPHER, J. P., PISTORIUS, E. K. & AXELROD, B. (1970). Isolation of an isoenzyme of soybean lipoxygenase. *Biochim. Biophys. Acta.*, **198**, 12–19.

CHRISTOPHER, J. P., PISTORIUS, E. K. & AXELROD, B. (1972). Isolation of a third isoenzyme of soybean lipoxygenase. *Biochim. Biophys. Acta.*, **284**, 54–62.

DAVIS, B. J. (1964). Disc electrophoresis. II. Method and application to human serum proteins. *Ann. N.Y. Acad. Sci.*, **121**, 404–27.

DIEL, E. & STAN, H. J. (1978). Purification and characterization of two isoenzymes of lipoxygenase from soybeans. *Planta*, **142**, 321–8.

EKPENYONG, T. E. & BORCHERS, R. L. (1980). The fatty acid composition of the oil of the winged bean (*Psophocarpus tetragonolobus* L.) seeds. *J. Amer. Oil Chem. Soc.*, **57**, 147–9.

ERIKSSON, C. E. & SVENSSON, S. G. (1970). Lipoxygenase from peas. Purification and properties of the enzyme. *Biochim. Biophys. Acta.*, **198**, 449–59.

ESKIN, N. A. M. & HENDERSON, H. M. (1974*a*). Lipoxygenase in *Vicia faba minor*. *Phytochemistry*, **13**, 2713–16.

ESKIN, N. A. M. & HENDERSON, H. M. (1974*b*). A study of lipoxygenase from small faba beans. *Proc. IV. Int. Congress Food Sci. and Technol.* (1974), Vol. I, pp. 263–9.

GARCIA, V. V., PALMER, J. K. & YOUNG, R. W. (1979). Fatty acid composition of the oil of winged beans, *Psophocarpus tetragonolobus* (L.) DC. *J. Amer. Oil Chem. Soc.*, **56**, 931–2.

GARDNER, H. W. & WEISLEDER, D. (1970). Lipoxygenase from *Zea mays*. 9 D-hydroperoxide-*trans*-10-*cis*-Octa-decadienoic acid from linoleic acid. *Lipids*, **5**, 678–83.

GUSS, P. L., RICHARDSON, T. & STAHMANN, M. A. (1967). Oxidation reduction enzymes in wheat. III. Isoenzymes in wheat fractions and soybean. *Cereal Chem.*, **44**, 607–10.

HAYDAR, M., STEELE, L. & HADZIYEV, D. (1975). Oxidation of pea lipids by pea seed lipoxygenase. *J. Food Sci.*, **40**, 808–14.

JOHNS, E. B., PATTEE, H. E. & SINGLETON, J. A. (1973). Lipoxygenase-mediated pentane production: Characterization of the system. *J. Agr. Food Chem.*, **21**, 570–3.

KALAC, J. (1973). Soybean lipoxygenase II. Identification of lipoxygenase isoenzymes in a purified product. *Biologia (Bratislava)*, **28**, 725. (*Chem. Abstr.*, **80**(5), 24128, 1974.)

KLEIN, B. P. (1976). Isolation of lipoxygenase from split pea seeds, snap beans and peas. *J. Agr. Food Chem.*, **24**, 938–42.

NATIONAL ACADEMY OF SCIENCES (1975). *The winged bean: A high protein crop for the tropics*, National Academy of Sciences (USA). Government printing office, Washington, DC.

RACKIS, J. J., HONIG, D. H., SESSA, D. J. & MOSER, H. A. (1972). Lipoxygenase and peroxidase activities of soybeans as related to the flavor profile during maturation. *Cereal Chem.*, **49**, 586–97.

SANDERS, T. J., PATTEE, H. F. & SINGLETON, J. E. (1975). Lipoxygenase isoenzymes of peanut. *Lipids*, **10**, 681–5.

SEKIYA, J., AOSHIMA, H., KAJIWA, T., TOGO, T. HATANAKA, A. (1977). Purification and some properties of potato tuber lipoxygenase and detection of linoleic acid radical in the enzymes reaction. *Agric. Biol. Chem.*, **41**, 827–32.

STEVENS, F. C., BROWN, D. M. & SMITH, E. L. (1970). Some properties of soybean lipoxygenase. *Arch. Biochem. Biophys.*, **136**, 413–21.

TRUONG, VAN DEN (1980). *Lipoxygenase isoenzymes of winged bean*, Psophocarpus tetragonolobus (*L.*) DC., PhD dissertation, University of the Philippines at Los Baños, College, Laguna, Philippines.

TRUONG, VAN DEN, RAYMUNDO, L. C. & MENDOZA, E. M. T. (1979). Cowpea lipoxygenase: Part I. Physicochemical characterization. *Bull. Philippine Biochem. Soc.*, **2**, 1–12.

TRUONG, VAN DEN, RAYMUNDO, L. C. & MENDOZA, E. M. T. (1982). Winged bean lipoxygenase—Part 2: Physicochemical properties. *Food chem.*, in press.

TRUONG, VAN DEN, RAYMUNDO, L. C. & MENDOZA, E. M. T. (1981). Lipoxygenase activity in germinating winged bean seed and its role in beany flavor formation. Second International Seminar on Winged Bean, Colombo, Sri Lanka, January 19–23, 1981.

WOLF, W. J. (1975). Lipoxygenase and flavor of soybean protein products. *J. Agric. Food Chem.*, **23**, 136–41.

WONG, K. C. (1978). Uses and current status of winged bean research in Malaysia. *Public forum and prospects and potential uses of the winged bean and the plans for its development*, Manila, Philippines, January 1978.

WORTHINGTON ENZYME MANUAL (1972). Peroxidase, Catalase. In: *Worthington enzyme manual*, Worthington Biochemical Corporation, New Jersey. Freehold.

YAMAMOTO, A., FUJII, Y., YASUMOTO, K. & MITSUDA, H. (1980). Partial purification and study of some properties of rice germ lipoxygenase. *Agric. Biol. Chem.*, **44**, 443–5.

YOON, S. & KLEIN, B. P. (1979). Some properties of pea lipoxygenase isoenzymes. *J. Agric. Food Chem.*, **27**, 955–62.

Food Chemistry 8 (1982) 203–213

PARTIAL PURIFICATION AND CHARACTERISATION OF POTATO PECTINESTERASE*

A. PURI, T. SOLOMOS & A. KRAMER

Food Science Program, University of Maryland, College Park, MD 20742, USA

(Received: 5 January, 1981)

ABSTRACT

Pectinesterase (EC 3.1.1.11) was extracted from potato (Solanum tuberosum *var. Russet Burbank) tissue and purified 9·6-fold by ammonium sulphate precipitation and chromatography on Sephadex G-100. The enzyme preparation thus obtained has a molecular weight of 25,000, an apparent* K_m *of 0·09% for citrus pectin and a pH optimum of 7·5. NaCl is a positive modulator of the enzyme. The energy of activation of the enzyme is 6200 calories. A* Q_{10} *of 1·33 is observed in the temperature range of 25 to 45°C, and the optimum temperature for the enzyme is 55°C.*

INTRODUCTION

Pectinesterase (PE; EC 3.1.1.11) catalyses the demethylation of methyl esters of polygalacturonic acid and is widely distributed in plants and microorganisms (Kertesz, 1951). Since PE seems to be present in all living tissues of higher plants, an essential function of this enzyme appears likely. However, the physiological role of the enzyme is not well understood. The activity of the enzyme has been implicated in the ripening mechanism of fruits such as tomatoes (Kertesz, 1938), bananas (Hultin & Levine, 1965), apples (Lee & Wiley, 1970) and peaches (Ben-Arie & Lavee, 1971). On the other hand, Nagel & Patterson (1967) observed a decrease in the PE activity of pears during maturation. A similar trend was observed in avocados (Zauberman & Schiffman-Nadel, 1972). PE activity has also been related to the problem of plant pathogenesis. Smith (1958) suggested that PE activity aggravates plant diseases, whilst Bateman & Millar (1966) suggested that PE activity prevents tissue disintegration.

* Based on a paper presented at the 40th Annual Meeting of the Institute of Food Technologists, New Orleans, LA., USA, 8–11 June, 1980.

Scientific paper No. A-2910, contribution No. 5967 of the Maryland Agricultural Experiment Station, Department of Horticulture (Food Science Program).

Food Chemistry 0308-8146/82/0008-0203/$02·75

Biochemical characteristics of PE have been studied in a number of plant tissues, including tomatoes (Lee & MacMillan, 1968; Nakagawa *et al.*, 1970; Pressey & Avants, 1972), bananas (Hultin & Levine, 1963), carrots (Markovic, 1978) and oranges (Versteeg, 1979). Vas *et al.* (1967) detected the presence of the enzyme in potatoes. Later, Roeb & Stegemann (1975) separated potato PE isoenzymes by gel electrophoresis. However, little information exists on the properties of potato PE. Thus, the main objective of this study was to purify potato PE and study some of its physical and kinetic characteristics.

MATERIALS AND METHODS

Materials

Potato tubers, *Solanum tuberosum* (var. Russet Burbank) were purchased from a local supermarket in September, 1979. The material was stored in a cold room at 5 °C until it was used.

Enzyme extraction

Potatoes were thoroughly washed with water, peeled and homogenised in extraction media containing 10 mM phosphate, 0·25 % cysteine hydrochloride and 1M NaCl, pH 7·5. The ratio of tissue to buffer used was 1:2. To aid removal of phenolics, 200 mg of polyvinylpolypyrrolidone was also added to the extraction media for every gramme of tissue homogenised. After extraction, the pH of the extract was adjusted to 8 and the extract was stirred overnight at 5 °C. Microbial contamination of the extract during this period was prevented by the addition of cycloheximide and chloramphenicol (50 μg of each per millilitre of homogenate). The extract was filtered through four layers of cheesecloth, centrifuged at 25,000 *g* for 50 min, and the supernatant obtained was designated as the 'crude extract'. All extraction steps were carried out at 5 °C.

Enzyme assay

PE activity was assayed by titrating the carboxyl groups formed by the de-esterification of pectin by the enzyme, as per the method of Kertesz (1955). A typical reaction mixture contained 20 ml of 0·5 % citrus pectin in 0·25M NaCl and 1 ml of enzyme. The pH of the reaction mixture was maintained at 7·5 by the addition of 0·01M NaOH. The temperature of the reaction was maintained at 30 °C. Non enzymatic de-esterification of pectin was determined in the absence of PE and corrections were made to obtain the correct rate of the enzymatic reaction.

A unit of PE is defined as the amount of enzyme required to liberate 1 micro equivalent of carboxyl groups in 10 min at 30 °C and at a pH of 7·5. The effects of time, enzyme and substrate concentration, NaCl, pH and temperature on PE activity were studied using purified enzyme.

Purification of potato PE

Solid ammonium sulphate was added slowly to the crude extract to a saturation level of 80%. The solution was allowed to stand for 1 h and was then centrifuged. The precipitate was dissolved in a small volume of 100 mM phosphate buffer, pH 7·5, and dialysed overnight against 10 mM phosphate buffer, pH 8. Three changes of the buffer were made during this period. The dialysate was concentrated in an ultrafilter apparatus using a PM-10 membrane (Amicon Corp.). Two millilitres of the concentrated extract (about 80 mg protein) were loaded onto a Sephadex G-100 column (35 × 2·5 cm) previously equilibrated with 20 mM phosphate buffer, pH 7·5, which was also used to elute the column. Fractions of 5 ml were collected and assayed for PE activity. Fractions with PE activity were combined and concentrated by ultrafiltration, using conditions previously described. The purified and concentrated enzyme was used for all characterisation studies. All purification steps were carried out at 5 °C.

Determination of energy of activation, Q_{10} values and rate constants

Energy of activation (E_a) was estimated from the slope of the Arrhenius plot obtained by plotting the logarithm of the initial enzyme velocity against the reciprocal of reaction temperature (absolute). Q_{10} values and rate constants were determined according to the method of Segal (1975).

Molecular weight determination

The molecular weight of potato PE was determined by gel filtration on Sephadex G-100, according to the method of Whitaker (1963). Bovine serum albumin (BSA), α-chymotrypsinogen and cytrochrome C were used as marker proteins.

Protein determination

Protein content was determined by the method of Lowry *et al.* (1951), using BSA as a standard. Protein in the column eluate was detected at 280 nm using a Beckman Model 25 recording spectrophotometer.

All chemicals used in this study were purchased from the Sigma Chemical Company, St. Louis, USA.

RESULTS AND DISCUSSION

A summary of the entire purification procedure is presented in Table 1. Ammonium sulphate precipitation increased the purity of the enzyme 2·3-fold. Chromatography on Sephadex G-100 increased the purity 9·6-fold. Further purification of the enzyme was attempted using ion exchange chromatography. However, this procedure was not adopted as it failed to improve the resolution of the enzyme, or increase its purity. The final enzyme recovery after purification was 43%. PE was eluted as a

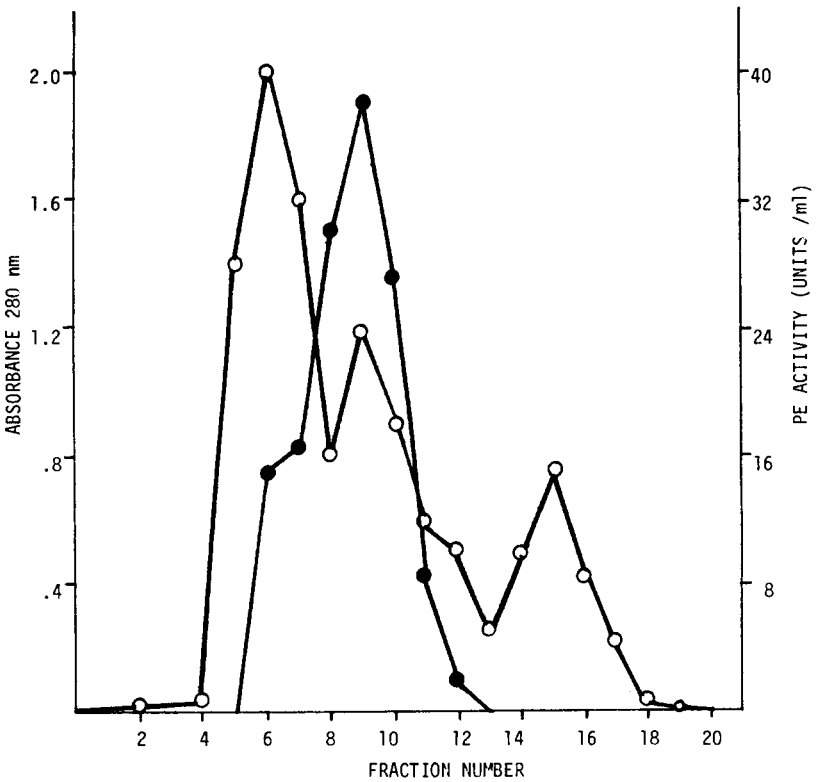


Fig. 1. Chromatography of potato PE on Sephadex G-100. The sample (80 mg protein) was applied to a 32 × 2·5 cm column and eluted with 20 mM phosphate buffer, pH 7·5. The flow rate of the buffer was 15 ml/h. The volume of each fraction was 5 ml. ○—○ Absorbance at 280 nm. ●—● PE activity.

single peak on Sephadex G-100 (Fig. 1), indicating the presence of only one enzyme species or more than one species with a somewhat similar molecular weight.

Figure 2 shows the time course of the reaction after the addition of PE. It can be seen that the reaction rate is linear with time for up to 15 min. This was found to hold good for the various enzyme concentrations used. Preliminary studies with the crude

TABLE 1
PURIFICATION SCHEME OF POTATO PE

Fraction	*Volume (ml)*	*Total PE activity (Units)*	*Specific activity (units/mg protein)*	*Yield (%)*	*Purification*
Crude extract	600	9301·2	5·4	100	1
Ammonium sulphate (80% precipitate)	118	5580·6	12·4	60	2·3
Sephadex G-100 Eluate	100	3999·5	52·3	43	9·6

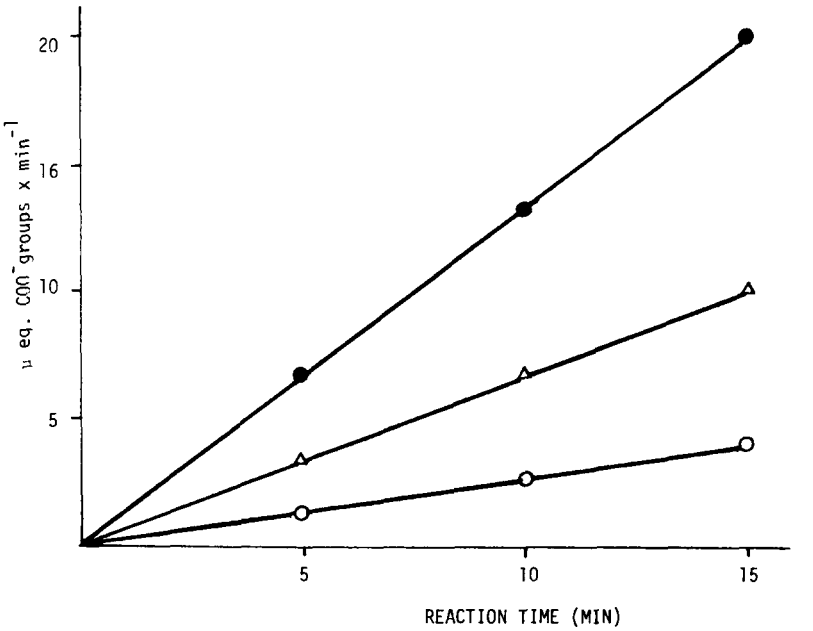


Fig. 2. Time course and effect of enzyme concentration on potato PE activity. Measurements were carried out using 20 ml of 0·5 % citrus pectin in 0·25M NaCl, with varying levels of enzyme (○—0·75 mg; △—1·88 mg and ●—3·75 mg of protein). The pH of the reaction was 7·5 and the temperature, 30 °C.

extract also indicated a linear response of the enzyme with respect to time, for up to 15 min.

The effect of citrus pectin concentration on the initial velocity of the reaction is presented in Fig. 3. From this double reciprocal plot of substrate concentration against reaction velocity, an apparent Michaelis–Menten constant (K_m) for citrus pectin was calculated to be 0·09 %. K_m values of plant PE vary considerably (Versteeg, 1979). The K_m being reported here for potato PE is close to the values of 0·075 % and 0·08 % which have been reported for tomato PE (Lee & MacMillan, 1968) and orange PE (MacDonnell *et al.*, 1945).

The pH and salt dependency of PE is well known (Lineweaver & Ballou, 1945; MacDonnell *et al.*, 1945). Figure 4 shows the effect of pH on potato PE activity. The optimum pH of the enzyme is 7·5. A pH optimum of 7·5 has also been reported for papaya PE (Chang *et al.*, 1965), cranberry PE (Arakji & Yang, 1969) and orange PE (MacDonnell *et al.*, 1945). The effect of NaCl on potato PE is presented in Fig. 5. Maximum activity was observed at a concentration of 0·25M NaCl, with activity being reduced at higher NaCl levels. The stimulatory effect of NaCl on plant PE varies considerably. However, these results are in close agreement with those reported for alfalfa PE (Lineweaver & Ballou, 1945) and pea PE (Collins, 1970).

The effect of temperature on potato PE is demonstrated in Fig. 6. The optimum

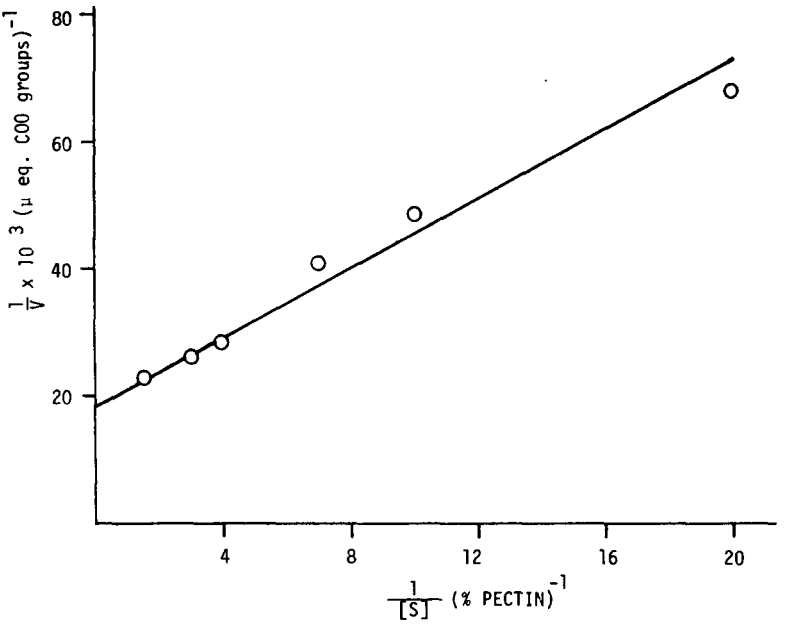


Fig. 3. Lineweaver–Burk plot of potato PE activity as a function of substrate concentration. Reaction mixtures contained varying levels of citrus pectin in 0·25M NaCl and 1 ml (1·5 mg of protein) of purified enzyme. The pH of the reaction was 7·5 and the temperature, 30°C.

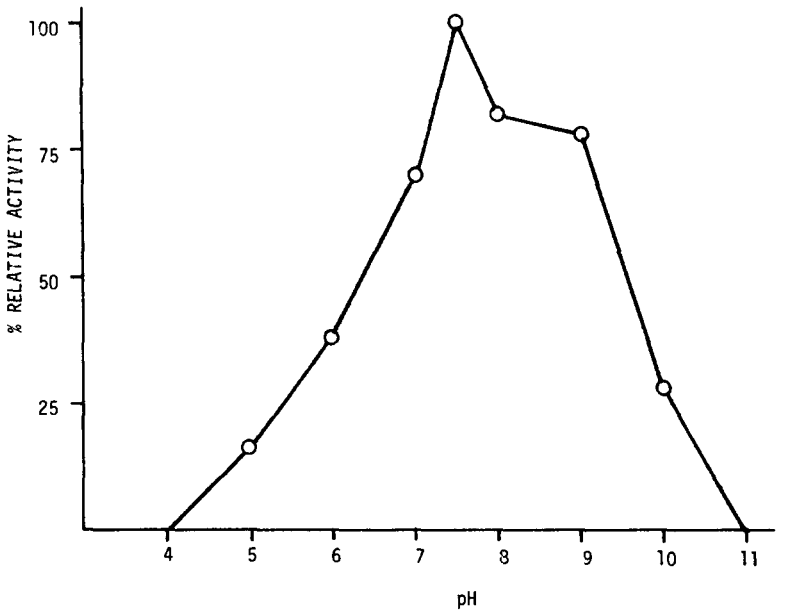


Fig. 4. The effect of pH on potato PE activity. The reaction mixture contained 20 ml of 0·5% citrus pectin in 0·25M NaCl and 1 ml (1·5 mg of protein) of purified enzyme. The temperature of the reaction was 30°C.

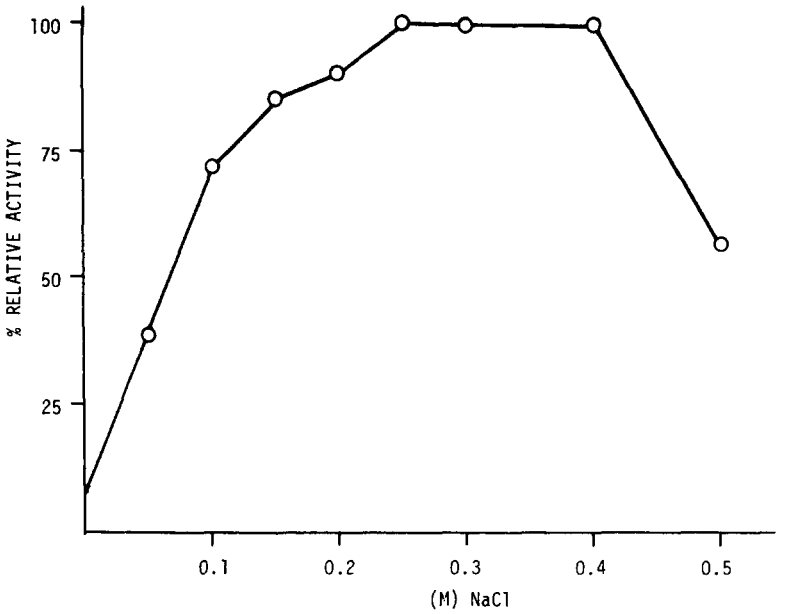


Fig. 5. The effect of NaCl on potato PE activity. Reaction mixtures contained 1 ml (1·5 mg of protein) of purified enzyme, 20 ml of 0·5 % citrus pectin and varying levels of NaCl. All reactions were carried out at pH 7·5 and at a temperature of 30 °C.

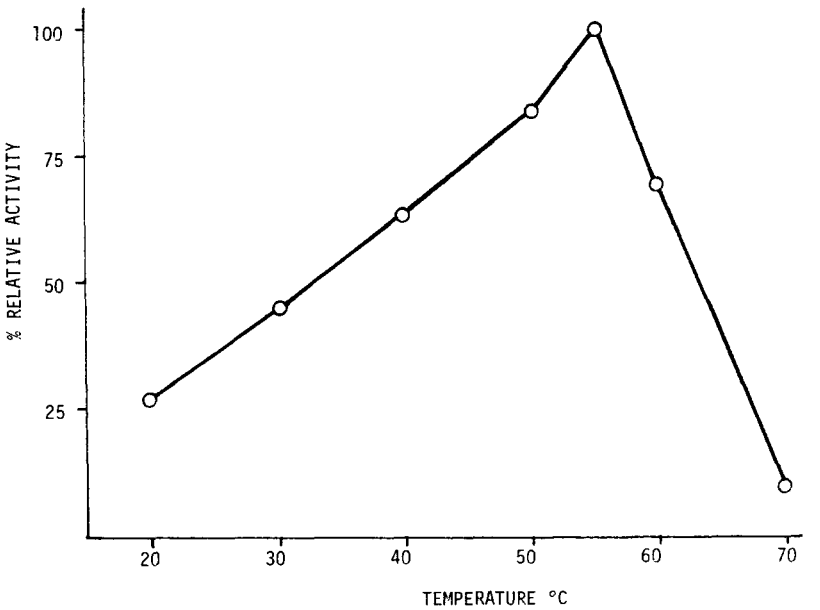


Fig. 6. The effect of temperature on potato PE activity. The velocity of the reaction was measured at various temperatures. The reaction mixture used was as described in the section in the text headed 'Materials and Methods'.

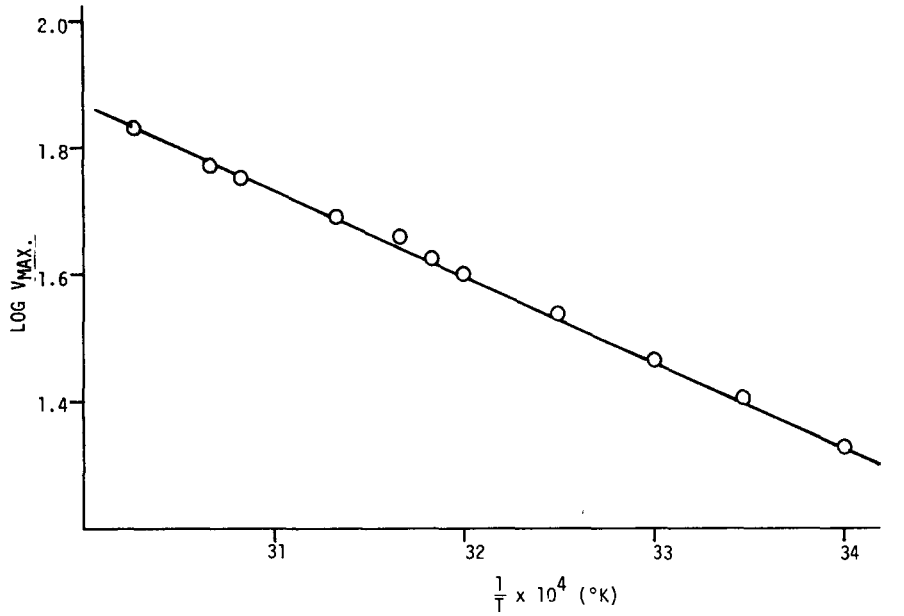


Fig. 7. Arrhenius plot of potato PE. The reaction velocity was determined at various temperatures and the plot was constructed as outlined in the section in the text headed 'Materials and Methods'. The slope of the plot is $-E_a/RT$.

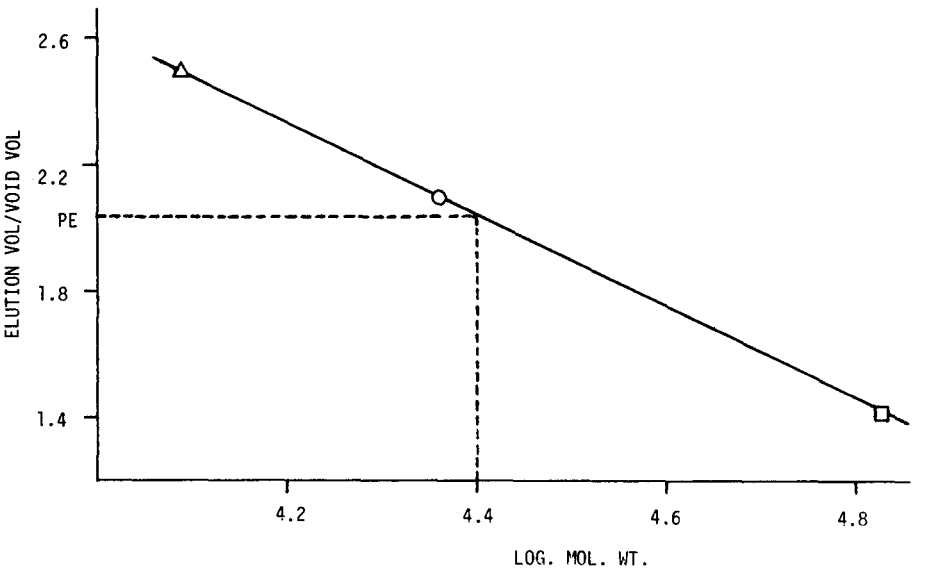


Fig. 8. Standard curve for estimating the molecular weight of potato PE. Elution pattern of proteins (□—BSA; ○—α—chymotrypsinogen; △—cytrochrome C) on Sephadex G-100. Column conditions were as those described in Fig. 1.

temperature for the enzyme is 55 °C. Similar temperature optima have been reported for apple PE (Lee & Wiley, 1970) and strawberry PE (Leuprecht & Schaller, 1968). Rate constants for de-esterification of citrus pectin by potato PE are presented in Table 2. A Q_{10} of 1·33 was observed in the temperature range 25 to 45 °C. Q_{10} values in the temperature range 45 to 55 °C are higher, while the Q_{10} drops sharply in the temperature range 55 to 65 °C. The Q_{10} in the temperature range 25 to 45 °C, being reported for potato PE, compares favourably with data reported for cucumber PE

TABLE 2
RATE CONSTANTS FOR THE DE-ESTERIFICATION OF CITRUS PECTIN BY POTATO PE

Reaction temperature (°C)	*Velocity* (*μeq. of COO^- groups formed/5 min*)	*Rate constants* (*μeq. COO^- groups × min^{-1} × mg protein*)	Q_{10}
25	24·0	4·8	—
35	31·9	6·38	1·33
45	42·7	8·54	1·34
55	62·9	12·6	1·47
65	32·5	6·5	0·52

(Bell *et al.*, 1951), pea PE (Collins, 1970) and strawberry PE (Leuprecht & Schaller, 1968). The energy of activation (E_a) of potato PE, as determined from the slope of the Arrhenius plot (Fig. 7), was estimated to be 6200 calories. No breaks were observed in the Arrhenius plot. These results are in agreement with those of Lee & Wiley (1970) who obtained an E_a of 5800 calories for apple PE. However, considerably higher E_a values with breaks in the Arrhenius plot have been reported for tomato PE by Nakagawa *et al.* (1970).

The apparent molecular weight of potato PE was estimated to be about 25,000 (Fig. 8). Except for a low molecular weight form from bananas, plant PE's have reported molecular weights in the range 24,000 to 37,000 (Versteeg, 1979). The molecular weight of potato PE is close to that of tomato PE, which has been reported to have a molecular weight around 24,000 (Pressey & Avants, 1972).

Although the enzyme preparation in this study was purified only 10-fold, comparisons of physical and kinetic properties with those obtained from PE's with a greater degree of purity (MacDonnell *et al.*, 1945; Lee & MacMillan, 1968; Pressey & Avants, 1972) were more or less similar. This gives us confidence that protein impurities present in the partially purified potato PE extract do not appreciably interfere with the determinations of the physical and kinetic parameters of the enzyme.

Based on the physical and chemical properties studied, the nature of potato PE is similar to that of other plant PE. Roeb & Stegemann (1975) separated potato PE into various isoenzyme species via polyacrylamide gel electrophoresis. This study failed to resolve isoenzymes of potato PE by gel filtration. This is probably because the isoenzymes have a somewhat similar molecular weight and differ only by virtue

of their electrical charge. The results reported in this study are thus likely to represent the action of multiple forms of PE, rather than a single form of the enzyme.

Some properties of potato PE, especially its response to temperature, have direct implications in the potato processing industry. Pre-heating tubers at 55 °C for 15–20 min prior to freezing has been reported to improve the texture of frozen potatoes (Lester & Williams, 1969). According to Bartolome & Hoff (1972) pre-heating tubers at 55 °C causes potato PE to act optimally on native potato pectin to produce free carboxyl groups which complex with divalent ions to form cross links between polyuronide chains—and hence improve firmness.

PE activity could also be of physiological importance in the softening of tubers during storage. The rôle of PE may be an initiating step in such a process as it would yield a substrate for the action of polygalacturonase (PG), which exists in potato tubers (Puri *et al.*, 1980). Present work in this laboratory is oriented towards studying the combined effects of potato PE and PG in relation to textural changes in potatoes.

REFERENCES

ARAKJI, O. A. & YANG, H. Y. (1969). Identification and characterization of the pectic enzymes in the McFarlain cranberry. *J. Food Sci.*, **34**, 340.

BARTOLOME, L. G. & HOFF, J. E. (1972). Firming of potatoes: Biochemical effect of preheating. *J. Agric. and Food Chem.*, **20**, 266.

BATEMAN, D. F. & MILLAR, R. L. (1966). Pectic enzymes in tissue disintegration. *Ann. Rev. Phytopath.*, **4**, 119.

BELL, T. A., ETCHELLS, J. L. & JONES, I. D. (1951). Pectinesterase in the cucumber. *Arch. Biochem. Biophys.*, **31**, 431.

BEN-ARIE, R. & LAVEE, S. (1971). Pectic changes occurring in Elberta peaches suffering from wooly breakdown. *Phytochemistry*, **10**, 531.

CHANG, L. W. S., MORITA, L. L. & YAMAMOTO, H. Y. (1965). Papaya pectinesterase inhibition by sucrose. *J. Food Sci.*, **30**, 218.

COLLINS, J. L. (1970). Pectin methylesterase activity in the southern pea (*Vigna senensis*). *J. Food Sci.*, **30**, 218.

HULTIN, H. O. & LEVINE, A. S. (1963). On the occurrence of multiple forms of pectinesterase. *Arch. Biochem. Biophys.*, **101**, 396.

HULTIN, H. O. & LEVINE, A. S. (1965). Pectin methylesterase in the ripening banana. *J. Food Sci.*, **30**, 917.

KERTESZ, Z. I. (1938). Pectic enzymes II. Pectic enzymes of tomatoes. *Food Research*, **3**, 481.

KERTESZ, Z. I. (1951). *The pectic substances*, Interscience Publishers Inc., New York, NY.

KERTESZ, Z. I. (1955). Pectic enzymes. In: *Methods in enzymology*, Vol. 1. Academic Press Inc., New York, NY, p. 158.

LEE, M. & MACMILLAN, J. D. (1968). Mode of action of pectic enzymes I. Purification and certain properties of tomato pectinesterase. *Biochemistry*, **7**, 4005.

LEE, Y. S. & WILEY, R. C. (1970). Measurement and partial characterization of pectinesterase in apple fruits. *J. Am. Soc. Hort. Sci.*, **95**, 465.

LESTER, R. & WILLIAMS, T. P. (1969): *Improving texture of frozen potatoes by preheating*. US Patent 3476573.

LEUPRECHT, H. & SCHALLER, A. (1968). Ergebnisse methodologischer Untersuchungen hinsichtlich der Messung der Pektinmethylesterase-Aktivitat in aufgetautem Tiefgefrier-Erdbeerpuree. *Fruchtsaft Ind.*, **13**, 50–74.

LINEWEAVER, H. & BALLOU, G. A. (1945). The effect of cations on the activity of alfalfa pectinesterase (pectase). *Arch. Biochem.*, **6**, 373.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**, 265.

MACDONNELL, L. R., JANSEN, E. F. & LINEWEAVER, H. (1945). The properties of orange pectinesterase. *Arch. Biochem.*, **6**, 389.

MARKOVIC, O. (1978). Pectinesterase from carrot (*Daucus carota* L.). *Experentia*, **34**, 561.

NAGEL, C. W. & PATTERSON, J. E. (1967). Pectic enzymes and development of the pear (*Pyrus communis*). *J. Food Sci.*, **32**, 294.

NAKAGAWA, H., YANAGAWA, Y. & TAKEHANA, H. (1970). Studies on the pectolytic enzyme. V. Some properties of purified tomato pectinesterase. *Agric. Biol. Chem.*, **34**, 998.

PRESSEY, R. & AVANTS, J. K. (1972). Multiple forms of pectinesterase in tomatoes. *Phytochemistry*, **11**, 3139.

PURI, A., SOLOMOS, T. & KRAMER, A. (1980). Polygalacturonase in potato tubers—Its presence and some of its properties. Paper presented at the 77th Annual Meeting of the American Society for Horticultural Science, Fort Collins, CO., July 27–Aug. 1. *Lebensm. Wiss. u. Technol.* (In press).

RÖEB, L. & STEGEMANN, H. (1975). Pectin methylesterases: Isoenzymes, properties and detection in polyacrylamide gels. *Biochem. Physiol. Pflanzen.*, **168**, 607.

SEGEL, I. H. (1975). *Biochemical calculations.* (2nd edn), John Wiley & Sons, Inc., New York.

SMITH, W. K. (1958). A survey of production of pectic enzymes by plant pathogenic and other bacteria. *J. Gen. Microbiol.*, **18**, 33.

VAS, K., NEDBALEK, M., SCHEFFER, H. & KOVACS-PROSZT, G. (1967). Methodological investigations on the determination of some pectic enzymes. *Fruchtsaft Ind.*, **12**, 164.

VERSTEEG, C. (1979). *Pectinesterases from the orange fruit—Their purification, general characteristics and juice cloud destabilizing properties.* PhD Dissertation. Wageningen, The Netherlands.

WHITAKER, J. R. (1963). Determination of the molecular weights of proteins by gel filtration on Sephadex. *Anal. Chem.*, **35**, 1950.

ZAUBERMAN, G. & SCHIFFMAN-NADEL, M. (1972). Pectin methylesterase and polygalacturonase in avocado fruit at various stages of development. *Pl. Physiol.*, **49**, 864.

Food Chemistry 8 (1982) 215–223

THE VITELLINE MEMBRANE: DYNAMICS OF CHOLESTEROL METABOLISM IN HENS' EGGS

TERESA SMOLIŃSKA & TADEUSZ TRZISZKA
Institute of Storage and Food Technology, Agricultural Academy, Norwida 25/28, 50-375 Wrocław, Poland

(Received: 25 November, 1980)

ABSTRACT

Changes in cholesterol levels in egg contents were studied to indicate trends and dynamics of processes of penetration through the vitelline membrane from yolk to egg white. Investigations were carried out on eggs stored for a period of 35 days at room temperature (22°C). Levels of free and esterified cholesterol were determined by the spectrophotometric method at 7-day intervals. The selective character of the vitelline membrane depends on conditions and period of storage. The study demonstrated that the vitelline membrane loses its selective nature between the twentieth and thirtieth days under particular conditions of storage. Following this period, a spontaneous penetration of cholesterol into the egg white is observed—i.e. the barrier equilibrium of the membrane becomes unstable. The presence of free cholesterol in egg white may be indicative of loss of egg freshness.

INTRODUCTION

Recently there have been numerous reports in the literature (Laskowski, 1966; Bartov *et al.*, 1971; Turk & Barnett, 1971; Teekell *et al.*, 1975; Branżowa Norma BN-72/8036-05, 1972) about the function of cholesterol in eggs. Cholesterol is considered to be the most important sterol found in all tissues of the animal organism, and is especially abundant in spinal cord, cerebrum and as bile acids. It is also one of the main components of cellular structure. Cholesterol shows high resistance to the action of harmful agents such as bacterial and chemical toxins. All raw materials of animal origin contain cholesterol and in eggs it is present in large quantities. As main constituents of the yolk, fats make up 64% of the dry matter and do not occur as homogeneous compounds.

Food Chemistry 0308-8146/82/0008-0215/$02·75

In relation to the total quantity of lipids of the yolk, triglycerides make up 65 %, lipids, 30 % and cholesterol, 4 %; it is to be noted that cholesterol is found in both the free and bound forms. Cholesterol is associated with phospholipids (lecithin) and the surface of the molecule is surrounded by protein (Solecki *et al.*, 1976).

Cholesterol level in egg is not stable; it depends on many factors such as the season, the strain, and the feeding and breeding system of the hens. It is maize—and mainly maize oil, as well as coconut oil—that, as nutritional agents, have the greatest effect on the increase of cholesterol level in egg yolk. Oils containing many unsaturated acids, such as rape oil or soybean oil, however, show a marked ability to lower the cholesterol level in blood and thus in the yolk of an egg (Becker *et al.*, 1977; Teekell *et al.*, 1975; Turk & Barnett, 1971).

Cholesterol plays an important role in the function of biological membranes. Investigations (Dowben, 1973; Gottleb, 1977) have demonstrated that cholesterol (mainly free) makes up 20–30 % of lipids extracted from membranes of mammalian erythrocytes as well as from other preparations of plasmatic membranes except for mitochondrial membranes which contain very little cholesterol. The established presence of lipids in the vitelline membrane (Trziszka, 1977; Trziszka & Smolińska, 1980) indicates that sterols—and specifically cholesterol, in view of its substantial level in the egg yolk—could also be a component of the membrane lipids. In the light of the above-mentioned investigations it may be interesting to demonstrate whether or not the vitelline membrane takes an active part in the binding and displacement of cholesterol.

In this paper an attempt is made to determine the changes in cholesterol level in the egg on the basis of its ability to penetrate through the vitelline membrane from the yolk to the white, as well as to establish whether cholesterol can be an indicator of the freshness of eggs.

MATERIAL AND TESTING METHODS

The experimental material comprised 240 hens' eggs (hybrid of New Hampshire × Sussex) of average weight of 60–65 g stored at room temperature (22 °C) and relative humidity 80 % (± 2) for 35 days. The investigations were carried out in the January–February period at 7-day intervals (Table 1). The aim of the investigation was to determine the level of free and esterified cholesterol in the egg yolk, vitelline membrane and egg white. For this purpose, eggs were broken and the egg white was mechanically separated from the egg yolk, exactly weighed and then the cholesterol content was determined (Fig. 1). The membranes were washed in NaCl solutions (Trziszka & Smolińska, 1980) and then solubilised in phosphate buffer (0·1 M) containing 5 % SDS, 1 % β-mercaptoethanol (Trziszka, 1971). The solution of vitelline membranes obtained in this way served for the determination of the cholesterol they contained.

TABLE 1
LAYOUT AND BALANCE OF MATERIALS

Period of investigations (days)	*Number of eggs in one sample*	*Number of replications* n	*Total number of eggs*	*Weight of sample* *Vitelline membrane dry matter (mg in 1 ml solubilate)*	*Yolk (g)*	*White (g)*
0	4	10	40	About 15	5	20
7	4	10	40	15	5	20
14	4	10	40	15	5	20
21	4	10	40	15	5	20
28	4	10	40	15	5	20
35	4	10	40	15	5	20
	Total		240			

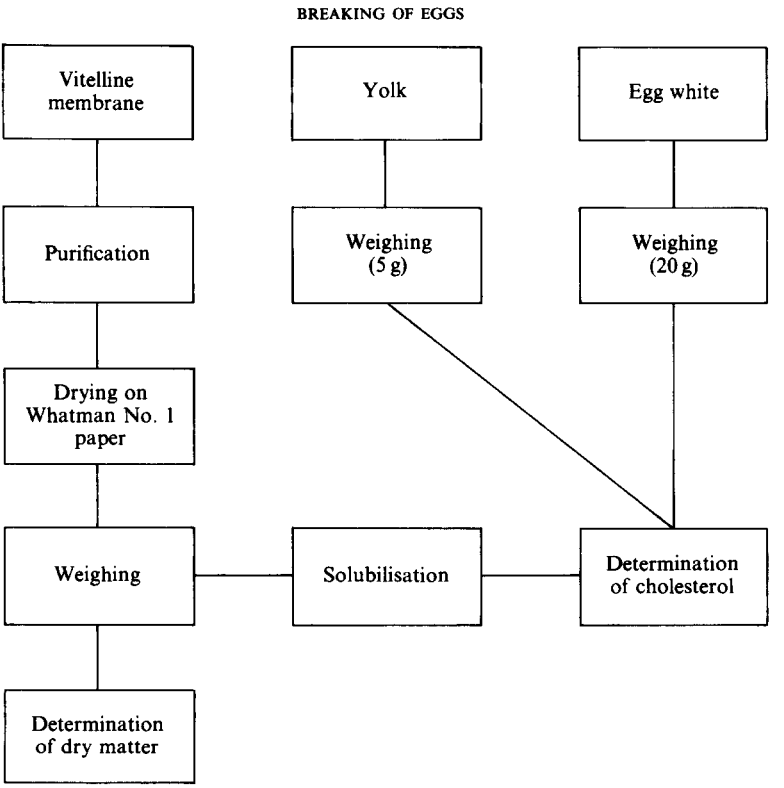


Fig. 1. Determination of cholesterol.

The extraction of lipids from the yolk and the white was conducted in a solvent mixture of chloroform:methanol (2:1). Five grammes of yolk and 20 g of egg white were homogenised with 20 cm^3 of solvent mixture and the homogenate was centrifuged. In the extracted lipids cholesterol was determined by the Lieberman–Burchardt method (Mejbaum-Katzenellenbogen & Mochnacka, 1968) consisting of the extraction of cholesterol with a mixture of absolute ethanol and acetone (1:1), cholesterol being precipitated by means of digitonin. The concentration of all forms of cholesterol in the coloured solutions was determined spectrophotometrically at a wavelength of $\lambda = 610$ nm. Cholesterol contents were calculated on a whole sample basis and the percentage content of cholesterol in dry matter was calculated from a standard curve. Dry matter in the white and yolk was determined at the same time by a refractometric method according to the analytical Polish Standards (Branzowa Norma BN-72/8036-05, 1972). The method consists in reading values of dry matter from Tables on the basis of the determined coefficient of refraction in an Abbé refractometer.

Dry matter in the vitelline membrane was determined by the drier method according to the analytical Polish Standards (Branzowa Norma BN-72/8036-05, 1972). The sample was dried at a temperature of 105 °C to constant value.

RESULTS AND DISCUSSION

On the basis of the present results and previous investigations (Turk & Barnett, 1971; Becker *et al.*, 1977; Trziszka, 1977) one can say that lipids play an essential rôle in conversions of a biochemical nature. It has been proved that lipids in the yolk of eggs stored at low temperatures are generally stable because of their complexing with albumens and because of lack of enzymes of an oxidising character (Evans *et al.*, 1958). At elevated (that is, room) temperatures, however, the stability period of lipids is proportionally shorter.

The results of the investigations (Figs. 2 and 3) show that the content of total cholesterol (first testing period) in the yolk averages 0·289 g, this making up 3 % of the dry matter of the yolk. The results presented are consistent with the literature findings (Turk & Barnett, 1971) quoted. 15·5 to 17·5 mg of cholesterol in 1 g of yolk, which corresponds to about 3 % of cholesterol in dry matter, and similar data are reported by Becker *et al.* (1977) who found an average value of about 0·3 g of cholesterol in the dry matter of yolk. In the current investigations, carried out during the storage of eggs, it was observed that cholesterol in the yolk showed a decreasing tendency. The content of total and free cholesterol in the yolk during the storage of eggs successively decreased, which proved statistically significant ($P < 0{\cdot}05$). Esterified cholesterol, however, remained at the same level in this period; thus, the cholesterol loss in the yolk, which intensified particularly after 21 days' of storage of

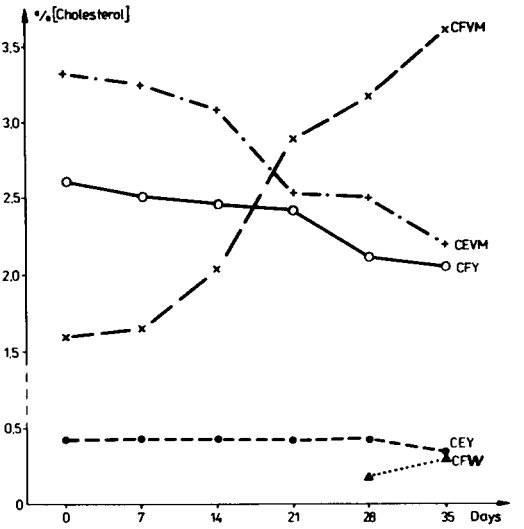


Fig. 2. Changes in the free and esterified cholesterol content of the dry matter.
CFVM—Free cholesterol of vitelline membrane (dry matter).
CEVM—Esterified cholesterol of vitelline membrane (dry matter).
CFY—Free cholesterol in the dry matter of the yolk.
CEY—Esterified cholesterol in the dry matter of the yolk.
CFW—Free cholesterol of the dry matter of the egg white.

eggs at a temperature of 22 °C, was the result of migration of non-esterified cholesterol in the direction of the vitelline membrane and egg white after 28 days.

Cholesterol and phospholipids show functional interaction. It was demonstrated that cholesterol and phospholipids of the erythrocytes exchange intensely *in vivo* and *in vitro* with cholesterol and serum lipids (Solecki *et al.*, 1976). Lipid structures built of phospholipids and free cholesterol in cellular membranes and lipoproteins of the plasma show some similarity (Solecki *et al.*, 1976). The transport of lipids from one structure to the other may take place, e.g. in the blood. In other investigations it was demonstrated that during the incubation of blood *in vitro*, free cholesterol penetrates from erythrocytes to the plasma, but no transport of lipids was detected. Cholesterol and phospholipids are displaced simultaneously when cell membranes are damaged (Gottleb, 1977; Sinha *et al.*, 1977).

Studies on the exchange of labelled cholesterol between the inside of cells and plasma demonstrated that there is some accumulation of cholesterol on the inner surface of the erythrocyte membrane and the penetration of cholesterol takes place at various rates according to the temperature of incubation (Lange *et al.*, 1977). Cell membranes can have an ability to synthesise and regenerate the components of phospholipids, and the molar proportion of cholesterol to phospholipids often approximates to unity.

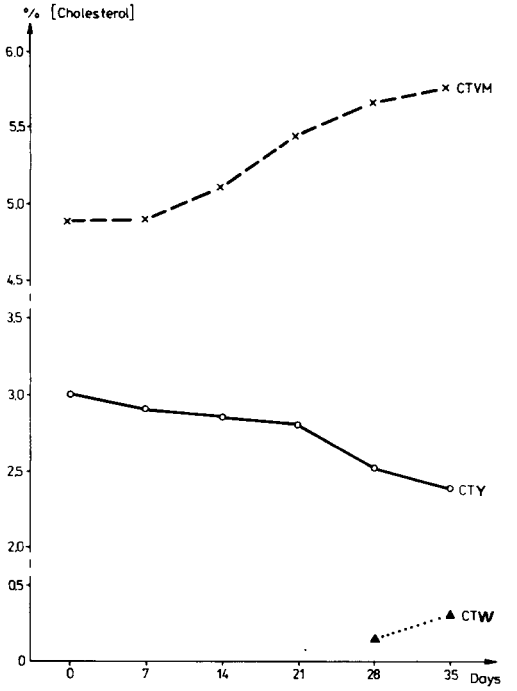


Fig. 3. Changes in the total cholesterol content in the dry matter of the eggs.
CTVM—Total cholesterol of vitelline membrane (in the dry matter).
CTY—Total cholesterol in the dry matter.
CTW—Total cholesterol of the dry matter of the egg white.

The presence of lipids and their role in cell membranes has already been described (Dowben, 1973). As is well known, they serve as a carrier for compounds soluble in fats. The role played by lipid compounds in the vitelline membrane, whose presence was detected in the investigations by Trziszka (1977), Trziszka & Smolińska (1979) and earlier by Britton (1973), however, is hardly clarified.

As a result of the current investigations one can conclude that, of the total quantity of lipids contained in the egg, cholesterol is bound with the lipids of the vitelline membrane in substantial amounts.

In the vitelline membrane of newly laid eggs there is a high content of total cholesterol amounting to about 4·90 % of the dry matter of the membrane and of this, 1·60 % is free cholesterol (non-esterified).

From the seventh to the twenty-first days of storage of eggs, under the conditions described, an increment of free cholesterol up to 2·92 % was observed which, at the same time, caused an increase of total cholesterol up to 5·43 %. After 35 days of storage of eggs further elevation of free cholesterol up to 3·55 % was noted, and thus an increase of total cholesterol up to 5·75 %. Statistical analysis of the changes in

cholesterol content in the vitelline membrane showed highly significant differences ($P < 0{\cdot}01$). The esterified cholesterol ranged from the initial value of 3·30 to 2·2% after 35 days' storage. From this it follows that structurally bound cholesterol in the vitelline membrane undergoes dissociation during the storage of eggs. However, a substantially greater increment of free cholesterol than would result from its dissociation from structural bonding of the vitelline membrane may be caused by the migration of free cholesterol from the egg yolk.

The reduction of cholesterol level in the egg yolk during the first weeks of egg storage leads to the displacement of cholesterol from the yolk to the vitelline membrane where it can be accumulated. It can be said that the phenomenon is analogous to the exchange of cholesterol and phospholipids from the erythrocyte shadow to the blood serum (Dowben, 1973; Gottleb, 1977). It is to be noted that the period starting with the twenty-first day of egg storage in the conditions discussed is critical for the resistance of the vitelline membrane. The elasticity of the membrane decreases because of changes occurring in its structure, and a more spontaneous exchange between the white and the yolk takes place. In the process of ageing this exchange is associated mainly with the displacement of water from the egg white to the yolk, as well as with intensification of proteolytic processes in the protein complexes of the membrane (Trziszka, 1977).

In his investigations Britton (1973) demonstrated that the vitelline membrane of eggs stored at room temperatures of 22 °C showed a great ability to accumulate water; however, one cannot exclude the possibility of the accumulation of cholesterol which can then be displaced to the egg white with the loss of natural selectivity of the vitelline membrane.

After 28 days of storage of eggs, the presence of cholesterol in the egg white was detected and it was demonstrated that this was exclusively free cholesterol. This fact may be of great importance in studies of the function of the vitelline membrane in ageing processes of egg content. The biological function of the membrane is associated with its selective permeability.

As metabolic processes of the egg content become more advanced, one can observe the migration of low-molecular weight compounds through the vitelline membrane—a fact confirmed by earlier investigations (Smolińska & Trziszka, 1979). In some cases it may include the infiltration of even ovalbumin (Teekell *et al.*, 1975) and glucose which is connected with substantial changes in the membrane (Trziszka, 1977). The detection of the presence of cholesterol in the egg white testifies to the changes of a biophysico–chemical nature taking place in the vitelline membrane, these resulting in an increase of permeability of the vitelline membrane for macromolecules.

Thus it appears that the presence of cholesterol in the egg white can serve as an indicator of functional changes of the vitelline membrane and may be useful also as an indicator of freshness in studies of stored eggs. The evaluation of its usefulness, however, would require further detailed investigations because of the variability of

cholesterol in the egg content which depends on many factors, such as strain of hens, age, season, feeding and breeding. Furthermore, the conditions under which eggs are stored and lose their freshness are also highly variable.

Investigations of the function of lipids, and especially of cholesterol, in the vitelline membrane have revealed significant variability of fat compounds within the range 1·75% to 16·5% on a dry weight basis (Trziszka & Smolińska, 1980). In addition, the role of cholesterol may be substantially more complex than it appears from the results here.

In conclusion, it should be emphasised that the course of changes of cholesterol in egg is of a dynamic character and is strictly associated with alterations of the natural process of ageing occurring in the vitelline membrane.

CONCLUSIONS

(1) After 21 days' storage of eggs at room temperature (22 °C) there is a migration of free cholesterol from the yolk and vitelline membrane to the egg white which testifies to the prior barrier equilibrium nature of the vitelline membrane.

(2) The presence of cholesterol in the egg white may be a useful parameter in freshness studies on eggs.

REFERENCES

BARTOV, I., BORNSTEIN, S. & BUDOWSKI, P. (1971). Variability of cholesterol concentration in plasma and egg yolks of hens and evaluation of the effect of some dietary oils. *Poultry Sc.*, **50**, 1357.

BECKER, W. A., SPENCER, J. V., VERSTRATE, J. A. & MIRISH, L. V. (1977). Genetic analysis of chicken egg yolk cholesterol. *Poultry Sc.*, **56**, 895.

BRANŻOWA NORMA BN-72/8036-05, 1972.

BRITTON, W. M. (1973). Vitelline membrane chemical composition in natural and induced yolk mottling. *Poultry Sc.*, **52**, 459.

DOWBEN, R. (1973). *Błony biologiczne*, PZWL, Warszawa.

EVANS, R. J., DAVIDSON, J. A., BAUER, D. H. & BANDENER, S. L. (1958). Distribution of proteins in fresh and stored shell eggs. *Poultry Sc.*, **37**, 81.

GOTTLEB, M. H. (1977). The reactivity of human erythrocyte membrane cholesterol with a cholesterol oxidase. *Biochim. Biophys. Acta*, **446**(3), 422.

LANGE, J., COHEN, C. M. & POZNANSKY, M. J. (1977). Trans-membrane movement of cholesterol in human erythrocytes. *Proc. Natl. Acad. Sc. USA*, **74**(4), 1538.

LASKOWSKI, K. (1966). *Poziom cholesterolu w jajach kurzych, mleku i śmietanie w Polsce.* Prace Inst. Przem. Mlecz. WSR, Olsztyn, 21.

MEJBAUM-KATZENELLENBOGEN, W. & MOCHNACKA, J. (1968). *Kurs praktyczny z biochemii*, PWN, Warszawa.

OKTABA, W. (1971). *Metody statystyki matematycznej w doświadczalnictwie*, PWN, Warszawa.

SINHA, A. K., SHATTIL, S. J. & COLMAN, R. W. (1977). Cyclic AMP metabolism in cholesterol rich platelets, *J. Biol. Chem.*, 252/10/3310.

SMOLIŃSKA, T. & TRZISZKA, T. (1979). Examination on selection of vitelline membrane for some electrolytes, *Zesz. Nauk. AR Wrocław, Technologia Żywności* I, 124, 65.

SOLECKI, A., BODZIO, T. & ROGULSKI, J. (1976). Transfer of cholesterol and lecithin between erythrocytes and serum *in vitro*. *Acta Biochim. Polonica*, **23**, 235.

TEEKELL, R. A., BREIDENSTEIN, C. P. & WATIS, A. B. (1975). Cholesterol metabolism in the chicken. *Poultry Sc.*, **54**, 1036.

TRZISZKA, T. & SMOLIŃSKA, T. (1981). Chemical characterisation of the vitelline membrane of hen eggs, *Food Chem.* (in press).

TRZISZKA, T. (1977). Changes in the structure of vitelline membrane of hen eggs from various periods of cold storage (dissertation). *Zesz. Nauk. AR, Wroclaw, Technologia Żywności* I, 124, 45.

TRZISZKA, T. & SMOLIŃSKA, T. (1980). The dynamics of lipid metabolism in hens' eggs, AR Wrocław. (Manuscript.)

TURK, D. E. & BARNETT, B. D. (1971). Cholesterol content of market eggs, *Poultry Sc.*, **50**, 1303.

Food Chemistry 8 (1982) 225–231

THE CHEMICAL AND PHYSICAL CHARACTERISTICS AND FATTY ACID COMPOSITION OF SEED OILS EXTRACTED FROM CRUCIFEROUS SPECIES CULTIVATED IN PAKISTAN

AMJAD ALI & JAMES E. MCKAY

Department of Food Science, The University, Leeds, Yorkshire LS2 9JT, Great Britain

(Received: 15 December, 1980)

ABSTRACT

The fatty acid compositions of the seed oils of yellow sarson (Brassica trilocularis), *brown sarson* (Brassica campestris *ssp.* dichotoma) *and taramira* (Eruca sativa) *grown in Pakistan were determined by gas chromatography. Characteristically high contents of docosenoic acid were observed. Compositions of the seed oils of some cruciferous species from other origins were examined for comparison. Other analytical characteristics have been determined.*

INTRODUCTION

The oils obtained from cruciferous seeds have attracted considerable interest during the last two decades, particularly since such crops may be grown successfully in the northern regions of Europe and North America. Such oils, often described conveniently but loosely as 'rapeseed' oils, have been subjected to considerable investigation and the properties of both seeds and oils have been reviewed by Appelqvist & Ohlson (1972).

The fatty acid composition of *Brassica* seed oils is not as simple as those of other common vegetable oils. Fifteen fatty acids, ranging in chain length from 16 to 24 carbon atoms and in number of double bonds from zero to three, are present at levels greater than 0·5%. The effects of variety (Delhaye & Guyot, 1970), environment (Dembinski *et al.*, 1967; Appelqvist, 1968*b*; Marquard, 1973) and extraction technique (Shavlo, 1973) on the fatty acid composition complicate the comparison, with respect to composition, of seed oils of different origin.

The production of cruciferous oil crops in Pakistan is considerable, e.g. 87% of the total edible oil seed production of the country (excluding cottonseed) in 1973–1974, and the oil produced amounted to 40·7% of the total vegetable oils produced in the

Food Chemistry 0308-8146/82/0008-0225/$02·75

TABLE 1
NAMES AND SOURCES OF THE CRUCIFERAE SPECIES STUDIED

Common name	*Systematic name*[a]	*Source*	*Nature of sample*
Yellow sarson	*Brassica trilocularis*	Food Technology Dept., Agricultural University, Faisalabad, Pakistan	Seed
Brown sarson	*Brassica campestris* ssp. *dichotoma*	Food Technology Dept., Agricultural University, Faisalabad, Pakistan	Seed
Brown sarson[b]	*Brassica campestris*	Tropical Products Inst., Grays Inn Road, London	Seed
Brown mustard	*Brassica juncea*	Reckitt and Colman, (Food Division), Norwich	Seed
White mustard	*Sinapis alba*	Reckitt and Colman, (Food Division), Norwich	Seed
Taramira	*Eruca sativa*	Private collection	Oil
Sarson	Unknown	Private collection	Oil

[a] According to the classification of Alam (1945).
[b] Nepali origin.

same year (Ministry of Food and Agriculture, 1975). The species which constitute these crops are mainly those listed in Table 1. The crops are grown and processed on the domestic scale and in the traditional manner, so that the edible oil industry itself is largely unorganised and traditional and thereby capable of considerable development.

Since the information concerning the composition of oils produced from the traditional varieties of Cruciferae grown in Pakistan is sparse (Tremazi *et al.*, 1968; Baluch & Natali, 1970; Ahmad & Ali, 1971) and since existing information may well include data derived from imperfectly characterised seed, this investigation of the composition of oil extracted from authenticated seed grown in Pakistan has been undertaken. A number of oils derived from other species, including commercial samples of sarson and taramira oils, have been examined for purposes of comparison.

EXPERIMENTAL

Materials

Seed samples were obtained from the sources indicated in Table 1. Solvents used were of a high degree of purity. The sodium methoxide–methanol mixture was prepared by mixing the appropriate amount of sodium methoxide (Koch–Light Laboratories Ltd) with dry methanol.

Extraction of the oil

Dry, clean seeds were crushed and extracted in a Soxhlet apparatus with

petroleum ether (b.p. 60–80 °C). After extraction the solvent was removed using a rotary vacuum evaporator at a temperature not exceeding 40 °C. On completion of evaporation the pressure within the evaporator was restored to the atmospheric level with nitrogen.

Analysis of the oils

The methods of the American Oil Chemists' Society (1975) were used for the determination of the following physical and chemical characteristics: relative density, refractive index, saponification, iodine and acid values and unsaponifiable matter.

Esterification procedure

The methyl esters of the fatty acids were prepared by methanolysis of the oil samples using sodium methoxide as catalyst (Christie, 1973) and were purified by thin layer chromatography using the solvent system: petroleum ether: diethyl ether: acetic acid (90:10:1, v/v). The purified methyl esters were stored in hexane solution in glass-stoppered tubes at 4 °C until analysed.

Gas chromatography

The instrument used was a Pye 104 gas chromatograph fitted with a flame ionisation detector. The column employed was glass (1·525 m × 4 mm i.d.) packed with 10 % ethylene glycol adipate on Chromosorb W (80/100 mesh, acid washed). Nitrogen was used as the carrier gas. The operating conditions were as follows: injection port temperature, 225 °C; column temperature, 200 °C: detector temperature, 225 °C; carrier gas flow, 50 ml/min; hydrogen flow, 50 ml/min; air flow, 750 ml/min. Fatty acid methyl esters of high purity were used to calibrate the detector response and to identify the sample components by relative retention time. Peak area was determined by triangulation.

RESULTS AND DISCUSSION

The seed weight, oil content and physical and chemical characteristics of the oils are given in Table 2. Generally, the seed weight is correlated with the oil content, i.e. the larger the seeds the higher the oil content, although, since the oil content of the seed is influenced by environmental conditions during crop growth, harvesting technique and length and conditions of the storage of the seed (Tremazi, 1954; Schuster, 1967), the absence of such correlations among samples of different age and origin is not unusual. Here the sarson samples show a marked correlation between seed weight and oil content. The other characteristics of the different seed oils are within the ranges prescribed in the international standards recommended for edible *Brassica* seed oils (FAO/Codex Alimentarius Commission, 1969, 1970) except for the slightly higher

TABLE 2
PHYSICAL AND CHEMICAL CHARACTERISTICS OF CRUCIFERAE SEED OILS

	White mustard	*Brown mustard*	*Brown sarson*	*Yellow sarson*	*Brown sarson*[a]	*Recommended standards for rapeseed*[b]
Seed weight (g)[c]	5·836	3·016	3·205	3·088	3·815	—
Petroleum ether extract (% w/w)	32·5	39·2	42·2	38·5	46·8	—
Relative density (20 °C/20 °C)	0·910	0·911	0·910	0·910	0·913	0·910–0·920
Refractive index (n_D40 °C)	1·474	1·476	1·474	1·475	1·474	1·465–1·469
Saponification value (mg KOH/g oil)	174	178	170	171	173	168–181
Iodine value (Wijs)	105·4	121·3	100·6	105·6	102·7	94–120
Unsaponifiable matter (% w/w)	0·85	0·82	0·83	0·85	0·89	2
Acid value (mg KOH/g oil)	1·23	3·59	0·92	1·16	4·43	4·0

[a] Five-year old sample from Nepal.
[b] FAO/Codex Alimentarius Commission (1969, 1970).
[c] Weight of 1000 dry seeds in grammes.

values for refractive index and for the acid value of the Nepali sarson sample which exceeds the prescribed range. The fatty acid compositions of the various oils are shown in Table 3 and a typical gas chromatogram is given in Fig. 1.

The fatty acid compositions of the sarson oils differ very little from one another in terms of the contributions of acids with different numbers of carbon atoms and double bonds as shown in Table 4. The sample of Nepali origin has a noticeably high linolenic acid content while no significant difference exists between the composition of brown sarson oils produced by solvent extraction and hydraulic pressure, respectively, contrary to the observations of Shavlo (1973).

The oils extracted from *Sinapis alba* and *Eruca sativa* are very similar to the sarson oils in fatty acid composition but possess rather higher contents of linolenic acid. That obtained from *Brassica juncea*, on the other hand, is markedly different with respect to the contributions of fatty acids in terms of different numbers of

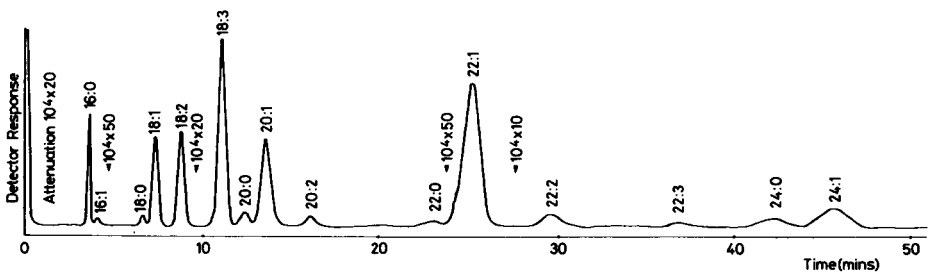


Fig. 1. Gas chromatogram of the fatty acid methyl esters derived from *B. trilocularis* oil with variation of the detector sensitivity to show the 22:3 peak. For gas chromatographic conditions see text.

TABLE 3

FATTY ACID COMPOSITION OF RAPESEED AND MUSTARD SEED OILS[a] (METHYL ESTERS AS % OF TOTAL METHYL ESTERS)

	Fatty acid esters													
	16:1	*16:1*	*18:0*	*18:1*	*18:2*	*18:3*	*20:0*	*20:1*	*20:2*	*22:0*	*22:1*	*22:2*	*22:3*	*24:0*	*24:1*
Yellow sarson	1·9	0·1	0·9	8·7	12·2	11·1	1·0	6·6	0·9	0·6	51·2	1·0	0·4	1·1	2·3
Brown sarson	1·7	0·2	1·0	13·2	12·5	7·8	0·9	7·9	0·6	1·0	51·5	0·8	Tr	Tr	1·0
Brown sarson[b]	1·8	0·2	1·0	11·7	14·7	7·3	0·9	7·0	0·9	1·1	50·0	1·3	Tr	0·5	1·6
Brown sarson[c]	1·6	0·2	0·9	9·6	11·1	15·1	0·9	6·0	0·5	Tr	52·5	0·8	Tr	Tr	0·8
Sarson oil[d]	2·9	0·2	1·3	13·0	11·7	8·8	1·0	9·3	0·6	0·7	48·1	0·9	Tr	Tr	1·5
Brown mustard	2·2	0·2	1·2	17·4	20·5	14·1	0·7	11·4	1·1	0·5	28·1	0·5	Tr	Tr	2·2
White mustard	3·1	0·2	0·7	9·1	11·7	12·5	0·7	10·8	0·7	Tr	46·5	0·4	Tr	Tr	3·6
Taramira oil[d]	5·5	0·2	1·2	15·1	7·6	12·9	0·6	11·4	0·4	Tr	43·1	0·3	Tr	Tr	1·7

[a] Average of three analyses.
[b] Mechanically expressed oil.
[c] Sarson sample from Nepal.
[d] Commercial oil samples.
Tr = trace.

TABLE 4
THE DISTRIBUTIONS OF FATTY ACIDS IN THE OILS ON THE BASES OF NUMBER OF CARBON ATOMS AND OF NUMBER OF DOUBLE BONDS (DB)

Oil sample	*Percentage of total fatty acids*							
	C_{18}	C_{20}	C_{22}	C_{24}	C_{18}–C_{24}	*1 DB*	*2 DB*	*3 DB*
Yellow sarson	32·9	8·9	52·8	3·4	98·0	68·9	14·1	11·4
Brown sarson	34·5	9·4	53·3	1·0	98·2	73·8	13·9	7·8
Brown sarson[a]	34·7	8·8	52·4	2·1	98·0	70·5	16·9	7·3
Brown sarson[b]	36·7	7·4	53·3	0·8	98·2	69·1	12·4	14·1
Sarson oil[c]	34·8	10·9	49·7	1·5	96·9	72·1	13·2	8·8
Duro[d]	36·4	10·3	50·0	1·1	97·8	72·1	14·9	9·1
Lembkes[d]	38·0	10·9	47·0	1·5	97·4	70·2	14·5	10·5
Brown mustard	53·2	13·1	29·1	2·2	97·6	59·3	22·1	14·1
White mustard	34·0	12·3	46·9	3·6	96·8	70·2	13·0	12·5
Taramira oil[c]	36·8	12·3	43·3	1·7	94·1	71·5	8·3	12·9

[a] Mechanically expressed.
[b] From Nepal.
[c] Commercial samples.
[d] From Appelqvist ;1968*b*, 1969).

carbon atoms and double bonds. The low erucic acid content suggests that this oil is of European rather than Asian origin (Appelqvist, 1970).

With the exception of that of *Brassica juncea*, all the oils are closely similar to those of the Swedish winter rape varieties, Duro and Lembkes, described by Appelqvist (1968*b*, 1969). The great predominance of fatty acids with 18 or more carbon atoms is marked in all the samples and docosatrienoic acid, reported to occur in mustard species (Appelqvist, 1968*a*) was detected in all the samples analysed.

ACKNOWLEDGEMENTS

The authors are grateful to Dr J. H. Merz, Reckitt and Colman (Food Division), Messrs. J. H. Broadbent and T. W. Hammonds, Tropical Products Institute, and the Head of the Department of Food Technology, Agricultural University, Faisalabad, for the provision of samples.

REFERENCES

AHMAD, R. & ALI, I. (1971). Study of fatty acid composition of rapeseed and mustard oils. *Sci. Ind.*, **8**, 304–7.

ALAM, Z. (1945). Nomenclature of oleiferous brassicas cultivated in the Punjab. *Indian J. Agric. Sci.*, **15**, 173–81.

AMERICAN OIL CHEMISTS' SOCIETY (1975). *Official and tentative methods*, 3rd edition (revised). A.O.C.S., Champaign, Illinois.

APPELQVIST, L-Å. (1968*a*). Lipids in Cruciferae I. Fatty acid composition in seeds of some Svalöf varieties and strains of rape, turnip rape, white mustard and false flax. *Acta Agr. Scand.*, **18**, 3–21.

APPELQVIST, L-Å. (1968*b*). Lipids in Cruciferae III. Fatty acid composition of diploid and tetraploid seeds of *Brassica campestris* and *Sinapis alba* grown under climatic extremes. *Physiol. Plant.*, **21**, 615–25.

APPELQVIST, L-Å. (1969). Fatty acid patterns in single seeds and seed populations of various Cruciferae and in different tissues of *Brassica napus*. *Hereditas*, **61**, 9–44.

APPELQVIST, L-Å. (1970). Lipids in Cruciferae VI. The fatty acid composition of seeds of some cultivated *Brassica* species and *Sinapis alba* L. *Fette, Seifen, Anstrichmittel*, **72**, 783–92.

APPELQVIST, L-Å. & OHLSON, R. (1972). *Rapeseed*. Elsevier, Amsterdam, London, New York.

BALUCH, M. A. A. & NATALI, A. H. (1970). Studies on the technological characteristics of some of the improved varieties of rapeseed and other cruciferous crops of West Pakistan. *Zesz. Probl. Postepow NaukRoln.*, No. 91, 83–9.

CHRISTIE, W. W. (1973). *Lipid analysis*. Pergammon Press, Oxford, pp. 30–40.

DELHAYE, D. & GUYOT, A. (1970). Contribution a l'etude de la variation de la composition en acides gras des huiles de Colza. *Bull. Rech. Agron. Gembloux*, **5**, 443–9.

DEMBINSKI, F., JARUSZEWSKA, H., KRZYWINSKA, F. & KRASNODEBSKI, P. (1967). Effect of soil moisture and of nitrogen fertilizer on the composition of fatty acids in seed oil of summer rape. *Pamiet. Pulawski*, **25**, 241–50 (C.A. **68**, 21282).

FAO/Codex Alimentarius Commission (1969). CAC/RS 24.

FAO/Codex Alimentarius Commission (1970). CAC/RS 34.

MARQUARD,[1] R. (1973). Changes in composition of different vegetable oils caused by breeding and environment. *Z. Lebensm.-Unters. Forsch.*, **153**, 354–62.

MINISTRY OF FOOD AND AGRICULTURE (1975). *Agricultural statistics of Pakistan*. Government of Pakistan, Islamabad.

SCHUSTER, W. (1967). Variation in fat content of various oil plants. I. Winter rape and sunflowers. *Fette, Seifen, Anstrichmittel*, **69**, 831–7.

SHAVLO, V. F. (1973). Change in the lipid fatty acids in relation to the method of lipid extraction. *Byull. Nauchno-Tekh. Inf. Maslichn. Kul't*, Part 2, 49–52 (C.A. **83**, 117571).

TREMAZI, S. A. (1954). A study of factors influencing the oil content of the seed of toria. *Texas J. Sci.*, **6**, 217–24.

TREMAZI, S. A., LOVEGREN, N. V. & FEUGE, R. O. (1968). Characteristics and evaluation of some rapeseed oils. *J.A.O.C.S.*, **42**, 78–81.

Food Chemistry 8 (1982) 233–235

BOOK REVIEWS

Chocolate, Cocoa and Confectionery. (Second edition). By Bernard W. Minifie. Science and Technology. AVI Publishing Co., Connecticut. 1980. x + 735 pp. Price: US$49·50.

The second edition of this valuable text still emphasises technology. References have been brought up to date and in response to many requests the sections on sugar and confectionery have been enlarged.

The book is in three parts. Part I (pp. 1–181) is on cocoa and chocolate. Part II (pp. 182–493) is on confectionery and includes an important section on flavour and flavouring materials, as well as their sensory evaluation. Part III (pp. 494–720) is on general technology, including bloom, pest control, wrapping, quality control, food value and research.

Finally, there is an appendix which includes special instruments, methods, a bibliography and analysis and a supplement on recent development, which contains a synopsis of new equipment.

The book is well illustrated and contains much valuable information for food chemists including sections on cocoa butter, replacement fats, confectionery sugars, etc., but the emphasis throughout is on their practical use in processing rather than their chemistry.

There are also brief mentions of taste and sweetness but, once again, no theory is offered.

This book is a valuable reference work for food scientists and technologists and possibly the best available text in this field.

G. G. BIRCH

The Safety of Foods. (Second edition). Edited by H. D. Graham, AVI Publishing Co., Connecticut, 774 pp., 1980.

The trend towards the consumption of more and more factory-processed foods distributed over ever increasing areas, and the realisation of the potential of such

Food Chemistry (8) (1982)—
Printed in Great Britain

Food Chemistry 8 (1982) 233–235

BOOK REVIEWS

Chocolate, Cocoa and Confectionery. (Second edition). By Bernard W. Minifie. Science and Technology. AVI Publishing Co., Connecticut. 1980. x + 735 pp. Price: US$49·50.

The second edition of this valuable text still emphasises technology. References have been brought up to date and in response to many requests the sections on sugar and confectionery have been enlarged.

The book is in three parts. Part I (pp. 1–181) is on cocoa and chocolate. Part II (pp. 182–493) is on confectionery and includes an important section on flavour and flavouring materials, as well as their sensory evaluation. Part III (pp. 494–720) is on general technology, including bloom, pest control, wrapping, quality control, food value and research.

Finally, there is an appendix which includes special instruments, methods, a bibliography and analysis and a supplement on recent development, which contains a synopsis of new equipment.

The book is well illustrated and contains much valuable information for food chemists including sections on cocoa butter, replacement fats, confectionery sugars, etc., but the emphasis throughout is on their practical use in processing rather than their chemistry.

There are also brief mentions of taste and sweetness but, once again, no theory is offered.

This book is a valuable reference work for food scientists and technologists and possibly the best available text in this field.

G. G. BIRCH

The Safety of Foods. (Second edition). Edited by H. D. Graham, AVI Publishing Co., Connecticut, 774 pp., 1980.

The trend towards the consumption of more and more factory-processed foods distributed over ever increasing areas, and the realisation of the potential of such

Food Chemistry (8) (1982)—
Printed in Great Britain

foods to harm large numbers of people has led to an increased concern with measures to ensure the safety of foods. This book—and the conference that preceded the first edition—is an expression of that concern.

It comprises twenty-four chapters by different authors and covers all aspects of food safety, ranging from microbiological and chemical hazards to irradiation and the role of legislation. As is almost inevitable in a book of this type, the quality and styles of the contributions are variable, but many of the chapters offer particularly extensive and well presented surveys of their topics. Amongst the microbiological chapters, that on *Clostridium botulinum* includes an excellent consideration of the potential hazards and their control in different kinds of foods, and that on *Staphylococcus aureus* is a clear summary of current information. A chapter on the control of food-borne diseases includes a nice integration of the various methods used and leaves the reader with a satisfying overview.

Amongst the chapters dealing with toxic chemicals, those on nitrosamines, mercury, polychlorinated biphenyls and polybrominated biphenyls, toxic plants and toxic marine animals are especially extensive and complete considerations of the hazards they represent.

Other topics covered adequately include salmonellae, viruses, mycotoxins, trace metals, pesticides, antibiotics and irradiation. The final chapters deal with aspects of additives in foods and approaches to the regulation of food safety in North and South America. These are useful in that they clearly illustrate the difficulties and judgements involved in determining safety and setting standards and guidelines.

The book is well produced, except for the rather poor quality of the photographs included and a relatively brief index. Overall, it offers a good introduction to most aspects of food safety, especially in relation to North American practice. It includes a number of informative and well written contributions that will be useful to educators and students of food technology, and many other persons in the food industry would, no doubt, find it helpful to expose themselves to some of the issues that are debated in its pages.

D. OWENS

Advances in Fish Science and Technology. Edited by J. J. Connell, Fishing News Books Ltd., Farnham, Surrey, Great Britain. 528 pp. Price: £39·50.

This book is a collection of the papers—80 in all—presented at the Jubilee Conference of the Torry Research Station, Aberdeen, Scotland, 23–27 July, 1979 and, in common with many books of this type, is a relatively expensive publication. However, unlike many bound collections of papers, the expense is well justified since nowhere else does one find such comprehensive and up to date reviews and research papers presented under one cover. The book is a must for university and college

foods to harm large numbers of people has led to an increased concern with measures to ensure the safety of foods. This book—and the conference that preceded the first edition—is an expression of that concern.

It comprises twenty-four chapters by different authors and covers all aspects of food safety, ranging from microbiological and chemical hazards to irradiation and the role of legislation. As is almost inevitable in a book of this type, the quality and styles of the contributions are variable, but many of the chapters offer particularly extensive and well presented surveys of their topics. Amongst the microbiological chapters, that on *Clostridium botulinum* includes an excellent consideration of the potential hazards and their control in different kinds of foods, and that on *Staphylococcus aureus* is a clear summary of current information. A chapter on the control of food-borne diseases includes a nice integration of the various methods used and leaves the reader with a satisfying overview.

Amongst the chapters dealing with toxic chemicals, those on nitrosamines, mercury, polychlorinated biphenyls and polybrominated biphenyls, toxic plants and toxic marine animals are especially extensive and complete considerations of the hazards they represent.

Other topics covered adequately include salmonellae, viruses, mycotoxins, trace metals, pesticides, antibiotics and irradiation. The final chapters deal with aspects of additives in foods and approaches to the regulation of food safety in North and South America. These are useful in that they clearly illustrate the difficulties and judgements involved in determining safety and setting standards and guidelines.

The book is well produced, except for the rather poor quality of the photographs included and a relatively brief index. Overall, it offers a good introduction to most aspects of food safety, especially in relation to North American practice. It includes a number of informative and well written contributions that will be useful to educators and students of food technology, and many other persons in the food industry would, no doubt, find it helpful to expose themselves to some of the issues that are debated in its pages.

D. OWENS

Advances in Fish Science and Technology. Edited by J. J. Connell, Fishing News Books Ltd., Farnham, Surrey, Great Britain. 528 pp. Price: £39·50.

This book is a collection of the papers—80 in all—presented at the Jubilee Conference of the Torry Research Station, Aberdeen, Scotland, 23–27 July, 1979 and, in common with many books of this type, is a relatively expensive publication. However, unlike many bound collections of papers, the expense is well justified since nowhere else does one find such comprehensive and up to date reviews and research papers presented under one cover. The book is a must for university and college

libraries catering for food science and food technology students and also for the fish processing industry.

The book is divided into two sections. The first reviews the state of the art of fish science and technology, dealing with changes in resources, fish handling, processing, marketing and quality assurance. The biological and nutritional aspects are also discussed and extensive reference sections are given at the end of each paper. Section 2 deals with recent advances in fish science and technology and only when reading these papers does one realise the amount of research work being carried out in this area of the food industry—an area which, according to many people, is dying. The impression, after reading the book, is that the fishing industry is alive and well and adapting rapidly to meet present demands.

Section 2 is divided into fourteen chapters dealing with such products as minced fish and novel krill products such as krill fingers and krill sausage. Chapters on smoked and frozen foods deal with problems and developments in these areas although, as is indicated in the paper concerned with smoked fish, the process today is used for the flavour it produces rather than its preservative effects. Quality assurance and microbiological aspects of fish are discussed and the use of the Torrymeter to assess the freshness of cod fillets is described although, as with most instrumental methods for fish freshness, conclusive results were not obtained. Several papers deal with the utilisation of less well known species of fish and perhaps in the future we may be eating tilapia and mandi with our chips. An important chapter on the topical subject of by-product utilisation in the fishing industry is included and the production of fish meal and protein concentrate from the unlikely source of big eye fish makes interesting reading. Seasonal changes in fish composition are described and eight papers cover the subject of protein studies in fish. Four short chapters on fish technology and its transfer, low molecular weight compounds, water in fish and histology complete the section.

Obviously even to mention every paper in the book would not do each the credit it deserved and I suggest that any interested parties borrow a copy of the book prior to purchase to obtain details of all the papers if they have any doubts as to the quality of the text.

MALCOLM W. KEARSLEY

ANNOUNCEMENT

ENZYMES IN FOOD TECHNOLOGY—INTERNATIONAL SYMPOSIUM

Versailles, France, is the venue for an International Symposium on the use of enzymes in food technology, to be held there from the 5th to the 7th of May, 1982.

Organised by the Centre National de Co-ordination des Etudes et Recherches sur la Nutrition et l'Alimentation (a laboratory of the French National Centre for Scientific Research), under the auspices of the French Ministry of Research and Technology, the symposium will include lectures on 'Enzymes in the Carbohydrate Industry', 'Biosensors for Food Process Control', 'Enzymes in the Protein Food Industry' and 'Enzymes in the Beverage Industry'.

In addition, there will be two 'round table' discussion meetings on the prospects for growth in enzyme use and on regulations governing the use of enzymes.

Further information may be obtained from Mrs Annie Hilaire, General Secretariat, Symposium Enzymes—AFN, 72 rue de Sèvres, 75007 Paris, France.

Food Chemistry (8) (1982)—
Printed in Great Britain

ERRATUM

McLellan, K. M. and Robinson, D. S., 'The Effect of Heat on Cabbage and Brussels Sprout Peroxidase Enzymes'. *Food Chemistry*, Volume 7 (1981), pp. 257–266.

We have been asked to point out that the data in Table 2 on p. 264 should read as follows:

TABLE 2
E_a FOR THE INACTIVATION OF PEROXIDASES
(calculated from Arrhenius plots)

Fraction	*Whole hearted cabbage*	*Spring cabbage*	*Brussels sprout*
	E_a ($kJ\ mole^{-1}$)		
Soluble	166·4	149·7	194·2
Ionically bound	133·1	249·6	212·1
Covalently bound	99·8	—	—

Food Chemistry (8) (1982)—
Printed in Great Britain

Food Chemistry 8 (1982) 239–246

IMMOBILISATION OF AMYLASES ON KRILL CHITIN

JÓZEF SYNOWIECKI, ZDZISŁAW SIKORSKI & MARIAN NACZK

Technical University Politechnika Gdańska,
Institute of Organic and Food Chemistry and Technology, Gdańsk, Poland

(Received: 11 February, 1981)

ABSTRACT

Krill chitin was used as a support for diastase. The enzyme was immobilised by simple adsorption or in the presence of 0·1 % glutaraldehyde. Alternatively, the enzyme was supported on chitin previously activated with glutaraldehyde. The best results were achieved by the first procedure using chitin fractions of 22–52 mesh, obtained by demineralisation of raw dried krill offal with 22% HCl (1:10) for 2 h at room temperature and deproteinisation with 28 % KOH solution for 2 h at 95 °C. Supporting diastase without any treatment with glutaraldehyde did not reduce the enzyme activity. The optimum pH for binding of the diastase on chitin preparations was 6·2 in the presence of glutaraldehyde or 6·7 without the crosslinking agent. Immobilisation shifts the optimum pH for the activity of diastase by 0·5 units towards the acid side.

INTRODUCTION

Several investigators have reported on the utilisation of chitin, isolated from shells of edible shellfish, as support for papain (Finley *et al.*, 1977), lactase, α-chymotrypsin, acid phosphatase (Stanley *et al.*, 1975) and glucose isomerase (Stanley *et al.*, 1976). For fixation of the enzymes on the support usually glutaraldehyde is used, in concentrations ranging from 0·1 to 2 % in the reaction mixture. In most cases supporting of the enzymes on crab chitin results in a negative shift of the optimum pH value for the enzyme activity by 0·7 to 1 pH units. However, binding of α-chymotrypsin is accompanied by a 1·2-unit alkaline shift, which is thought to be caused by the low diffusion rate of the carboxylic groups released during hydrolysis of the protein (Stanley *et al.*, 1975).

Recently there is a growing interest in commercial exploitation of the vast resources of Antarctic krill. Processing of krill to various edible products yields

Food Chemistry 0308-8146/82/0008-0239/$02·75

from 8 to 10 % of offal (Sikorski *et al.*, 1979), which can be a good source of high-quality chitin and chitosan (Brzeski *et al.*, 1980). The use of krill chitin in biochemical reactors could be one of several factors influencing the overall economy of krill fisheries. Chitin obtained from krill offals, contrary to that isolated from fresh water crayfish, was found to have a highly porous surface and therefore could be suitable to bind enzymes strongly by noncovalent bonds (Popowicz, pers. comm.).

MATERIALS AND METHODS

Raw dried krill offal was demineralised by treating with 22 % HCl (1:10) for 2 h at room temperature and deproteinised with KOH solution of different concentrations during 2 h at 95 °C. The raw chitin was further purified by extraction with methanol (1:5), air dried at 80 °C, and separated into fractions of different mesh sizes. For comparative purposes also, Fluka chitin and Kyowa chitosan were used. Characteristics of the supports are given in Table 1.

Riedel diastase used in the experiments had maximum activity at 50–52 °C and pH 5·7. The results of thermal inactivation and of inhibitory action of Cu^{2+} salts indicate that the enzyme preparation did not exhibit other amylolytic activity except that of β-amylase. The same can also be concluded when the optimum pH and temperature of the enzyme are compared with those of published data.

The enzyme was supported by adsorption on 50 mg of the chitin preparation during 1 h at 25 °C with intermittent mixing, followed by 20 h of standing at 4 °C, and by washing of the free diastase with 800 cm^3 of distilled water. Covalent binding of the enzyme was accomplished in the presence of 0·1 % and 0·3 % glutaraldehyde

TABLE 1
THE CHARACTERISTICS OF SUPPORTS USED IN THE EXPERIMENTS[a] (FRACTION 22–52 MESH)

Support	*Concentration of KOH solution* %	*Chemical characteristics*			*Activity of bound diastase (rate of reaction mg/min)*
		Ash (%)	*Nitrogen*[b] (%)	*Treatment of available NH_2 groups* (μM/g *chitin*)	
Krill chitin	0·5	0·08	6·39	211	1·62
	3·0	0·06	6·27	190	1·64
	10·0	0·04	6·17	232	1·90
	28·0	0·05	6·23	408	2·18
Commercial chitin (Fluka)	—	3·62	7·73	—	0·19
Chitosan	—	0·30	—	—	1·36

[a] Mean values of results obtained in 4–6 experiments. The coefficient of variation was ≤ 5 %.
[b] For demineralised dry chitin.

in the mixture, all other parameters being equal. Alternatively, the diastase was supported on chitin previously activated with glutaraldehyde (50 mg of chitin in 5 cm^3 2% glutaraldehyde and 0·5 cm^3 concentrated acetic acid for 24 h at room temperature).

The quantity of the enzyme bound to the support was assayed by determining the amount of starch hydrolysed by the diastase supported on 50 mg of chitin, measured according to Heinkel (1956); however, the quantity of the reagents was five times greater than in the original procedure. The quantity of hydrolysed starch (S) was calculated using the formula:

$$S = \frac{A_0 - A}{A_0} 100\%$$

where A_0 and A is the extinction of the starch solution prior to and after hydrolysis, respectively. For comparative purposes the activity of the immobilised enzyme was expressed as the degree of hydrolysis of starch or as the rate of reaction.

RESULTS AND DISCUSSION

The binding of the enzymes

The most active preparations were obtained by simple adsorption of the enzyme on krill chitin deproteinised by 28% KOH solution (Table 1). Glutaraldehyde brings about a decrease in activity of the bound diastase, both when used for pretreating of the support or for crosslinking of the enzyme (Table 2). The activity of diastase fixed with glutaraldehyde depends upon the concentration and time of action of the crosslinking agent and on the concentration of the enzyme (Table 3). About 39 to 46% of the initial amount of diastase can be supported on chitin by simple adsorption without any significant loss in activity (Table 4). The yield of bound diastase depends upon the quality of the support, i.e. the molecular weight of

TABLE 2
COMPARISON OF THE ACTIVITY OF DIASTASE IMMOBILISED ON CHITIN BY DIFFERENT TREATMENTS[a]

Treatment	*The relative activity of immobilised diastase* (%)
Adsorption	100
Adsorption and binding by glutaraldehyde	56·9
Adsorption after pretreatment of chitin with glutaraldehyde	53·8

[a] Mean values of results obtained in six experiments. The coefficient of variation was ≤5%.

TABLE 3
INFLUENCE OF THE CONCENTRATION OF DIASTASE AND GLUTARALDEHYDE IN THE REACTION MIXTURE ON THE ACTIVITY OF THE BOUND ENZYME

Concentration of glutaraldehyde in the reaction mixture (%)	*Concentration of diastase in the reaction mixture* (% *of the weight of chitin*)		
	20	*30*	*40*
	Activity of bound diastase (%)		
0	39	56	73
0·1	31	34	41
0·3	20	21	31

[a] Mean values of results obtained in six experiments. The coefficient of variation was ≤5%.

the polymer as well as the content of minerals and residual proteins, which in turn depend on parameters of demineralisation and deproteinisation of the krill offals.

The increase in the activity of amylase adsorbed on chitin preparations, obtained by demineralisation of the offal with 22% HCl and deproteinisation with more concentrated KOH solutions, suggests that for effective binding of the enzyme the availability of deacetylated amino groups of chitin is necessary. The best results were obtained on chitin fractions of 22–52 mesh (Fig. 1).

The optimum range of pH for binding of the enzyme on chitin preparations is 6·2 in the presence of glutaraldehyde or 6·7 without the crosslinking agent (Fig. 2). The pH value, 6·2, is close to that of the isoelectric point, reported for different preparations of diastase. It is possible that isoelectric aggregation is partially responsible for the increased binding of the enzymes on chitin at this pH. However at pH 6·7 ionic attraction phenomena seem to be additionally responsible for binding of the enzyme, At the optimum pH value most efficient immobilisation of diastase was accomplished at very low ionic strength (Fig. 3). At optimum pH and ionic strength the efficiency of binding or activity of the preparations decreased linearly with increasing temperature of the mixture (Fig. 4). After 1 h at 25 °C and a

TABLE 4
DISTRIBUTION OF DIASTASE BETWEEN THE SUPPORT AND SOLUTION

	The quantity of enzyme (mg)[a]		
1	*2*	*3*	*4*
Initial in the mixture	*Immobilised on 50 mg of chitin without treatment with glutaraldehyde*	*Residual in the solution*	$\sum(2+3)$
5	2·3	3·3	5·6
10	4·1	5·3	9·4
15	6·0	8·9	14·9
20	7·9	12·8	20·7

[a] Mean values of results obtained in six experiments. The coefficient of variation was ≤8%.

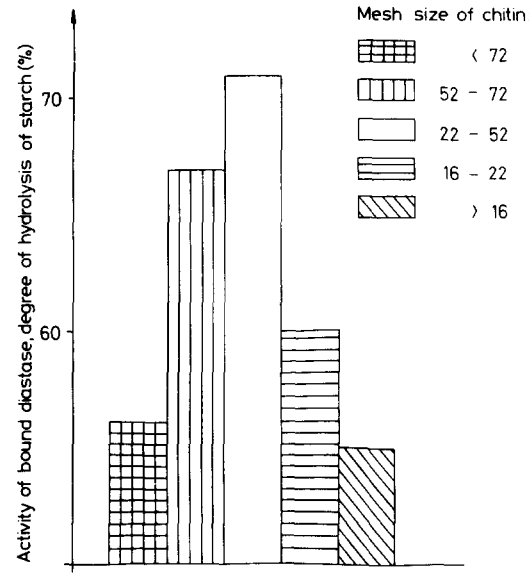


Fig. 1. Activity of diastase preparations adsorbed on chitin as influenced by the mesh size of the support (pH = 6·7, $T = 25\,°C$, ionic strength = 0·05).

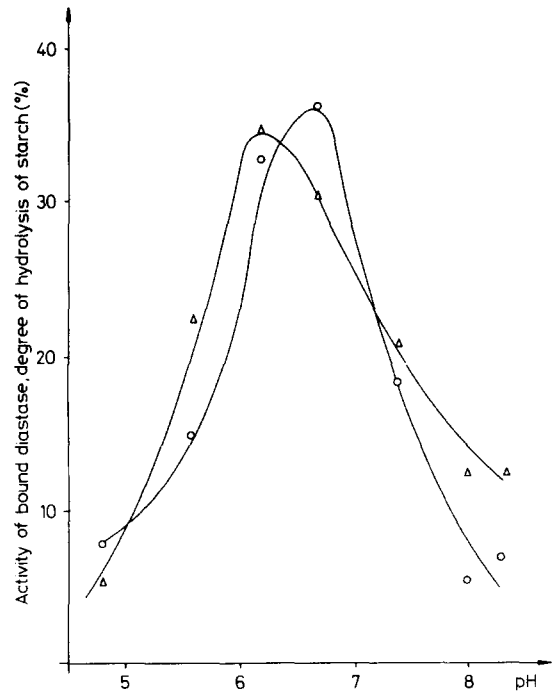


Fig. 2. Activity of diastase immobilised on chitin at different pH values ($T = 25\,°C$). Immobilisation by adsorption (○—○), adsorption in the presence of 0·1 % glutaraldehyde (△—△).

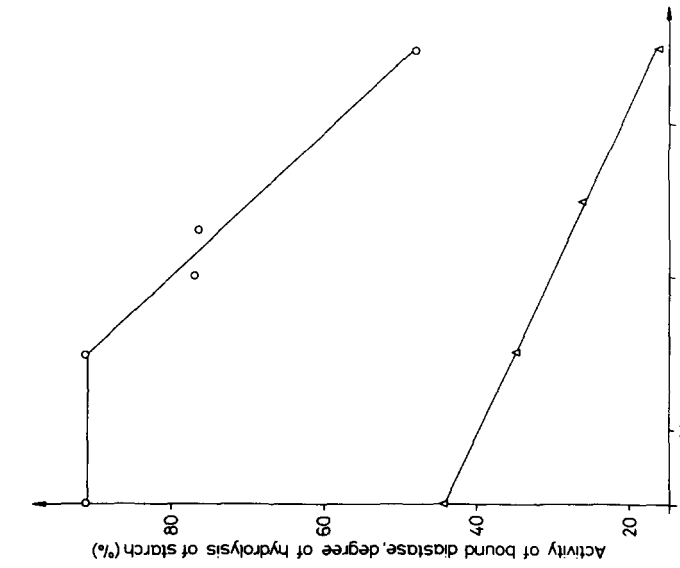


Fig. 4. Activity of diastase immobilised on chitin at different temperatures (optimum pH for enzyme binding = 6·2 or 6·7, ionic strength = 0·05). Immobilisation by adsorption (○—○), adsorption in the presence of 0·1 % glutaraldehyde (△—△).

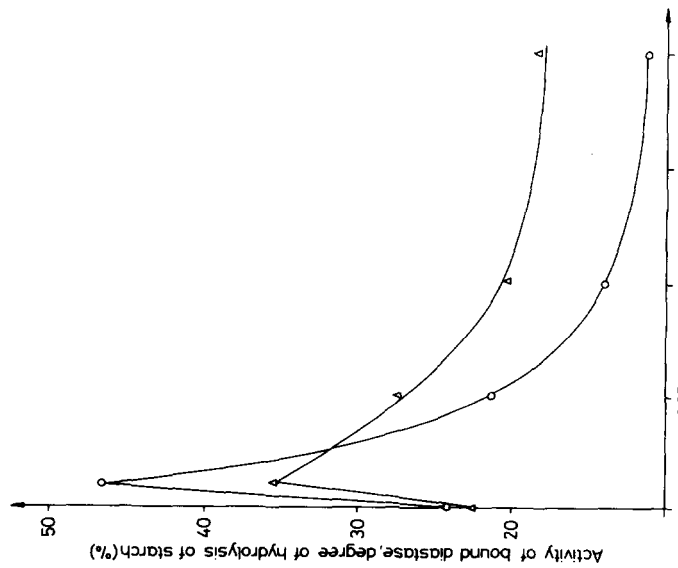


Fig. 3. Activity of diastase immobilised on chitin at different ionic strengths (optimum pH enzyme binding = 6·2 or 6·7, $T = 25\,°C$). Immobilisation by adsorption (○—○), adsorption in the presence of 0·1 % glutaraldehyde (△—△).

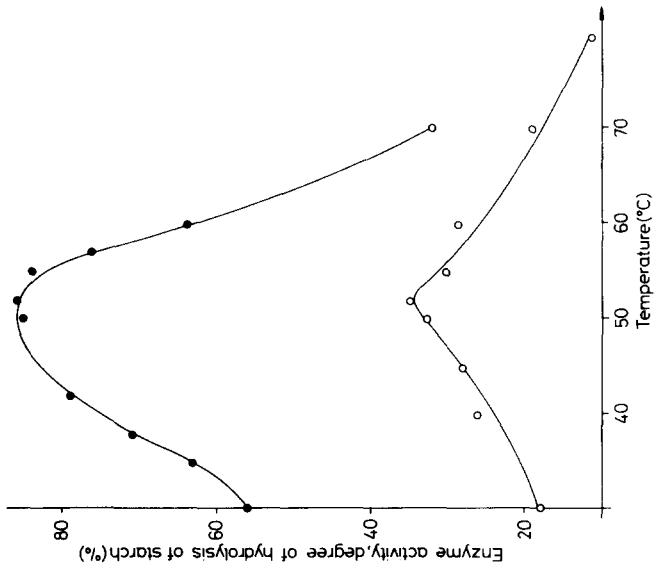


Fig. 6. The influence of temperature on the activity of native diastase (●—●) and diastase immobolised on chitin (○—○).

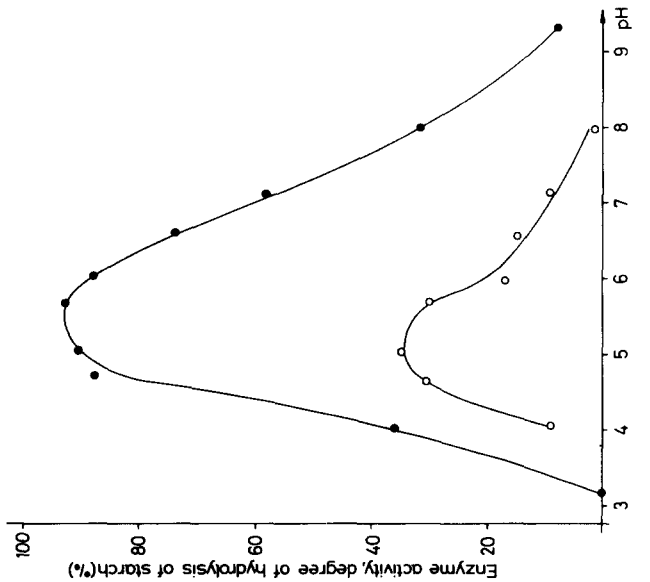


Fig. 5. The influence of pH on the activity of native diastase (●—●) and diastase immobilised on chitin (○—○).

pH of about 7 the activity of the enzyme supported by adsorption was 60 % and that fixed with glutaraldehyde was 36 %. After an additional holding time of 20 h at 4 °C the activity increased only by about 4 %.

Properties of the immobilised enzyme

Immobilisation on chitin brings about a shift of the optimum pH for the activity of diastase towards the acid side by about 0·5 units (Fig. 5). A similar effect of binding on chitin has been reported in the case of glucose isomerase (Stanley *et al.*, 1976) and acid phosphatase (Stanley *et al.*, 1975).

There is no change in the optimum temperature for the activity of the enzyme, although the bound diastase is slightly more heat resistant (Fig. 6).

The activity of diastase supported on krill chitin was three to five times greater than that supported on samples of Fluka chitin and Kyowa chitosan of the same mesh size supplied by the producers. Further treatment of the Fluka preparation with HCl and NaOH, identical to that applied for preparing the krill chitin, did not improve the result of enzyme binding.

ACKNOWLEDGEMENTS

The authors acknowledge with thanks the financial support obtained under the research grant PR-Kryl administered by the Sea Fisheries Institute in Gdynia. The authors also express their thanks to Kyowa Oil and Fat Co. Ltd for supplying free samples of chitosan.

REFERENCES

BRZESKI, M., MIECZKOWSKA, M., STOLZ, H., WOJTASZ, A., SOWA, K. & NEUGEBAUER, W. (1980). Technologia otrzymywania chityny i chitosanu. Paper presented at the *Seminar on Chitin and Chitosan*. Sea Fisheries Institute, Gdynia, January.

FINLEY, J. W., STANLEY, W. L. & WATTERS, G. G. (1977). Removal of chill haze from beer with papain immobilized on chitin. *Biotechnol. Bioeng.*, **19**, 1895.

HEINKEL, K. (1956). Uber die Vergleichbarkeit erschiedener-Bestimmungsmethoden der Blutdiastasen. *Klinische Wochenschrift*, **34**, 155.

SIKORSKI, Z. E., BYKOWSKI, P. & KNYSZEWSKI, J. (1980). The utilization of krill for food. In: *Food Process Engineering, Vol. 1. Food Processing Systems*. (Linho, P. *et al.* (Eds)), Applied Science Publishers, London, pp. 845–55.

STANLEY, W. L., WATTERS, G. G., CHAN, B. & MERCER, J. M. (1975). Lactase and other enzymes bound to chitin with glutaraldehyde. *Biotechnol. Bioeng.*, **17**, 315.

STANLEY, W. L., WATTERS, G. G., KELLY, S. H., CHAN, B. G., GARIBALDI, J. A. & SCHADE, J. E. (1976). Immobilization of glucose isomerase on chitin with glutaraldehyde and by simple adsorption. *Biotechnol. Bioeng.*, **18**, 439.

Food Chemistry 8 (1982) 247–251

LEVELS OF SOME TRACE ELEMENTS IN HOSPITAL DIETS

I. O. AKINYELE & O. OSIBANJO

Department of Human Nutrition and Department of Chemistry, University of Ibadan, Ibadan, Nigeria

(Received: 24 September, 1981)

ABSTRACT

Composite diets served to patients at the University College Hospital, Ibadan were collected and analysed for their concentration of zinc, copper, iron, manganese and cadmium. Data collected indicate that the concentration of zinc for breakfast foods was highest in bread and fried egg and lowest in bread alone. Copper was highest in yam and corned beef stew but not detected in any of the bread-containing breakfast foods. Iron and manganese were highest in hot pap and moinmoin and lowest in yam, stew and meat.

For lunch and dinner diets, the concentration of zinc was highest in dodo, stew and meat and lowest in dodo alone; copper was highest in eba, melon soup and meat and was non-detectable in jollof rice and meat, pounded yam, melon soup and meat. Iron was highest in amala, melon soup and meat and lowest in dodo alone. Manganese was highest in agidi, vegetable soup and fish and was non-detectable in pounded yam, okro soup and fish. The levels found appear sufficient to meet daily dietary requirements.

INTRODUCTION

Considerable variations exist in the composition and nutritional value of foods due to genetic and climatic conditions, cooking and other processing operations to which the food materials have been subjected before consumption. It is therefore essential to know the nutritive value of foods if established dietary standards are to be translated into applicable practical information for the qualitative and quantitative selection of the diet. Analytical data in nutrition are used to establish the essentiality and requirement of a nutrient, identify adequate, sub-adequate or marginal intakes in selected populations and prevent non-optimal intakes (Mertz, 1974).

Food Chemistry 0308-8146/82/0008-0247/$02·75

In recent times, there has been considerable interest in the levels of trace metallic elements in foods, possibly because of the deleterious effects on human health caused by the contamination of foods with trace amounts of toxic metals such as lead and cadmium, and also to ascertain the beneficial and nutritional requirements of those elements known to be essential to human or animal life. Though various food composition tables are available for use in developing countries, most of them are incomplete and only deal with the composition of raw uncooked items and contain only scattered information on the trace element content. Moreover, there is a dearth of information on the composition of cooked mixed Nigerian diets as served traditionally in homes and hospitals.

Due to the known relationship between trace metals and disease (Prasad *et al.*, 1961; 1963), this study was carried out to provide data on the trace metals composition of diets served to patients in a hospital. The University College Hospital (UCH) situated in Ibadan was chosen as the study centre because of the recognition by the hospital that balanced diets served to patients in the wards play a significant role in the rapid recovery from various ailments and diseases.

MATERIALS AND METHODS

Food samples from UCH wards were collected daily in quantities identical to those served to the patients in the hospital. The samples were weighed to the nearest gramme and then transferred from the serving plates into clean, weighed, plastic bags for transportation to our laboratory. The sampling lasted a period of four weeks so as to cover the various combinations of foods served in the hospital at breakfast, lunch and dinner times.

Each diet sample collected was thoroughly mixed in a quartz mortar with a pestle. After mixing, the paste formed was transferred to clean, dry plastic plates and dried in a moisture extraction oven at about 80 °C for 4 h. The temperature was later increased to 105 °C until the sample was dried to constant weight. The dried sample was mixed with a clean dry quartz pestle before storage in polythene bags. About 2-g sample aliquots from each dried food sample were accurately weighed into clean, dry silica crucibles and redried at 104 °C in the oven followed by dry-ashing in a muffle furnace at 450 °C for 16 h. The residual white ash obtained was dissolved in about 2 cm^3 of concentrated nitric acid and the solution diluted with de-ionised water. Two cubic centimetres of 5 % lanthanum chloride solution was added (as a releasing agent to circumvent cation–anion interference effects) and the solution transferred to a 250 cm^3 volumetric flask and made up to the mark with deionised water.

Stock standard solutions for zinc, copper, manganese, iron and cadmium for the calibration curves were prepared from Analar salts of the metals dissolved in deionised water in 250 cm^3 volumetric flasks followed by the addition of 2 cm^3 of

TABLE 1
SOME TRACE MINERAL CONSTITUENTS OF UCH BREAKFAST DIETS

Item	*Zn*	*Cu*	*Fe*	*Mn*	*Cd*
	(mg/100 g dry matter)				
Bread and fried egg	1·73	nd	3·15	0·43	0·09
Bread, stew and sardine	1·06	0·62	2·55	0·50	0·15
Bread alone	1·00	nd	3·00	0·38	0·05
Yam and corned beef stew	1·35	1·22	2·00	nd	nd
Hot pap[a] and moinmoin[b]	1·63	0·53	3·98	0·66	nd
Bread and boiled egg	1·24	nd	3·06	0·47	0·12
Yam, stew and meat	1·26	0·43	2·00	nd	nd

[a] This is derived by mixing hot water with soluble starches from maize obtained by fermentation.
[b] This is made from cowpea flour mixed with oil and various condiments.
nd, Not detectable.

concentrated nitric acid and 2 cm^3 of 5 % lanthanum chloride solution. The atomic absorption measurements were taken on the Perkin-Elmer Atomic Absorption Spectrophotometer, Model 305B using the appropriate hollow cathode lamps.

RESULTS AND DISCUSSION

The results of the trace metal analysis for foods commonly served at breakfast time are presented in Table 1 whilst those for lunch and dinner diets are shown in Table 2. These results indicate that the breakfast diets are good sources of iron but moderate sources of zinc, and poor sources of copper and manganese. Copper was not detected in four of the dietary samples whilst manganese was not detectable in two

TABLE 2
SOME TRACE MINERAL CONSTITUENTS OF UCH LUNCH AND DINNER DIETS

Item	*Zn*	*Cu*	*Fe*	*Mn*	*Cd*
	(mg/100 g dry matter)				
Amala,[a] melon soup and meat	2·09	0·08	8·40	0·26	0·13
Dodo,[b] stew and fish	1·08	1·31	2·16	0·49	nd
Dodo,[b] stew and meat	2·43	0·52	3·00	0·52	0·05
Pounded yam, okro soup and fish	1·61	0·38	3·00	nd	0·03
Eba,[c] melon soup and meat	1·40	0·58	3·85	0·48	nd
Agidi,[d] vegetable soup and fish	2·28	0·51	3·00	0·76	0·05
Mashed beans	2·30	0·39	5·15	0·91	0·14
Jollof rice and meat	1·31	nd	2·00	0·50	0·29
Pounded yam, melon soup and meat	1·53	nd	2·79	0·59	0·09
Dodo,[b] alone	0·95	0·40	1·00	0·47	0·02

[a] Dried yam flour mixed with hot water into a paste.
[b] Ripe plantain fried in red palm oil.
[c] Cassava flour mixed with hot water into a paste.
[d] Maize starch obtained through fermentation mixed with hot water and allowed to cool.
nd, Not detectable.

of the samples. Cadmium was found to be present at ultra-trace levels in all the samples. This is not surprising since cadmium is a toxic metal which can only be present in foods in large quantities due to contamination from various sources during processing or cooking.

Hot pap and moinmoin contained the highest amount of iron while yam, stew and meat or corned beef contained the least (Table 1). Zinc was found to be highest in bread and fried egg and least in bread alone. It is pertinent to note that frying or boiling of the egg affected the total dietary zinc content. In addition, hot pap and moinmoin served in the hospital contained high amounts of zinc, copper, iron and manganese when compared to other breakfast foods. Moinmoin is normally made from beans, a good source of iron (see mashed beans, Table 2), enriched with oil, sauces, meat, eggs and fish.

The lunch and dinner diets indicated that copper was not detectable in jollof rice and meat and in pounded yam, melon soup and meat. Manganese was also not detectable in pounded yam, okro soup and fish. Cadmium was not detected in dodo, stew and meat, and in eba, melon soup and meat (Table 2). Iron was highest in amala, melon soup and meat and least in dodo. The zinc content of dodo, stew and meat was significantly higher than dodo alone. Except for dodo stew and fish, there was little variation in the copper content of lunch and dinner diets. The copper content of the amala diet was considerably lower than the other diets. Nonetheless, the amala, melon soup and meat diet is a good source of iron and a moderate source of zinc. The manganese content of these diets followed no particular trend except that all the diets are poor sources of the element (Table 2).

The importance of zinc, copper, iron and manganese in human nutrition has long been recognised. The amount of zinc necessary in the diet appears to be related to its availability and evidence by O'Dell *et al.* (1972) suggests that zinc is more readily available from animal products than diets of plant origin. This is due primarily to the phytate and fibre content of the latter diets which inhibit the intestinal absorption of zinc by forming insoluble chelates. The greater portion of the diets analysed in this study are of plant origin and the animal products like fish and meat included in the diets constitute small portions. It is therefore reasonable to investigate the availability of zinc in these diets.

Aside from its function as a biocatalyst, copper is essential in the maintenance of a healthy central nervous system, pigmentation, prevention of anaemia and is interrelated with the functions of zinc and iron. A typical adult man has been shown to consume between 2 to 5 mg of copper daily (O'Dell, 1976). This daily intake of copper could easily be met by the population consuming the diets analysed in this study.

Iron assumes a significant importance in the diet because of its role in haemoglobin formation. Deficiency of dietary iron therefore leads to anaemia especially in children and pregnant women. The most frequent cause of iron deficiency in the population is that physiological requirements for iron exceed iron

absorption capabilities. While most of the dietary sources analysed contain substantial amounts of iron, their availability is questionable. It is therefore not known whether the iron present in these diets would be sufficient to prevent a deficiency condition in Nigeria without resorting to the provision of supplementary iron.

Data from several sources suggest that manganese intakes of 0·035–0·070 mg per kilogramme body weight per day would result in a balance of manganese intake with excretion. The results from this study show that manganese deficiency would not occur in patients consuming most of the diets studied. Recently reported concentrations of cadmium in foods in the USA (Mansko & Corneliussen, 1974) show that dairy products, potatoes, leafy vegetables, grain and cereals contain fairly high amounts. Variations were also reported geographically. This shows that a possible source of cadmium in foods is the soil in which these foods are grown. Based on our results, the intake of cadmium by the patients in UCH is considerably lower than that reported for the United States or Canada. In addition, the level of industrialisation is very low in Nigeria, hence the chance of contamination of agricultural land with industrial wastes containing cadmium is remote.

CONCLUSIONS

Diets consumed by patients at the UCH have been shown by analysis to be good sources of iron and zinc and moderate sources of copper and manganese. Cadmium, a toxic metal, was present at less than trace levels.

ACKNOWLEDGEMENT

The authors are grateful to the UCH, the Departments of Human Nutrition and Chemistry, University of Ibadan for providing the necessary facilities for this research.

REFERENCES

MANSKO, D. D. & CORNELIUSSEN, P. E. (1974). Pesticide residues in total diet samples (VII). *J. Pestic. Monit.*, **8**, 110–24.

MERTZ, W. (1974). Trace element review. *Proceedings of the Western Hemisphere Nutrition Congress* IV, pp. 141–7.

O'DELL, B. L. (1976). Biochemistry and physiology of copper in vertebrates. In: *Trace elements in human health and disease*. (Prasad, A. S. (Ed.)), Academic Press, N.Y., pp. 391–413.

O'DELL, B. L., DURPO, C. E. & SAVAGE, J. E. (1972). Evaluation of zinc availability in foodstuffs of plant and animal origin. *J. Nutr.*, **102**, 653–60.

PRASAD, A. S., HALSTEAD, J. A. & NADIMI, M. (1961). Syndrome of iron deficiency anaemia, hepatosplenomegaly hypogonadism, dwarfism and geographia. *Amer. J. Med.*, **31**, 532–6.

PRASAD, A. S., MIALE, A., FARID, Z., SAMSTEAD, H. N., SCHULET, A. R. & DARBY, W. J. (1963). Biochemical studies on dwarfism, hypogonadism and anaemia. *Arch. Intern. Med.*, **111**, 407–28.

Food Chemistry 8 (1982) 253–262

EFFECT OF GERMINATION ON THE NITROGENOUS CONSTITUENTS, PROTEIN FRACTIONS, *IN VITRO* DIGESTIBILITY AND ANTINUTRITIONAL FACTORS OF FENUGREEK SEEDS (*TRIGONELLA FOENUM GRAECUM* L.)

A. RAFIK EL-MAHDY

Food Science Department, Faculty of Agriculture, University of Alexandria, Alexandria, Egypt

&

L. A. EL-SEBAIY

Food Technology Department, Kafr El-Sheikh, Faculty of Agriculture, University of Tanta, Egypt

(Received: 16 February, 1981)

ABSTRACT

This study was designed to determine the effect of germination on nitrogenous constituents, protein fractions, in vitro *digestibility and antinutritional factors (namely trypsin inhibitor and haemagglutinin) of the Egyptian fenugreek seeds Geiza 2 variety. After 96 h of germination, there was 18% decrease in the dry weight of seeds, a slight increase of total nitrogen (TN), a decrease of protein nitrogen (PN) and a marked increase of both non-protein nitrogen (NPN) and free amino acid nitrogen (FAAN). Non-protein nitrogen other than FAAN, amido nitrogen and ammonia nitrogen also increased. The protein fractions (namely albumin, globulin, prolamin and glutelin) were separated according to their solubilities in different solvents. The ratio between the four protein fractions in ungerminated seeds was 4:3·5:2·8:1 and became 2:6·5:7·7:1 after germination as calculated on the basis of their PN.*

Trypsin inhibitor activity per gramme of fenugreek was found to be higher by 66% in germinated fenugreek than ungerminated seeds. Both ungerminated and germinated fenugreek was found devoid of the haemagglutinin activity.

Germination resulted in a slight increase in pancreatic digestibility, 33·7% decrease in digestibility by pepsin followed by pancreatin, while a small decrease was found in peptic digestibility.

Food Chemistry 0308-8146/82/0008-0253/$02·75

INTRODUCTION

The fenugreek (*Trigonella foenum graecum* L.) is an annual herbaceous plant of the leguminous family, widespread in certain countries of the Mediterranean basin and Asia (Rosengarten, 1969). The seeds are rich in protein which ranges from 20 to 30 % with an average of about 27 % (Kolousek & Coulson, 1955; Shankarcharya & Natarajan, 1973; El-Madfa, 1975).

The first to study the protein in the fenugreek was Wunschendorff (1919). He determined the value of 27 % of protein in the seed and separated the different protein fractions (globulins, albumins, nucleoproteins) of which the amino acid compositions were partially established. Hassan & Basha (1932) isolated a prolamin soluble in boiling 70 % alcohol.

No available information concerning the presence of trypsin inhibitor, haemagglutinin, *in vitro* digestibility of fenugreek and the effect of germination exists in the literature. On the other hand there are copious data concerning these subjects in other pulses.

During germination of legumes, the proteins are hydrolysed with increased proteolytic activity (Beevers, 1968). Previous studies have indicated that germination of black kidney beans (Pusztai & Duncan, 1971; Palmer *et al.*, 1973) and other pulses (Desikachar, 1947; Evenson *et al.*, 1964) results in improved digestibility, although contrary findings have been published for common beans and peas (Chattapadhgay & Bannerjee, 1953). The view that trypsin inhibitor activity is largely responsible for the low digestibility of black kidney beans has been challenged by Palmer *et al.* (1973), who observed an increase in trypsin inhibitor content accompanied by an increase in digestibility.

El-Hag *et al.* (1978), found an apparent relationship between the improved digestibility of red kidney bean after sprouting and the decline in trypsin inhibitor activity. Whether such changes occur in germinated fenugreek seed, and affect *in vitro* digestibility is not established. Thus, this work is aimed to study the effect of germination on the different nitrogenous constituents, protein fractions, *in vitro* digestibility and trypsin inhibitor and haemagglutinin activities of fenugreek seeds.

MATERIALS AND METHODS

Materials

Seed type: The Fenugreek seeds Geiza 2 variety were purchased from an Egyptian market.

Germination procedure: The seeds were sorted, cleaned, washed and dried in an air draught oven at 40 °C. The seeds were surface-sterilised by soaking in alcohol for 1 min, washed with tap water, then soaked in five volumes of water for 6 h at room

temperature (30–35 °C). The water was then removed and the germination begun on a screen in the dark for 96 h. The seeds were washed twice every 12 h with tap water. The dry weight of seedlings at the end of the germination period was determined. The seedlings were dried overnight in a flowing air oven at 40 °C. Germination for antitrypsin and haemagglutinin activities was done by the same method using 1 g of seeds. After germination the sprouts were taken fresh for the assay of antitrypsin and haemagglutinin activities.

Preparation of samples for analysis: Ungerminated and germinated dried seeds were ground in a laboratory Willey mill to pass through a 40 mesh sieve. The ground samples were stored at 5 °C until analysed.

Methods

Samples were analysed for dry weight by drying at 115 °C to constant weight.

Moisture and total nitrogen (TN) were determined by the methods of the Association of Official Agricultural Chemists (1975). The micro-Kjeldahl method was used for the TN estimation and the crude protein was calculated by multiplying total nitrogen by the factor 6·25.

Total non-protein nitrogen (TNPN) was determined by the method of Becker *et al.* (1940), 1 g of the sample was extracted with 0·8 M trichloroacetic acid and the filtrates were analysed for non-protein nitrogen by the micro-Kjeldahl method.

Free amino acid nitrogen (FAAN) was determined by the modified colorimetric method of Rosen (1957). Protein nitrogen (PN) was calculated as follows: TN – TNPN = PN.

Non-protein nitrogen other than FAAN was also obtained by calculation (TNPN – FAAN). Amido nitrogen was calculated after the determination of albuminoid nitrogen as described by the methods of the Association of Official Agricultural Chemists (1960) (percentage TN – percentage albuminoid nitrogen).

Protein fractions were determined by the method of McGuire & Earle (1958) with some modifications. One gramme of the dry and lipid-free sample was extracted with 100 ml portions of each of the following solvents: distilled H_2O, lactic acid of 4 % concentration, ethanol of 70 % concentration and finally 0·01 N potassium hydroxide. The suspension was shaken using a Gallenkamp flask shaker for 1 h at room temperature (30–32 °C). The suspension was centrifuged at 3000 rpm for 10 min, then the residue was extracted three times each with 40 ml portions of the same solvent and the supernatants were combined with the original extract. The total soluble nitrogen, NPN and FAAN were determined in the supernatants as described before. The results were expressed as a percentage of either the total nitrogen or the protein nitrogen in the sample.

The digestibility of fenugreek proteins was determined by using pepsin, pancreatin, and pepsin followed by pancreatin. The digests were prepared as described by Akeson & Stahman (1964). Casein was used as a reference protein and was exposed to similar treatments. The digestibility of protein was expressed as a

percentage of trichloroacetic acid soluble nitrogen referred to the total nitrogen and to the protein nitrogen content of the samples.

The trypsin inhibitor activity of fenugreek extracts was determined by the caseinolytic procedure described by Kunitz (1947). The trichloroacetic acid soluble digestion products were analysed by the method of Lowry *et al.* (1951). The fenugreek extracts were prepared by the homogenisation of a 1 g sample in 50 ml 0·1 M phosphate buffer (pH 7·6) for 3 min and centrifugation at 4000 rpm for 20 min. One millilitre of extract was diluted to 50 ml with phosphate buffer (pH 7·6) for protein estimation. The trypsin inhibitor unit (TIU) was expressed in terms of the tryptic units inhibited either per milligramme of inhibitor's protein or per gramme of sample (Kunitz, 1947). The tryptic unit (TU) was defined as the amount of enzyme which produces in 1 min, at the optimum conditions, products of digestion which contain one micromole of tyrosine not precipitable by trichloroacetic acid (Greenberg, 1955).

The haemagglutinin activities of the samples were determined by the method of Liener & Hill (1953) using a suspension of red blood cells from human whole blood type O (Chen *et al.*, 1977).

RESULTS AND DISCUSSION

Nitrogenous constituents

The moisture content of fenugreek seeds was found to be 10·9 %, while the moisture content of germinated fenugreek was 80·0 %. Germination of fenugreek seeds for 96 h decreased the dry weight by 18 % of the original ungerminated seeds.

The results illustrated in Table 1 show the different nitrogenous constituents of ungerminated and germinated fenugreek. It is clear that about 21 % of the total

TABLE 1

DIFFERENT NITROGENOUS CONSTITUENTS[a] OF UNGERMINATED AND GERMINATED FENUGREEK SEEDS

Nitrogenous constituents % *(on dry weight basis)*[b]	*Ungerminated seeds*	*Germinated seeds*
Total nitrogen	3·92	4·74
Total non-protein nitrogen	0·82	2·49
Protein nitrogen	3·11	2·26
Albuminoid nitrogen	2·80	2·90
Amido nitrogen	1·12	1·84
Free amino acids nitrogen	0·50	1·99
Ammonia nitrogen	0·02	0·07
Non-protein nitrogen other than free amino acid nitrogen	0·31	0·50
Crude protein (N × 6·25)	24·5	29·6

[a] Mean of three determinations.

[b] The dry matter contents of ungerminated and germinated seeds were 89·1 % and 20 %, respectively, of the wet weight.

nitrogen of ungerminated fenugreek was found in the form of NPN. When the total nitrogen of ungerminated fenugreek was compared with that of germinated seeds, there was an apparent increase amounting to 20·9 % which is attributed to the loss in dry weight of germinated seeds. On the other hand there was a decrease of PN amounting to 27 % of the original. A noteworthy observation is that the decrease in PN content (0·85 g/100 g) is approximately equal to the increase in both amido nitrogen and NPN other than FAAN (0·91 g/100 g). This may be the final result of the digestion of proteins, by the action of enzymes on amino acids, and the storage of a proportion of the ammonia nitrogen in the form of amido nitrogen. After germination FAAN increased by 298 % of the original amount. TNPN showed a high increase amounting to 204 % after germination. This increase was the result of the increase in the FAAN; the latter contributed 80 % of TNPN for germinated fenugreek. This increase is mainly due to the proteolytic action of enzymes which break down the protein molecules into simpler units of amino acids. There was also an increase in NPN other than FAAN which is equal to 38 % of the original amount in the ungerminated seeds.

The aforementioned results accord with those found in the literature and reported by Chen & Thacker (1978). They found that there was a net increase of the FAAN at the fifth day of pea seeds germination. Various investigators have shown that the reserve proteins of seeds are hydrolysed to their constituent amino acids before they are used for the formation of proteins of newly developing parts (Huber & Zalik, 1963; Ingle *et al.*, 1964; Deshumshi & Sohonie, 1966; Palmiano & Juliano, 1972; Hegazi, 1974). There is not complete utilisation of the free amino acids liberated after germination, however, since there is a net increase of the FAAN.

Protein fractions

The seed proteins could be separated for the purposes of crude classification by successive use of the following solvents: water for albumin, lactic acid for globulin, ethyl alcohol for prolamin and dilute alkali for glutelin (Altschual *et al.*, 1966). As shown in Table 2, albumin amounted to 48·5 % of the TN of ungerminated seeds, globulin came next as it represented 27·1 %, then glutelin at 18 %. Prolamin was found to be the lowest fraction, all of its total nitrogen being of protein nature.

A more realistic picture may be obtained when the ratio between the four protein fractions of germinated and ungerminated fenugreek was calculated on the basis of their PN. The ratio between albumin, globulin, glutelin and prolamin of ungerminated seeds was found to be 4:3·5:2·8:1 and became 2:6·5:7·7:1 after germination.

The results showed that the germination process lowered the albumin, globulin, and prolamin fractions by 80, 24 and 59 % respectively of their original values found in the ungerminated seeds. The drop in the PN of the albumin fraction was parallel to an increase in the FAAN. It is clear that after 96 h of germination 54 % of the fenugreek protein fractions were hydrolysed to non-protein nitrogen and the FAAN contributed 80 % of the latter. On the other hand the glutelin fraction increased by

TABLE 2
NITROGENOUS CONSTITUENTS OF DIFFERENT PROTEIN FRACTIONS OF UNGERMINATED AND GERMINATED FENUGREEK SEEDS[a]

Nitrogenous constituent per fraction	*Protein fractions*							
	Albumin		*Globulin*		*Prolamin*		*Glutelin*	
	UGS[b]	*GS*[c]	*UGS*	*GS*	*UGS*	*GS*	*UGS*	*GS*
Total nitrogen[d]	48·5	50·5	27·1	21·4	6·32	2·67	18·1	22·5
Total non-protein nitrogen[d]	23·5	45·4	4·82	4·6	0·00	0·00	0·60	2·53
Protein nitrogen[d]	25·0	5·10	22·3	16·8	6·32	2·67	17·5	19·9
Free amino acid nitrogen as a percentage of non-protein nitrogen	29·4	50·9	47·4	32·4	0·00	0·00	38·10	28·2

[a] Mean of three determinations.
[b] Ungerminated seeds.
[c] Germinated seeds.
[d] Expressed as percentage of the total nitrogen in the sample.

14 % after germination. Smith *et al.* (1959) found that the nitrogen extracted by 70 % ethyl alcohol, in most seeds of the legume family, was only of non-protein nature; thus there was no evidence for any appreciable amount of prolamin. It seems that fenugreek is an exception because this work proved the presence of a 70 % ethyl alcohol soluble protein, a result which agrees with that found by Hassan & Basha (1932) who isolated a prolamin soluble in ethyl alcohol from fenugreek seeds.

Trypsin inhibitor activity and haemagglutinating factors in the fenugreek seeds

No haemagglutinating activity is detected in the variety of fenugreek used in the present work. On the other hand the fenugreek extracts depress the tryptic digestion of casein. The results illustrated in Fig. 1 indicate that there was a positive correlation between the protein contents of the fenugreek extracts and their inhibitory effects on the tryptic digestion of casein. It is observed that germinated fenugreek extract caused stronger inhibition than that of ungerminated seeds when compared at the same level of protein concentration. The results obtained show

TABLE 3
TRYPSIN INHIBITOR ACTIVITY OF UNGERMINATED AND GERMINATED FENUGREEK SEEDS

Fenugreek	*T.I.U.* $\times 10^{-3}$ *per gramme*	
	Wet basis[a]	*Dry basis*
Ungerminated	27860	31303
Germinated	10571	52859

T.I.U. = Trypsin inhibitor unit.
[a] Moisture content of ungerminated seeds = 10·9 % and for germinated seeds = 80·0 %.

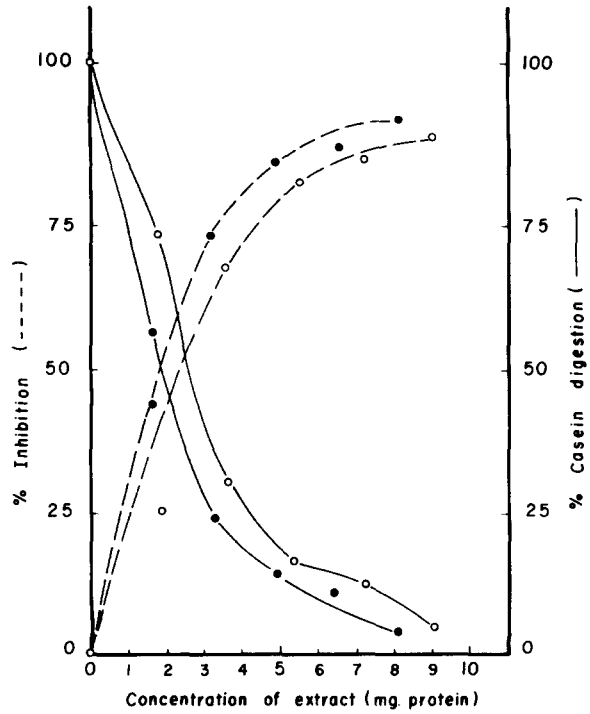


Fig. 1. Inhibition of the tryptic digestion of casein as a function of the level of ungerminated (○) and germinated (●) fenugreek extracts.

clearly that germination increased trypsin inhibitor activity by 66% of the original amount of the ungerminated seeds (Table 3). This finding is contrary to that reported by El-Hag *et al.* (1978). They found that germination of red kidney beans (*Phaseolus vulgaris*) decreased the trypsin inhibitor activity.

The concentration of trypsin inhibitor per gramme of ungerminated fenugreek is equal to that present in soybeans, *Glycine max* (Borchers *et al.*, 1947) and twice the amount found in *Vicia faba* beans (El-Mahdy *et al.*, 1981).

Extracts of ungerminated fenugreek seeds inhibited 90% and germinated extracts inhibited 94% of the trypsin activity (Table 4). Heat treatment of both extracts resulted in a decrease of the inhibition. As the time of heating increased the trypsin inhibitor activity decreased. After 30 min of heating two thirds of the inhibitor activity remained in the extracts of fenugreek. Thus, the fenugreek trypsin inhibitor, like those of soybean and faba beans (Bhatty, 1975), was thermostable.

No available data are found in the literature concerning the presence of haemagglutinin and trypsin inhibitor activities in the fenugreek seeds except that mentioned by Sauvaire *et al.* (1976). They reported that autoclaving does not modify the nutritional value of the protein concentrate of the fenugreek. They

TABLE 4
THERMOSTABILITY OF THE CRUDE TRYPSIN INHIBITOR OF FENUGREEK EXTRACTS

Heating time (min)	*T.I.U. × 10^{-3} per milligramme protein*		*% Inhibition of trypsin assay*	
	UGS	*GS*	*UGS*	*GS*
0	649	761	90	94
15	564	647	78	80
30	466	502	64	62

Extracts of fenugreek were heated under reflux in a boiling water bath, cooled to room temperature and used for trypsin inhibitor assay.

attributed this result to either the presence of thermostable factors such as protease inhibitor, and/or haemagglutinin or the fact that heat treatment given was not sufficient to eliminate eventual toxic substances.

The digestibility of fenugreek proteins as affected by germination

As found before, germination enhanced the amount of NPN fraction, so the digestibility expressed as a percentage of TN gives higher results. It is obvious that when the digestibility is expressed on the basis of protein nitrogen, a more realistic picture is obtained. Pancreatin digestibility of ungerminated seed proteins is very low as compared to either pepsin or pepsin followed by pancreatin digestibilities.

As shown in Table 5, germination does not show the expected improvement in digestibility. A slight increase in the pancreatic digestibility was observed, while a small decrease was found in the peptic digestibility. On the other hand germination resulted in a drastic decrease in the digestibility of seeds by pepsin followed by pancreatin, amounting to 33·7%. The low pancreatic digestibilities of fenugreek proteins may be attributed to the presence of trypsin inhibitor in both ungerminated

TABLE 5
In vitro DIGESTIBILITY OF FENUGREEK PROTEINS AS AFFECTED BY GERMINATION

Enzyme used	*Digestibility %*				
	Casein	*UGS*		*GS*	
	TN = PN	*TN*	*PN*	*TN*	*PN*
Pepsin	40·1	77·0	71·5	72·4	70·0
Pancreatin	84·9	38·6	21·8	52·9	23·7
Pepsin followed by pancreatin	80·8	74·1	40·4	76·4	26·8

TN = total nitrogen.
PN = protein nitrogen.
Non-protein nitrogen of ungerminated seeds = 0·82%, non-protein nitrogen of germinated seeds = 2·49% on a dry weight basis.

and germinated seeds. These results agree with those found by Venkataraman *et al.* (1976) on different pulses. They found that germination did not show any improvement of biological value, of green gram, cowpea, and chick pea. They found also that the *in vivo* digestibility of chick pea and cowpea decreased after 72 h germination while green gram showed an increase after the same period of germination.

REFERENCES

AKESON, W. R. & STAHMAN, M. A. (1964). A pepsin pancreatin digest index of proteins. *J. Nutr.*, **83**, 257–61.

ALTSCHUAL, A. M., YATSU, L. Y., ORY, R. L. & ENGLEMAN, E. M. (1966). Classification of major proteins. *Ann. Rev. Plant Physiol.*, **17**, 113–36.

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS (1960). *Official methods of analysis*, A.O.A.C., Washington, DC.

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS (1975). *Official methods of analysis*, A.O.A.C., Washington, DC.

BECKER, H. C., MILNER, R. T. & NAGEL, R. H. (1940). A method for the determination of non-protein nitrogen in soybean meal. *Cereal Chem.*, **17**, 447–57.

BEEVERS, L. (1968). Protein degradation and proteolytic activity in the cotyledons of germinating peas. *Phytochem.*, 7, 1837.

BHATTY, R. S. (1975). Trypsin inhibitors of faba beans (*Vicia faba* L. var. minor). I—Preliminary studies. *Canad. J. Bot.*, **53**, 3075–7.

BORCHERS, R., ACKERSON, C. W. & SANDSTEDT, R. M. (1947). Trypsin inhibitor. III—Determination and heat destruction of the trypsin inhibitor of soybeans. *Arch. Biochem.*, **12**, 367–74.

CHATTAPADHGAY, H. & BANNERJEE, H. (1953). Effect of germination on the biological value of protein and trypsin inhibitor activity in legumes. *Indian J. Med. Res.*, **41**, 185.

CHEN, L. H. & THACKER, R. R. (1978). Germination and nitrogenous constituents of pea seeds (*Pisum sativum*). *J. Fd Sci.*, **43**, 1884–5.

CHEN, L. H., THACKER, R. R. & PAN, S. H. (1977). Effect of germination on haemagglutinin activity of pea and bean seeds. *J. Fd Sci.*, **42**, 1666–7.

DESHMUSHI, A. D. & SOHONIE, K. (1966). Proteins of green gram. 3—Effect of germination on the nitrogeneous constituents. *J. Nutr. Dietet.*, **3**, 1.

DESIKACHAR, H. S. (1947). Role of inhibitors in soybeans. *Science*, **106**, 421–2.

EL-HAG, N., HAARD, N. F. & MORSE, R. E. (1978). Influence of sprouting on the digestibility coefficient, trypsin inhibitor and globulin proteins of red kidney beans. *J. Fd Sci.*, **43**, 1874–5.

EL-MADFA, I. E. (1975). Ueber das Trigonella protein (Trigonella *Foenum-Graecum*). *Die Nahring*, **19**, 683–6.

EL-MAHDY, A. R., MOUSTAFA, E. K. & MOHAMED, M. S. (1981). Trypsin inhibitor activity in the *Vicia faba* beans. *Food Chem.*, 7, 63–71.

EVENSON, G. J., STEENBACK, D. L. & PARSON, H. T. (1964). The effect of germination, the stage of maturity, and the variety upon the nutritive value of soybean protein. *J. Nutr.*, **27**, 225.

GREENBERG, D. N. (1955). Plant proteolytic enzymes. In: *Methods in enzymology*, Vol. II. (Colowick, S. P., and Kaplan, N. O. (Eds)), Academic Press, New York, p. 54.

HASSAN, A. & BASHA, M. K. (1932). A preliminary note on a prolamine isolated from a legume seed. *Biochem. J.*, **26**, 1843–5.

HEGAZI, S. M. (1974). Effect of germination on the carbohydrate, protein and amino acid contents of broad beans. *Zeit. Ernahrungswiss*, **13**, 200–3.

HUBER, R. E. & ZALIK, S. (1963). Lipid and protein in germinating and developing flax seed *Linum usitatissimum* L. *Can. J. Biochem. Physiol.*, **41**, 745.

INGLE, J., BEEVERS, L. & HAGEMAN, R. H. (1964). Metabolic changes associated with the germinating corn 1. Changes in weight and metabolites and their redistribution in the embryo axis, scutellum, and endosperm. *Plant Physiol.*, **39**, 735.

KOLOUSEK, J. & COULSON, C. B. (1955). Plant proteins. II—Amino acid content of the seed protein of *Trigonella–Foenum Graecum* L. and of the seed proteins and thallus protein of *Calega Officianalis* L. *J. Sci. Food Agric.*, **6**, 455–61.

KUNITZ, M. (1947). Crystalline soybean trypsin inhibitor II—General properties. *J. Gen. Physiol.*, **30**, 291–310.

LIENER, I. E. & HILL, E. C. (1953). The effect of heat on the nutritive value and haemagglutinating activity on soybean oil meal. *J. Nutr.*, **49**, 609–20.

LOWRY, O. H., ROSENBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). Protein estimation with the folin ciocateu reagent. *J. Biol. Chem.*, **193**, 265–75.

MCGUIRE, T. A. & EARLE, F. R. (1958). Changes in the solubility of corn protein resulting from the artificial drying of high moisture corn. *Cereal Chem.*, **35**, 179–88.

PALMER, R., MCINTOSH, A. & PUSZTAI, A. (1973). The nutritive evaluation of kidney beans. The effect of nutritional value of seed germination and changes in trypsin inhibitor content. *J. Sci. Fd Agric.*, **24**, 937–44.

PALMIANO, E. P. & JULIANO, B. O. (1972). Biochemical changes in the rice grain during germination. *Plant Physiol.*, **49**, 751.

PUSZTAI, A. & DUNCAN, I. (1971). Changes in proteolytic enzyme activities and transformation of nitrogenous compounds in the germinating seeds of kidney beans. *Planta (Berl.)*, **69**, 317.

ROSEN, H. (1957). A modified ninhydrin colorimetric analysis for amino acids. *Arch. Biochem. Biophys.*, **67**, 10–13.

ROSENGARTEN, F., JR. (1969). In: *The book of species*. Livingston Publishing Company, Wynewood, Pennsylvania.

SAUVAIRE, Y., BACCOU, J. C. & BESANCON, P. (1976). Nutritional value of the proteins of a leguminous seed: fenugreek (*Trigonella foenum-graecum* L.). *Nutr. Rep. Inter.*, **14**, 527–37.

SHANKARACHARYA, N. B. & NATARAJAN, C. P. (1973). Chemical composition of raw and roasted fenugreek seeds. *J. Food Sci. Technol.*, **10**, 179–81.

SMITH, C. R., EARIL, F. R. & WALFF, I. A. (1959). Seed protein solubility by comparison of solubility characteristics of selected seed protein. *J. Agric. Food Chem.*, **7**, 133–6.

VENKATARAMAN, L. V., GENESHKUMAR, K. & JAYA, T. V. (1976). Studies on the changes of proteins, carbohydrates, trypsin inhibitor, amylase inhibitor and haemagglutinins of some legumes during germination. *Proc. Soc. Biol. Chem.*, **34**.

WUNSCHENDORFF, M. H. E. (1919). Les matières proteique de la graine de fenugreec. *J. Pharm. Chem. Paris*, **20**, 86–8.

Food Chemistry 8 (1982) 263–268

NUTRITIVE VALUE OF SOME NIGERIAN EDIBLE MUSHROOMS

S. K. OGUNDANA & O. E. FAGADE

Department of Microbiology, University of Ife, Ile-Ife, Nigeria

(Received: 26 January, 1981)

ABSTRACT

Samples of Termitomyces robustus, T. clypeatus *and* Pleurotus tuber-regium *were analysed for their nutrient and toxic substances. The* Termitomyces *spp. contained as much as 31% proteins and about 32% carbohydrates, of which at least 26% were reducing sugars.* Pleurotus tuber-regium *contained 14·6% protein and 18·6% carbohydrates, of which only about 2·9% were reducing sugars. There was little difference in their crude fibre and ash content, while the fat content of* T. robustus *was a little higher than those of the other samples. The ascorbic acid content of each of the* Termitomyces *spp. (10 mg % and 14·3 mg %) was much higher than that of* Pleurotus *sp. (3·3 mg %). All the mushroom samples were low in hydrocyanic acid (HCN) and oxalate contents.*

INTRODUCTION

Termitomyces robustus has been described as the most delicious edible mushroom by the Yoruba people of Nigeria (Oso, 1975). It makes its appearance after heavy rains (Alasoadura, 1967), growing in large numbers in contact with termite nests in forest soils from August to October. *T. clypeatus* also grows in contact with termite nests but makes its appearance at the beginning of the rainy season around April, while *Pleurotus tuber-regium* is often found around breadfruit trees (Zoberi, 1973). In Nigeria, wild mushrooms are collected for food and, when plentiful, are frequently sold along the roadside to passers-by. Although *Termitomyces* and *Pleurotus* spp. are widely distributed in Nigeria (Alasoadura, 1967), very little work has been done on any edible mushrooms. Mycologists in Nigeria (Alasoadura, 1966; Zoberi, 1972, 1973) have only used taxonomic characters to determine edible ones. A pioneer work (Oke, 1966*a*) has been carried out to determine the nutritive value of mixed edible mushroom samples. The present investigations were,

Food Chemistry 0308-8146/82/0008-0263/$02·75

therefore, carried out to determine the nutritive value of the widely accepted *T. robustus*, *T. clypeatus* and *P. tuber-regium*.

MATERIALS AND METHODS

Raw materials

T. robustus was bought from sellers along Ife–Ibadan road and *T. clypeatus* was collected from the University of Ife maize plantation, while *P. tuber-regium* was harvested from a decaying log at the University of Ife staff quarters. All specimens were oven dried and ground into fine particles before use.

Determination of nutrient and toxic substances

Crude fibre and ash contents were determined according to the AOAC methods of analysis (AOAC, 1970); and the crude protein, ether extract and hydrocyanic acid were also determined by the methods of the AOAC (1975).

The total oxalate content was estimated using Oke's method (1966*a*) whereby 2 g of sample were digested with 190 ml of distilled water and filtered into a conical flask, and diluted to 250 ml. To a 50 ml portion of the extract were added 10 ml 6N HCl; the solution was then filtered and the precipitate washed with hot water. The filtrate and the wash were combined and titrated against 6N NH_4OH, using methyl red indicator. The solution was heated to about 90 °C and 10 ml of 5 % w/v $CaCl_2$ solution added to precipitate the oxalate. The precipitate formed overnight was washed free of calcium with distilled water; and then put with about 10–15 ml hot 25 % H_2SO_4 into a 50 ml graduated conical flask, and diluted to 20 ml with distilled water. The resultant solution was finally warmed to about 80 °C and titrated against 0·05 N $KMnO_4$. The calculation was made as recorded by Vogel (1961).

Total nitrogen determinations were based on the Kjeldahl method (Kirk, 1950) and the ascorbic acid content was determined by the methods of Everest (1960). The methods used for the total sugars were those of Dubois *et al.* (1951) and the reducing sugars estimated by Somogyi–Nelson procedures (Hestrin *et al.*, 1955).

The qualitative analysis of the sugars was then carried out using the methods of Dubois *et al.* (1956) as modified by Faparusi (1970) whereby 0·01 ml of the sugar extract of each mushroom was spotted in triplicate on Whatman No. 1 chromatography paper and the chromatogram developed for 72 h in 1-butanol–acetic acid–water (4:1:1, by volume). After drying, one of the triplicate columns was cut off and the sugars on it located. The sugar spots were used to mark the regions of these spots on the duplicates, and corresponding areas removed and eluted for 3 h with 5 ml distilled water. To 2 ml of the eluate was added 0·05 ml of 80 % (w/v) aqueous phenol reagent followed by a rapid addition of 5 ml of concentrated H_2SO_4. After the sample had stood at room temperature (25 °C) for 30 min, the optical density was determined at 480 nm, and the concentration of each sugar was calculated from the

standard curve. All blanks were read against a blank containing distilled water in place of the sugar solution.

RESULTS

Table 1 shows a typical proximate analysis of the mushroom samples. The results show that *P. tuber-regium* contains the highest amount of dry matter while *T. clypeatus* contains the least. There are few differences in the ash and crude fibre

TABLE 1
PROXIMATE ANALYSIS OF SOME NIGERIAN EDIBLE MUSHROOMS[a]

Sample	*Dry matter*	*Ash*	*Crude fibre*	*Nitrogen*	*Crude protein*	*Ether extract*	*Total sugar*	*Reducing sugar*
Termitomyces robustus	12·7	12·1	8·7	5·83	36·4	7·83	32·3	28·2
Termitomyces clypeatus	7·30	11·0	7·9	5·02	31·4	4·96	32·4	26·9
Pleurotus tuber-regium[b]	16·5	9·1	7·4	2·34	14·6	4·48	18·6	2·88

[a] All values expressed as percent of dry matter.
[b] Values recorded were those of the pileus only.

TABLE 2
QUALITATIVE ANALYSIS OF SUGAR CONTENT OF SOME NIGERIAN EDIBLE MUSHROOMS

Sample	*Fructose*	*Galactose*	*Glucose*	*Lactose*	*Maltose*	*Sucrose*
Termitomyces robustus	+	+ +	+ +	+	+ +	+
Termitomyces clypeatus	+	+ +	+ +	+	+ +	+
Pleurotus tuber-regium[a]	−	+ +	+	−	+	−

Key: + + = high concentration; + = low concentration; − = absent.
[a] Values recorded were those of the pileus only.

contents. The nitrogen and, consequently, the crude protein content of the two *Termitomyces* spp. are quite high in contrast to the low values obtained for *Pleurotus*. The amounts of fats and oil contained in *T. clypeatus* and *P. tuber-regium* are virtually the same (4·96 % and 4·48 %, respectively) while the amount in *T. robustus* alone is almost equal to the total amount possessed by the other two mushroom samples.

Termitomyces spp. contain high amounts of total and reducing sugars. *Pleurotus* sp. contains about half the sugar content of any of the *Termitomyces* spp., and also has a considerably lower reducing sugar content, 2·88 %. The qualitative analysis of the sugar content of mushroom samples is given in Table 2, while Table 3 shows the oxalic, hydrocyanic and ascorbic acid contents. All determinations are based on percentage of dry matter.

TABLE 3
OXALATE, HYDROCYANIC AND ASCORBIC ACID CONTENTS OF SOME NIGERIAN EDIBLE MUSHROOMS[a]

Sample	*Total oxalate*	*Hydrocyanic acid*	*Ascorbic acid*
	(%)	(*mg* %)	(*mg* %)
Termitomyces robustus	3·75	2·80	10·0
Termitomyces clypeatus	2·25	3·38	14·3
Pleurotus tuber-regium[b]	5·62	1·99	3·3

[a] All values expressed as percent of dry matter.
[b] Values recorded were those of the pileus only.

DISCUSSION

The values recorded for *P. tuber-regium* were those of the pileus only. This is because *P. tuber-regium* has a subterranean globose sclerotium which, although also edible, is disproportionately fatter than the pileus. For instance, a whole fruiting body may weigh up to 20 g, of which the pileus may weigh just about 1 g. Since all attempts to work with the whole fungus by combining various proportions of the pileus and sclerotium did not yield reliable results, attention was therefore focused on the pileus (the part harvested and eaten most).

In the present study, the Nigerian edible mushrooms investigated contain large amounts of water and are consequently low in dry matter content. This agrees with the results obtained by Oke (1966*a*) in his study of mixed mushroom samples; but the ash and cellulose contents obtained in this study are higher than those obtained for maize, sorghum and rice (Oke, 1965). The crude fibre of *Pleurotus* sp. expected to be highest, because of its tough texture, was lowest; and the proximate analysis of this mushroom only adds up to approximately 50% of the dry matter. This is probably due to the waxy substance it contains (Zoberi, 1973).

Although the protein content of *Pleurotus*, 14·6%, was much lower than those of *Termitomyces* spp. (36·4% and 31·4%), it is still higher than the value (13%) obtained for *Hydnum imbricatum* (Mlodecki *et al.*, 1974), which was, on its own, considered to be sufficiently nutritious. In general, the protein values obtained for the mushrooms lend further support to the assertion of some workers (Fink & Hoppenhaus, 1958; Rafalski *et al.*, 1968) that the protein contents of some mushrooms are as good as animal proteins and even surpass most vegetable proteins.

The oxalic acid content has been used as an index of toxicity in vegetables since large amounts would lower the nutritive value (Oke, 1966*a*). It is interesting to note that the oxalate contents of the mushroom samples investigated were low. The mushroom also showed low values of hydrocyanic acid compared with those recorded for poisonous varieties, and well below the toxic dose of 60 mg for man (Nicholls, 1951).

The various sugars present in the mushrooms (Table 2) are similar to those obtained in *Agaricus campestris* (Hughes *et al.*, 1958). The high reducing sugar content in *Termitomyces* spp., which could provide easily absorbed nutritious food materials, probably accounts for the easy attack by flies leading to the development of maggots within 24 h of emergence of the *Termitomyces* spp. from soil. Conversely, the relatively lower total sugar and very small amount of reducing sugar in *Pleurotus* sp. could therefore account for its not being infested by maggots.

Finally, the ascorbic acid content obtained for *Termitomyces* spp. in the present study compares favourably with those of some animal organs. For instance, Oke (1966*b*) found that cooked beef kidney contained 15 mg %; pig spleen, 11 mg %; while the uncooked goat lung and heart contained 9 and 10 mg %, respectively. This means, therefore, that the *Termitomyces* spp. can serve both as a delicious food for man and as a livestock feed (Gawecki & Frelich, 1967).

From the results obtained in this study, the *Termitomyces* spp. have been shown to be nutritionally better than *Pleurotus* sp., and to meet body requirements, possessing higher protein, carbohydrate, fat and vitamin C contents. This provides scientific support for the preference of *Termitomyces* to *Pleurotus* spp. and other edible mushrooms.

ACKNOWLEDGEMENTS

The authors are grateful to Professor O. L. Oke for providing laboratory facilities and to Mr John Dunkwu for his technical assistance.

REFERENCES

ALASOADURA, S. O. (1966). Studies in the higher fungi of Nigeria. II—Macrofungi associated with termite nests, *Nova Hediwigia Z. Kryptogamenkd.*, **II**, 387–93.

ALASOADURA, S. O. (1967). Studies in the higher fungi of Nigeria. I–The Genus *Termitomyces* Heim, *J. West Afr. Sci. Assoc.*, **12**(2), 139–46.

AOAC (1970). *Official Methods of Analysis*, 11th Edn, Association of Official Analytical Chemists, Washington, DC, USA.

AOAC (1975). *Official Methods of Analysis*, 12th Edn, Association of Official Analytical Chemists, Washington, DC, USA.

DUBOIS, M., GILLIES, K., HAMILTON, J. K., REBERS, P. A. & SMITH, F. (1951). Quantitative analysis of sugars by paper chromatography, *Nature (London)*, **168**, 167.

DUBOIS, M., GILLIES, K., HAMILTON, J. K., REBERS, P. A. & SMITH, F. (1956). Colorimetric method for the determination of sugars and related substances, *Anal. Chem.*, **28**, 350–56.

EVEREST, D. F. (1960). Determination of ascorbic acid in highly coloured solutions with *N*-bromosuccinimide, *Analyst*, **85**, 515–16.

FAPARUSI, S. I., (1970). Sugar changes during the preparation of Burukutu beer, *J. Sci. Food Agric.*, **21**, 79–81.

FINK, H. & HOPPENHAUS, K. W. (1958). Peculiar observations in the estimation of biological quality of the proteins of edible boletus (*Boletus edulis*) and mushroom (*Psalliota biospora*) with reference to dietetics and therapeutics, *Nutr. Abstr. Rev.*, **28**, Abs. 4886.

GAWECKI, K. & FRELICH, A. (1967). Mushroom meal for sheep, *Nutr. Abstr. Rev.*, **37**.

HESTRIN, S., FEINGOLD, D. S. & SCHRAMN, D. (1955). Somogyi–Nelson method for reducing sugars, in *Methods in Enzymology*, Academic Press, New York, USA.

HUGHES, D. W., LYNCH, D. L. & SOMNERS, G. F. (1958). Chromatographic identification of the amino acids and carbohydrates in the cultivated mushroom (*Agaricus campestris* L. ex. Fries), *J. Agric. Food Chem.*, **6**, 850–3.

KIRK, P. L. (1950). Kjeldahl method for total Nitrogen, *Anal. Chem.*, **22**, 254–8.

MLODECKI, H., LASOTA, W. & MATYS, J. (1974). Hygienic evaluation of *Hydnum imbricatum*. 1—Values for amino acids, minerals and fats, *Nutr. Abstr. Rev.*, **44**, Abs. 2184.

NICHOLLS, L. (1951). *Tropical Nutrition and Dietetics*, 4th Edn, London.

OKE, O. L. (1965). Chemical studies on some Nigerian cereals, *Cereal Chem.*, **42**(3), 299–302.

OKE, O. L. (1966*a*). Nutritive value of mushrooms, *West Afr. Pharm.*, **8**(3), 51–4.

OKE, O. L. (1966*b*). Ascorbic content of some animal organs, *Nutrition (London)*, **20**(3), 110–12.

OSO, B. A. (1975). Mushrooms and the Yoruba people of Nigeria, *Mycologia*, **67**(2), 311–19.

RAFALSKI, H., MLODECKI, H., SALM, J., LASOTA, W. & SWITONIAK, T. (1968). Nutritive value of field agaric, *Nutr. Abstr. Rev.*, **38**, Abs. 4755.

VOGEL, A. I. (1961). *A Textbook of Quantitative Analysis including Elementary Instrumental Analysis*, 3rd Edn, Longmans, London.

ZOBERI, M. H. (1972). *Tropical Macrofungi*, Macmillian, London.

ZOBERI, M. H. (1973). Some edible mushrooms from Nigeria, *Niger. Field*, **38**(2), 81–90.

Food Chemistry 8 (1982) 269–276

UTILISATION OF MANGO SEED KERNEL (*MANGIFERA INDICA*) AS A SOURCE OF OIL

YEHIA G. MOHARRAM & A. M. MOUSTAFA

Food Science and Technology Department, Faculty of Agriculture, University of Alexandria, Egypt

(Received: 26 March, 1981)

ABSTRACT

Fat extracted from mango seed kernel (Mangifera indica) *was analysed for its chemical and physical constants, triglycerides, fatty acids and phospholipids; and these were compared with those of cotton seed oil, cocoa butter and two of its substitutes, namely, Croklaan Special 555 and Wesco Special E. The effects of incorporation of mango seed kernel oil in butterscotch toffee on its organoleptic properties were also studied. It was found that mango kernel oil and cocoa butter were almost identical in several of their constants, triglycerides, fatty acids and effects on taste, odour and texture of the toffee.*

INTRODUCTION

As a result of the shortage in the production, and rising price of cocoa butter, different types of cocoa butter substitutes are now produced. These substitutes are made mainly by hydrogenation of certain fractions of vegetable fats. Croklaan Special 555 and Wesco Special E butters are commercial substitutes. The first, Croklaan 555, being produced by the Croklaan Company, Holland, and the second, Wesco Special E by the Friwesso Company, Holland, from refined, hydrogenated and deodorised palm kernel oil. Wesco Special E contains the following percentage of fatty acids: 55·5, lauric; 21, myristic; 8·5, palmitic; and 9·5, stearic. The iodine values, saponification numbers, free fatty acids and melting points of Croklaan Special 555 and Wesco Special E ranged from 0·0–1·5, 240–250, 0·0–0·1% and 33–35°C, and 0·0–1·0, 245–255, 0·0–0·1 and 35–36, respectively.

Mango seed kernel is one of the by-products of canning factories and so far is considered as a waste product. To the best of our knowledge there are no reports on

Food Chemistry 0308-8146/82/0008-0269/$02·75

TABLE 1
CONSTANTS AND FATTY ACID CONTENTS OF MANGO SEED KERNEL OIL

	References		
	Dhingra et al. (*1948*)	*Bruno & Goldberg* (*1963*)	*Foda* et al. (*1971*)
Physical and chemical constants			
Refractive index at 40 °C	1·4604	—	1·4598–1·4602
Melting point	—	—	25 °C
Specific gravity	—	—	0·9005–0·9018
Saponification value	188–195	—	189·62
Iodine value	39–48	—	50·67
Acid value	—	—	2·05
Thiocyanogen	—	—	43·14
Reichert–Meissl	0·12	—	—
Unsaponifiable matter	2·3–2·9	—	1·22
Fatty acids			
Palmitic (16:0)	11·2	4·4	7·48
Palmitoleic (16:1)	—	—	1·19
Stearic (18:0)	31·2	42·5	41·18
Oleic (18:1)	43·8	44·7	40·86
Linoleic (18:2)	4·1	5·4	7·19
Linolenic (18:3)	—	—	1·59

the utilisation of it in foodstuff preparation. It has an oil content in the range of 6–12 % (Dhingra *et al.*, 1948). The physical and chemical constants as well as the fatty acids of mango seed kernel are presented in Table 1. Foda *et al.* (1971) found that starch constituted about 92 % of the carbohydrates of mango seed kernel.

The chemical composition of mango seed kernels, the characteristics and structure of its oil as well as the utilisation of this oil in preparing butterscotch toffee are investigated in this study. Included also is a comparative study of cotton seed oil, cocoa butter, two cocoa butter substitutes, namely Croklaan Special 555 and Wesco Special E, and the oil recovered from mango seed kernel.

MATERIALS AND METHODS

Materials

The mango seeds remaining as waste after the manufacture of mango juice were obtained from the Edfina Company for Food Preservation, Alexandria, Egypt. The seeds were dehulled manually to free the kernels which were ground in a blender to pass through a 100 mesh sieve. The crude oils of mango kernel and cotton seed were extracted directly with petroleum ether (b.p. 40–60 °C) for 16 h in a Soxhlet apparatus.

Cocoa butter and the two cocoa butter substitutes (Croklaan Special 555 and Wesco Special E) were obtained from the Royal Company for the production of chocolates and confectionery products, Alexandria, Egypt.

Methods

Technological methods: Crude mango seed kernel oil and cocoa butter were used for preparing butterscotch toffee according to the method described by Lees & Jackson (1973).

Analytical methods: Moisture, fats crude protein, ash and carbohydrate were determined according to AOAC (1975). Fibre content was calculated by difference. The refractive index, specific gravity, melting point, iodine value, saponification number and acid value were estimated as described by AOAC (1975). The fractionation of oils into different classes was carried out by thin layer chromatography (TLC) according to the method of Mangold & Malins (1960). The solvent system used was petroleum ether (b.p. 40–60 °C), diethyl ether, acetic acid (70:30:2 v/v). Silica gel G-60 of Merk was used. The TLC method of Barrett *et al.* (1962) as described by Osman *et al.* (1977*a*) with silver nitrate, silica gel G-60 was followed in the separation of the different triglycerides of the oils. The solvent system used was carbon tetrachloride, chloroform, acetic acid, ethanol (60:40:0·5: 1·5 v/v). The different methyl esters of the fatty acids of the oils were prepared (Anon., 1966) and a thin layer of silica gel G impregnated with silver nitrate was used to fractionate the different fatty acid methyl esters with a solvent system of petroleum ether: diethyl ether (80:20 v/v) according to Privett *et al.* (1961). Quantitative determination of the fatty acids was accomplished by the graphical method described by Purdy & Truter (1962) and Osman *et al.* (1977*b*). All the TLC methods were applied to the mango seed kernel oil, cotton seed oil, cocoa butter and both cocoa substitutes. The phospholipids of the mango kernel oil were separated by TLC according to the method developed by Bunn *et al.* (1969).

The taste and texture of the butterscotch toffee prepared using the aforementioned fats, except cotton seed oil, were assessed subjectively by a panel of 11 members. The results were analysed statistically according to the rank method described by Kramer & Twigg (1962).

RESULTS AND DISCUSSION

Chemical composition of mango seed kernel

The seed in mango fruit was found to represent about 16–32·7 % of the whole fruit, with an average of 25 %. This wide range depends upon the size and stage of ripening of the mango fruit. The kernel was 50 % and 12·5 % of the seed and the whole mango fruit, respectively. The kernel was found to contain 13·8 % moisture and 72·6 % total carbohydrate, 13·7 % oil, 7·08 % crude protein (total N × 6·25), 1·75 % ash and 2·95 % crude fibre on a dry weight basis. These results indicate that mango seed kernel contains a large amount of carbohydrate and a small amount of crude protein, ash and crude fibre.

TABLE 2

CONSTANTS AND AREA PERCENTAGE OF SPOTS OF FATTY ACID ESTERS OF MANGO SEED KERNELS AND COTTON SEED OILS AND COCOA BUTTER AND TWO SUBSTITUTES

Oil type	*Specific gravity*[a]	*Refractive index*[a]	*Melting point* (°C)	*Saponification value*	*Iodine value*	*Acid value*	*Fatty acids (areas in mm² from chromatograms)*			
							Saturated	*Unsaturated*		
								(Double bonds)		
								One	*Two*	*Three*
Mango seed kernel oil	0·912	1·4583	35·0	187·7	49·4	1·93	39·6	52·9	6·21	1·35
Cocoa butter	0·917	1·4572	35·0	190·2	38·4	1·75	50·2	44·8	3·55	1·51
Croklaan Special 555	0·915	1·4521	40·0	245·0	2·50	0·54	95·4	2·20	2·40	—
Wesco Special E	0·915	1·4521	40·0	255·0	2·40	0·54	96·2	2·65	1·20	—
Cotton seed oil	0·925	—	194·0	104·5	—	—	—	—	—	—

[a] Specific gravity and refractive index were determined at 20°C.

Characterisation of mango seed kernel oil

Table 2 gives the values of some constants and analytical characteristics of the crude oil extracted from mango seed kernel and cotton seed as well as the butter of cocoa and the substitutes (Croklaan Special 555 and Wesco Special E). From these results the following observations may be made.

(a) Cotton seed oil had higher specific gravity, refractive index and iodine values than the other types of oil, while the saponification number was similar to that of mango seed kernel oil and cocoa butter. Generally, the obtained values of the constants and characteristics of cotton seed oil in this study are in agreement with the results obtained by El-Khalafy & Meara (1970) and Osman *et al.* (1977*a*).

(b) The cocoa butter substitutes (Croklaan Special 555 and Wesco Special E) had similar constants and characteristics. They had higher melting points and saponification numbers, and lower iodine and acid values than the cocoa butter and mango seed kernel oil. Generally, they contained more free fatty acids and had higher iodine values and higher melting points than the values specified by the producer.

(c) Mango seed kernel oil and cocoa butter had nearly the same values of constants and characteristics except that the iodine value was relatively higher in the mango seed kernel oil than in cocoa butter. This may be due to the higher content of saturated fatty acids in cocoa butter than in mango seed kernel oil. The constants and characteristics were found to be similar to those obtained by Dhingra *et al.* (1948) and Foda *et al.* (1971) for mango seed kernel oil and by Kinke (1976) for cocoa butter.

Identification of the separated lipid classes

It was found by Osman *et al.* (1977*a*) that crude cotton seed oil could be separated into nine lipid classes which appeared on TLC plates in the following sequence from the front to the base line: hydrocarbons, triglycerides, unknown X_1, free fatty acid, unknown X_2, steroids, diglycerides, monoglycerides and phospholipids. However fresh crude cotton seed oil was fractionated into only seven of the aforementioned classes (copies of chromatographs obtainable from authors). Both the monoglycerides and free fatty acids were absent. On the other hand, both the mango seed kernel oil and cocoa butter contained the same nine classes of lipid fractions, indicating their similarity. In the case of the butter substitutes (Croklaan Special 555 and Wesco Special E) the triglyceride class was clearly separated, whereas the other classes appeared faintly on the TLC plate. That may be attributed to the refining process utilised for preparing these products from the vegetable fats.

Separation of the different triglycerides

Six groups of triglycerides were found in cotton oil by Barrett *et al.* (1962), Chakrabarty & Bhattacharyya (1967), Wessels and Rajagopal (1969) and Osman *et*

al. (1977*a*). These groups were also found in the crude cotton seed oil (copies of chromatographs obtainable from authors). They were the triglycerides with one, two, three, four, five and six double bonds. Five separated triglyceride groups were obtained from mango seed kernel oil and cocoa butter (Fig. 2). Triglyceride groups with one, two, three and four double bonds were present in mango seed kernel oil and cocoa butter. According to Jurriens & Kroesen (1965) and Avancini *et al.* (1966), the triglycerides of cocoa butter were a saturated group, and mono-, di-, tri- and poly-unsaturated fractions. Only one triglyceride group was separated from Croklaan Special 555 and Wesco Special E butters. This group was saturated and identical in these two butters.

Fatty acids content of the oil

Table 2 gives the percentage of the area in mm^2 of the fatty acid methyl esters of the mango seed kernel oil, the cocoa butter and its two substitutes (Croklaan Special 555 and Wesco Special E) determined quantitatively from the chromatogram spots. The results in this table reveal that:

(a) Saturated fatty acids constituted nearly all of the fatty acid content of both cocoa butter substitutes;

(b) Saturated and unsaturated fatty acids were about 40 and 60 % in mango seed kernel oil and 50 and 50 % in cocoa butter, respectively. About 90 % of the unsaturated fatty acid in both mango seed kernel oil and cocoa butter was oleic acid. The concentration of linoleic acid in mango seed kernel oil was about twice that found in cocoa butter. Although linolenic acid is not given in the literature as one of the fatty acids of cocoa butter, it was found in this study in both cocoa butter and mango seed kernel oil. The presence of this acid in cocoa butter is perhaps a result of the limitation of the TLC method as compared with gas–liquid chromatography (GLC).

From the above results it can be seen that mango seed kernel oil and cocoa butter possess similar characteristics and structure. There were no differences between the two cocoa butter substitutes (Croklaan Special 555 and Wesco Special E) in their characteristics and structure. The constants and composition of cocoa butter differed completely from its substitutes.

Tentative identification of phospholipids

A sample of rat liver lipid extract which has a known pattern of phospholipid composition was used as a reference during the tentative identification of the phospholipids of mango seed kernel oil. The 11 fractions separated from the rat liver lipid extract were also obtained from the mango seed kernel oil (copies of chromatographs obtainable from authors). These fractions included the following components, starting from the base line: phosphatidyl serine, lysophosphatidyl choline, phosphatidyl inositol, sphingomyelin, phosphatidyl choline, phosphatidyl

ethanolamine, phosphatidic acid, cardiolipin, glycerophosphatidyl compounds, glycolipids, sterols and neutral lipids. Several of the phospholipids, such as phosphatidyl choline (lecithins), are used in food technology as emulsifiers and as antispattering agents in frying fats. The characteristics and composition as well as the presence of phospholipids in mango seed kernel oil recommend its utilisation in the food industry, especially in butterscotch toffee, which is a common sugar confectionery product in Egypt.

Utilisation of mango seed kernel oil in preparing butterscotch toffee

It was found from the taste panel tests that there were no differences in the taste, texture and odour of toffee prepared from both types of fats, mango seed kernel oil and cocoa butter.

From the above data the use of mango seed kernel oil as a cocoa butter substitute or partial replacer in the preparation of confectionery products can be suggested.

REFERENCES

ANON. (1966). Preparation of methyl esters of long chain fatty acids, *J. Am. Oil Chem. Soc.*, **43**, R.A.

AOAC (1975). *Official Methods of Analysis*, Association of Official Agricultural Chemists, Washington, DC, USA.

AVANCINI, D., PEDRONI, G. and DE FRANCESCO, F. (1966). Structure of glycerides determined by chromatographic techniques, *Riv. Ital. Sostanze Grasse.*, **43**, 450.

BARRETT, C. B., DOLLOS, M. S. J. & PODLEY, F. B. (1962). The separation of glycerides by TLC on silica impregnated with silver nitrate, *Chem. Ind. (London)*, 1050.

BRUNO, A. & GOLDBERG, P. H. (1963). The morphology and chemical composition of some Nigerian mango, *Trop. Agric.*, **40**, 143.

BUNN, C. R., KEERE, B. B. & ELKIN, T. G. H. (1969). A technique for improved thin layer chromatography of phospholipids, *J. Chromatogr.*, **45**, 326.

CHAKRABARTY, M. M. and BHATTACHARYYA, D. (1967). Detection of rearrangement reactions by silver nitrate TLC, *J. Chromatogr.*, **31**, 556.

DHINGRA, D. R., KAPOOR, S. N. & CHANDRU, G. (1948). Analysis of mango seed kernel, *Proc. Annu. Conv. Oil Technol. Assoc. (India)*, **3**, 39–44 (*CFCA*, **46**, 8273, 1948).

EL-KHALAFY, R. H. & MEARA, M. E. (1970). Fatty acids composition of Egyptian cotton seed oils, *Grasas Aceites (Seville)*, **21**, 66.

FODA, Y. H., HAMED, M. G. E. & ABD-ALLAH, M. A. (1971). Utilization of mango seed kernel. II—Studies on the oil obtained from mango seed kernel, *Research Bulletin* 455 (13); Ain Shams University, Faculty of Agriculture, Cairo, Egypt.

JURRIENS, G. & KROESEN, A. C. J. (1965). Determination of glycerides composition of several oils and liquid fats, *J. Am. Oil Chem. Soc.*, **42**, 9.

KINKE, A. (1976). The fatty acids composition of cocoa butter, *Gordian*, **76**, 324–326 (*CF Food Sci. Technol. Abs:* **9**, 5 K 26, 1977).

KRAMER, A. & TWIGG, B. A. (1962). *Fundamenpals of Quality Control in the Food Industry*, Avi. Publ., Co., Westport, Connecticut, USA.

LEES, R. & JACKSON, B. (1973). *Sugar Confectionery and Chocolate Manufacture*, Chemical Publ., Co., Inc., New York, USA.

MANGOLD, H. K. & MALINS, D. C. (1960). Fractionation of fats, oil, and waxes by thin layers of silicic acid, *J. Am. Oil Chem. Soc.*, **37**, 576.

OSMAN, H. O. A., MOUSTAFA, E. K. & BAKER, M. R. M. (1977*a*). Studies on oils and fats in Egypt. 6—Fractionation and identification of cotton seed crude oil major classes and within the triglyceride classes, *Egypt. J. Food Sci.*, **5**, 1.

OSMAN, H. O. A., MOUSTAFA, E. K. & BAKER, M. R. M. (1977*b*). Studies on oils and fats in Egypt. 7—Quantitative determination of palmitic, oleic, and linoleic acids in cotton crude oil by TLC, *Egypt. J. Food Sci.*, **5**, 1.

PRIVETT, O. S., BLANK, M. L. & LUNBERG, W. O. (1961). Determination of mono-, di- and triglycerides by molecular distillation and TLC, *J. Am. Oil Chem. Soc.*, **48**, 312.

PURDY, S. J. & TRUTER, E. V. (1962). The weight–area relationship in thin film chromatography, *Chem. Ind. (London)*, 506.

WESSELS, H. & RAJAGOPAL, N. S. (1969). TLC separation of mixture of isomeric and natural triglycerides, *Fette, Seifen; Anstrichm.*, **71**, 543.

Food Chemistry 8 (1982) 277–289

WINGED BEAN LIPOXYGENASE—PART 2: PHYSICOCHEMICAL PROPERTIES

TRUONG VAN DEN,† LEONCIO C. RAYMUNDO & EVELYN MAE T. MENDOZA‡

University of the Philippines at Los Baños (UPLB), Laguna, Philippines 3720

(Received: 27 February, 1981)

ABSTRACT

Winged bean lipoxygenase (linoleate: oxygen oxidoreductase EC 1.13.11.12) isoenzymes FI and FII were isolated and purified according to the method of Truong et al. *(1980).*

FI and FII were both highly specific for linoleic acid. They exhibited optimal activity at pH 6·0 and 5·8, respectively at 30°C. An activation energy of 4·5 kcal mol^{-1} was calculated for this lipoxygenase within the temperature range of 30–50°C.

At 0·075% Tween 20, FI and FII had K_m values for linoleic acid of 0·44 and 0·37 × 10^{-3} M, respectively, compared to 0·4 × 10^{-3} M for the crude enzyme. Maximal activity was obtained at 1·6 × 10^{-3} M. Higher levels of Tween 20 inhibited the lipoxygenase activity.

Both isoenzymes had identical average molecular weight of 80 000 daltons by gel filtration and SDS gel electrophoresis.

FI and FII isoenzymes were strongly inhibited by Hg^{++}, Mn^{++}, Mg^{++} and Fe^{+++} and activated by Zn^{++}, Co^{++} and Fe^{++}. A difference in the degree of inhibition or activation was observed between FI and FII response. Ca^{++} inhibited both FI and FII but the former was more sensitive to Ca^{++}. KCN also inhibited the two isoenzymes.

Among the antioxidants tested, butylated hydroxytoluene and butylated hydroxyanisole most effectively inhibited both FI and FII at only 10^{-6} M. Sulphydryl reagents such as iodoacetamide and dithiothreitol have little effect on the lipoxygenase isoenzyme activity.

The lipoxygenase isoenzymes were more stable at neutral pH. The enzyme in the crude extract and especially in situ *was more stable to heat treatment.*

† Present address: National Institute of Biotechnology and Applied Microbiology (BIOTECH), University of the Philippines at Los Baños College, Laguna, Philippines 3720.
‡ To whom correspondence should be addressed.

Food Chemistry 0308-8146/82/0008-0277/$02·75

INTRODUCTION

Lipoxygenase (linoleate: oxygen oxidoreductase EC 1.13.11.12) catalyses the oxidation of *cis,cis*-1,4-pentadiene systems in unsaturated fatty acids to conjugated *cis,trans*-hydroperoxides which are then subsequently degraded to form a variety of secondary products involved in the off- and beany flavour of food legumes (Gardner, 1975). This enzyme has been well studied in soybean, pea and peanut (Christopher *et al.*, 1972; Sanders *et al.*, 1975; Yoon & Klein, 1979). A number of isoenzymes which differ in physicochemical properties as well as mode of action on their substrates have also been reported.. In Part I of this series of studies, we reported the isolation and purification of lipoxygenase from winged bean (*Psophocarpus tetragonolobus* (L.) DC) (Truong *et al.*, 1982). Two major isoenzymes of similar R_f of 0·25 on polyacrylamide gel electrophoresis were separated by DEAE ion exchange chromatography.

The present investigation was undertaken to characterise the winged bean lipoxygenase isoenzymes in order to be able to understand their mechanism of action and role in beany flavour formation. Furthermore, the results obtained from this study can hopefully provide information on developing techniques for the inactivation of this enzyme for processing of winged bean food products.

MATERIALS AND METHODS

Materials

Dried seeds of the winged bean line, Batangas medium, the most widely grown variety in the Philippines, were used in this work. The seeds were ground in a Wiley mill to pass through a 40 mesh screen. Defatted flour was prepared from winged bean meal by extracting with acetone three times (1:10 meal to solvent) at 4°C.

Purification of lipoxygenase from winged bean

The lipoxygenase isoenzymes FI and FII were purified according to the methods described by Truong *et al.* (1982). Briefly, the purification method consisted of obtaining the 30–50 % ammonium sulphate precipitate, gel filtration on Sephadex G-150, ion-exchange chromatography on DEAE Sephadex A-50 column and finally, hydroxyapatite chromatography to give FI and FII active fractions. These fractions were shown to have a major protein band ($R_f = 0{\cdot}25$) and minor bands at R_f 0·28 and 0·21 for FI and FII, respectively, on polyacrylamide gel electrophoresis.

Assay for lipoxygenase activity

Lipoxygenase activity was determined spectrophotometrically by measuring the increase in absorbance at 234 nm due to the formation of conjugated diene hydroperoxide (Ben-Aziz *et al.*, 1970). The assay conditions are described in the previous paper (Truong *et al.*, 1982).

Assays were performed at 30 °C and at pH 5·8 in a Gilford Model 250 recording spectrophotometer. The reaction mixture had a final concentration of $1{\cdot}61 \times 10^{-3}$ M linoleic acid, 0·075 % Tween 20 in 0·1 M sodium phosphate buffer (pH 5·8).

One unit of enzyme activity is defined as the amount of enzyme which produces a change of one unit of absorbance at 234 nm per min.

Protein determination

Protein content was determined by the method of Lowry *et al.* (1951) or by measuring absorbance at 280 nm. Bovine serum albumin was used as the standard.

Gel filtration chromatography

Partially purified lipoxygenase from the ammonium sulphate step was applied on a Sephadex G-150 column (2·5 × 90 cm), eluted with 0·05 M phosphate buffer (pH 7·0) at a flow rate of about 17 ml h^{-1}. Fractions of 5 ml were collected. The column was calibrated with catalase, globulin, soybean lipoxygenase, bovine serum albumin, ovalbumin, chymotrypsinogen and cytochrome c.

Sodium dodecyl sulphate (SDS) gel electrophoresis

The molecular weights of the lipoxygenase isoenzymes were determined by SDS gel electrophoresis using 10 % acrylamide in the small pore gel according to the method of Weber & Osborne (1969). The standard proteins used were lysozyme, β-lactoglobulin, trypsinogen, pepsin, ovalbumin and bovine plasma albumin.

Kinetic constants

The apparent Michaelis–Menten constant (K_m) and maximal velocity (V_{max}) for linoleic acid were calculated using the Lineweaver–Burk method. A molar extinction coefficient of $2{\cdot}80 \times 10^4$ was used in all calculations (Privett *et al.*, 1955). In the aforementioned lipoxygenase assay, a one unit increase in absorbance equalled the oxidation of 0·107 μmol of linoleic acid.

The activation energy required to oxidise linoleic acid between 30 and 50 °C was determined by an Arrhenius plot (log V v. $1/K \times 10^3$), using the slope to calculate the activation energy.

Effect of metal ions and inhibitors

Metal ions (1 mM) were added to the enzyme preparations and allowed to stand at 30 °C for 30 min at pH 7·0. The residual activities were assayed. The enzyme was incubated with inhibitor in the reaction mixture for 5 min at 25 °C, and the substrate was then added to start the reaction.

Effect of pH on stability

The concentrated Sephadex G-150 pooled fraction was diluted with appropriate buffer (0·1 M citrate–phosphate buffer for pH 3 and 6; 0·1 M sodium phosphate

buffer for pH 6·5 and 8; 0·1 M Tris-HCl for pH 9 and 10) and allowed to stand at room temperature for 30 min. The remaining lipoxygenase activity was assayed as described above.

RESULTS AND DISCUSSION

Optimum pH and temperature

Purified winged bean lipoxygenase isoenzymes FI and FII had pH optima at pH 6·0 and 5·8, respectively, at two substrate concentrations, 0·5 mM and 1·6 mM linoleic acid. These values are close to the pH 6·2 value reported for soybean lipoxygenase-2 and -3 (Christopher *et al.*, 1972; Diel & Stan, 1978) and peanut lipoxygenase-2 and -3 (Sanders *et al.*, 1975) and pH 6·0 for pea lipoxygenase (Yoon & Klein, 1979).

Partially purified lipoxygenase from Sephadex G-150 exhibited optimal activity at 30 °C and showed greater sensitivity to temperature changes than the enzyme in the crude extract. Optimal temperature close to that of winged bean has been reported for soybean (Koch *et al.*, 1958). However, Lulai & Baker (1976) reported optimal temperature of 47 °C for barley lipoxygenase.

An activation energy of 4·5 kcal mol^{-1} was calculated for winged bean lipoxygenase within the temperature range of 30–50 °C. Pea and wheat lipoxygenases have an activation energy of 2·5 kcal mol^{-1} (Irvine & Anderson, 1953; Svenson & Erickson, 1972). Horse bean lipoxygenase has been reported to have an activation energy of 7·17 kcal mol^{-1} (Nicolas & Drapron, 1977).

Substrate dependence

The effect of substrate concentration on the activity of the purified winged bean lipoxygenase FI and FII at 0·075 % Tween 20 was studied. Apparent K_m values of $0{\cdot}44 \times 10^{-3}$ M and $0{\cdot}37 \times 10^{-3}$ M linoleic acid for FI and FII, respectively, were obtained. These values are quite close to the $0{\cdot}4 \times 10^{-3}$ M value for the enzyme in the crude extract and Sephadex G-150 pooled fraction (Truong, 1980). Maximal activity was attained at $1{\cdot}6 \times 10^{-3}$ M linoleic acid. Higher substrate concentration produced only a slight increase in enzyme activity. In contrast, crude barley lipoxygenase has been shown to attain optimal activity at only $5{\cdot}4 \times 10^{-5}$ M linoleic acid followed by a rapid decrease in activity at greater substrate concentrations. Truong *et al.* (1979) have reported a K_m of $0{\cdot}195 \times 10^{-3}$ M for crude cowpea lipoxygenase with V_{max} at $1{\cdot}6 \times 10^{-3}$ M; a slight increase in activity beyond this value was also observed.

The concentration of 0·075 % Tween 20 in the reaction mixture was found optimal for winged bean lipoxygenase. Higher levels of Tween 20 drastically reduced its activity. Ben-Aziz *et al.* (1970) had previously found that Tween 20 acted as a competitive inhibitor of soybean lipoxygenase. Unlike winged bean lipoxy-

genase, soybean lipoxygenase exhibited different K_m values which were much lower than those of winged bean lipoxygenase when the enzyme was assayed in various Tween 20 concentrations (Ben-Aziz *et al.*, 1970).

Bubbling oxygen into the buffer for 10–20 min at 15 p.s.i. pressure before adding the substrate and the enzyme solution in the reaction mixture had no effect on the activity. The dissolved oxygen in the buffer mixture appeared to be sufficient to saturate the enzyme for its optimum activity.

Substrate specificity

Table 1 shows that linoleic acid is the most suitable substrate for winged bean lipoxygenase. The activities of FI and FII with linolenic acid as substrate were only 17

TABLE 1
SUBSTRATE SPECIFICITY OF WINGED BEAN LIPOXYGENASE

Substrate	*Relative activity* (%)	
	FI	*FII*
Oleic acid	0	0
Linoleic acid	100	100
Linolelaidic acid	0	0
Linolenic acid	17	23
Monolinolein	0	0
Trilinolein	0	0
Linoleic methyl ester	0	0
Linoleic ethyl ester	0	0

and 23 % of their respective activities using linoleic acid as substrate. Monolinolein, trilinolein, linoleic methyl ester, linoleic ethyl ester were not oxidised by the enzyme preparations. In contrast, soybean lipoxygenase isoenzymes differ markedly in their activities toward free linoleic acid, linoleate methyl ester and trilinolein. Based on their preferential activity, the terms 'linoleic acid' lipoxidase, 'triglyceride' lipoxidase and 'ester' lipoxidase were suggested for different isoenzymes (Koch *et al.*, 1958; Verhue & Francke, 1972). Haydar *et al.* (1975) reported that pea lipoxygenase has high activity with either free linoleic acid or trilinolein. The inability of FI and FII to oxidise oleic acid, a mono-unsaturated fatty acid, and linolelaidic acid, a *cis-trans* isomer of linoleic acid (Table 1) strongly confirms the lipoxygenase nature of the two enzyme preparations.

Molecular weight

Winged bean lipoxygenase isoenzymes FI and FII were observed to elute from the Sephadex G-150 column at a volume corresponding to a molecular weight of 78 000 daltons. The molecular weight was obtained from a plot of K_{av} versus molecular weight of standard proteins (Fig. 1). Peanut and soybean lipoxygenase isoenzymes

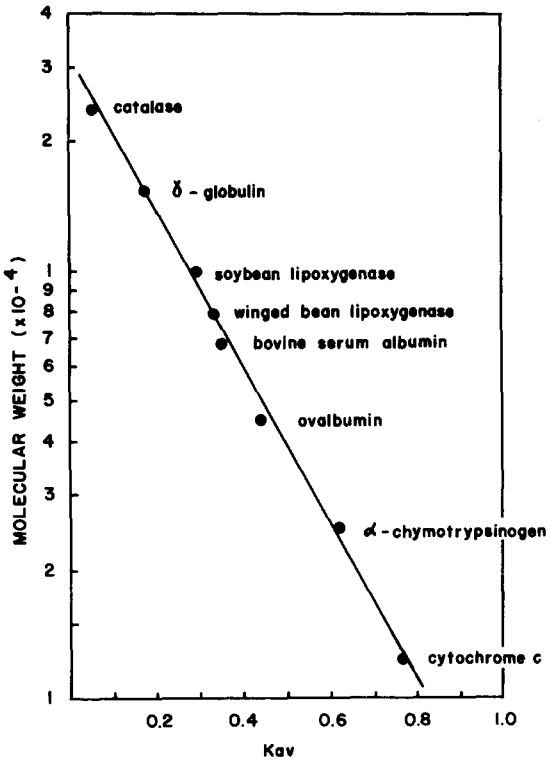


Fig. 1. Calibration curve for determination of molecular weight of winged bean lipoxygenase using Sephadex G-150 column chromatography (column dimensions: 2·5 × 90 cm). $K_{av} = V_e - V_0/V_t - V_0$; where V_e = elution volume; V_t = total volume; V_0 = void volume.

exhibit molecular weights of 73 000 and 105 000 daltons, respectively (Sanders *et al.*, 1975; Diel & Stan, 1978).

Using the SDS gel electrophoresis method, the molecular weight of FI and FII were found to be identical, about 82 000 daltons (Fig. 2), which is close to the value obtained from the gel filtration technique. Diel & Stan (1978) also obtained the same molecular weights of 100 000 for both soybean lipoxygenase-1 and -2 using this method.

TABLE 2
EFFECT OF DIFFERENT IONS ON THE STABILITY OF WINGED BEAN LIPOXYGENASE[a]

Enzyme fraction	*Relative activity* (%)										
	None	Ni^{++}	Ba^{++}	Hg^{++}	Cu^{++}	Zn^{++}	Mn^{++}	Mg^{++}	Co^{++}	Fe^{++}	Fe^{+++}
FI	100	70	84	0	87	144	65	61	127	194	17
FII	100	85	52	0	100	124	50	50	144	220	0

[a] Values shown are means of four replicates.

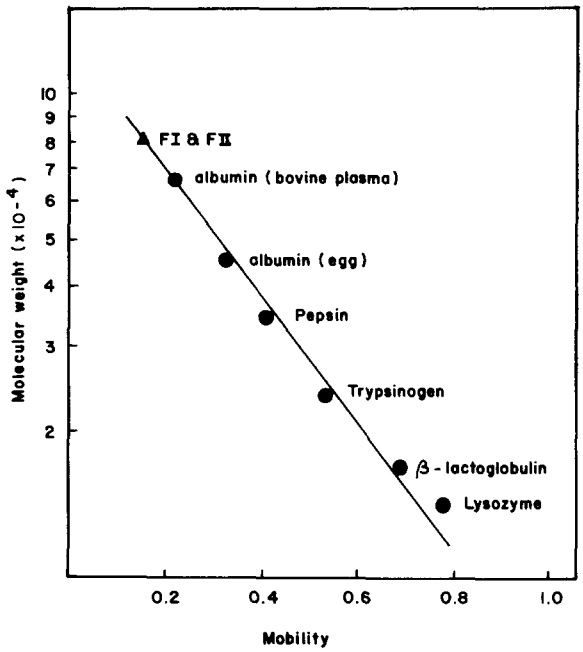


Fig. 2. Calibration curve for determination of the molecular weights of FI and FII using SDS polyacrylamide gel electrophoresis.

The SDS–PAGE protein patterns of FI and FII showed a major protein band at a mobility of 0·14 which was considered to be the protein of winged bean lipoxygenase, and four and six faint bands for FI and FII, respectively.

Effect of various metal ions on winged bean lipoxygenase isoenzymes

Table 2 shows the strong activation of FI and FII by Zn^{++}, Co^{++} and Fe^{++} and strong inhibition by Hg^{++}, Mg^{++} and Fe^{+++}. Although the trend in the response of FI and FII to the ions was similar in all cases, a difference in the degree of inhibition or activation by the metal on the isoenzyme activity was apparent.

The inhibitory effect of Fe^{+++} probably resulted from conformational changes in the enzyme affecting its active or binding site. Pistorius & Axelrod (1974) have reported the occurrence of one atom of Fe per mole of soybean lipoxygenase and that iron directly participated in the catalytic action of the enzyme.

Effect of antioxidants and inhibitors

In general, antioxidants inhibited the winged bean lipoxygenase reaction (Table 3). Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) showed complete inhibition at 10^{-6} M whereas ascorbic acid, hydroquinone and pyrogallol greatly inhibited the reaction at higher concentrations (10^{-4} to 10^{-3} M). Similar

TABLE 3
EFFECT ON WINGED BEAN LIPOXYGENASE OF DIFFERENT INHIBITORY REAGENTS AT VARIOUS CONCENTRATIONS

Effector	*% Inhibition*				
	10^{-7}M	10^{-6}M	10^{-5}M	10^{-4}M	10^{-3}M
Ascorbic acid					
FI	0	10	62	100	—
FII	8·8	33	69	100	—
H_2O_2					
FI	0	0	8	13	56
FII	0	0	0	37	93
Pyrogallol					
FI	15	10	21	30	100
FII	0	14	54	90	100
Propyl gallate					
FI	10	43	73	100	—
FII	16	31	70	100	100
Hydroquinone					
FI	11	16	36	76	—
FII	0	28	26	88	—
BHA					
FI	4	100	—	—	—
FII	17	100	—	—	—
BHT					
FI	0	100	—	—	—
FII	18	100	—	—	—
EDTA					
FI	0	0	0	0	0
FII	0	0	0	0	0
Cystein					
FI	0	0	0	0	10
FII	—	—	0	15	54
Dithiothreitol					
FI	0	10	25	25	42
FII	0	0	30	34	43
Iodoacetamide					
FI	—	—	—	0	0
FII	—	—	—	0	4

actions of antioxidants have been shown for other lipoxygenases (Beaux & Drapron, 1974; Palla & Verrier, 1974; Bonnet & Crouzet, 1977).

Ascorbic acid at a concentration of 10^{-4} M completely inhibited the two winged bean lipoxygenase fractions. Ascorbic acid is used to regulate the bleaching ability and rheological properties of lipoxygenase in the dough. Irvine & Anderson (1953) reported that ascorbic acid is a competitive inhibitor of durum wheat lipoxygenase.

Sulphydryl reagents like iodoacetamide had little or no effect on the winged bean lipoxygenase FI and FII although dithiothreitol at 10^{-5} M inhibited the isoenzymes by 25% and 34%, respectively.

Action of calcium ion and ethanol

Calcium ions were found to inhibit FI and FII activities at Ca^{++} concentrations

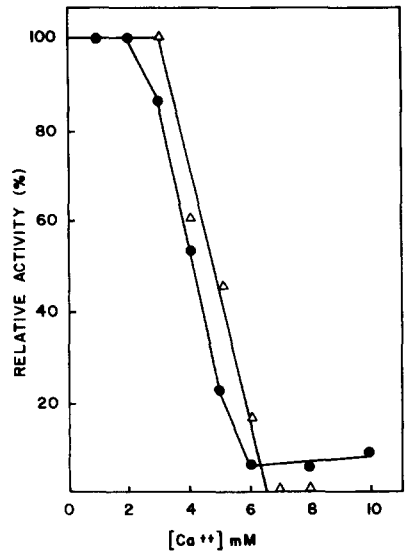


Fig. 3. Effect of calcium ions on winged bean lipoxygenase activity; ●—●, FI; △—△, FII.

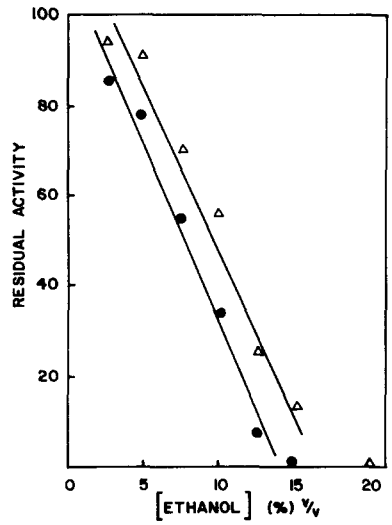


Fig. 4. Effect of ethanol on winged bean lipoxygenase activity; ●—●, FI; △—△, FII.

higher than 2 and 3 mM, respectively (Fig. 3). However, at Ca^{++} concentrations higher than 6 mM, FI still had about 7% of the control activity while FII was completely inactivated. In contrast, activation by Ca^{++} of soybean lipoxygenase-2 in the absence of Tween 20 was observed by Zimmerman & Snyder (1974). The assay of winged bean lipoxygenase without Tween 20 is not possible because if ethanol were used as solubilising agent, the high amount of ethanol (about 30%) needed to clarify the reaction mixture of pH 5·8 will inactivate the enzyme completely (see below).

However, even with Tween 20 in the reaction mixture, Sanders *et al.* (1975) were able to observe different responses of peanut lipoxygenase isoenzymes to various Ca^{++} concentrations. Inhibitory effects of Ca^{++} on soybean lipoxygenase-3 and pea lipoxygenase have also been reported (Christopher *et al.*, 1972; Haydar *et al.*, 1975).

The action of ethanol on FI and FII is shown in Fig. 4. FI was observed to be more sensitive to ethanol than FII. A complete inactivation of FI activity was obtained at 15% ethanol but about 12% activity of FII still remained at this concentration. Saturated monohydric alcohols inhibit lipoxygenase by hydrophobic bond formation between alcohol and enzyme (Mitsuda *et al.*, 1967). Steeping or wet milling of whole legumes with aqueous ethanol has been used to inactivate lipoxygenase in order to improve the flavour of legume products (Eldridge *et al.*, 1977). The above results suggest that ethanol treatment of winged bean seeds might be employed before processing to produce acceptable food products of this legume.

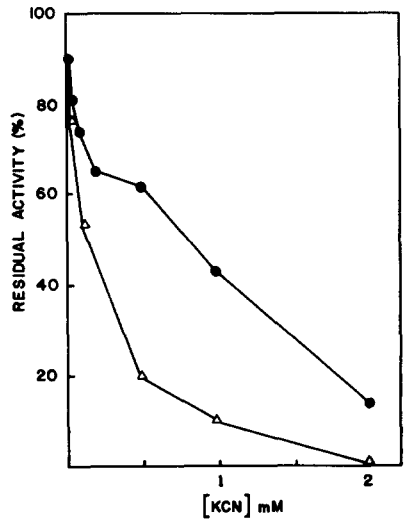


Fig. 5. Effect of KCN on winged bean lipoxygenase activity; ●—●, FI; △—△, FII.

Holden (1970) distinguished the peroxidation by lipoxygenase and by haeme protein based on their response to ethanol. Peroxidation by haeme protein was unaffected by ethanol concentrations up to 20% whereas drastic reduction of activity was already evident with lipoxygenase-catalysed reactions at this concentration.

Effect of KCN

Figure 5 shows the inactivation of winged bean lipoxygenases FI and FII by varying amounts of KCN. FI was less sensitive to cyanide than FII.

Cyanide ions have traditionally been used to differentiate haeme-catalysed from lipoxygenase-catalysed oxidation. However, contradictory results have been reported on the KCN effect on the various lipoxygenases (Grossman *et al.*, 1972; Sanders *et al.*, 1975; De Lumen, 1978; Flick *et al.*, 1978); thus the value of this test has become questionable.

Stability

The partially purified enzyme from the Sephadex G-150 step was found to retain 50% of its activity at pH 4·5 and pH 8·8 and was maximal at pH 7·2. At pH 3 and 10, activity was lower than 20%. The pH stability and pH activity profile of winged bean lipoxygenase could help in preventing the action of this enzyme during processing. An improvement in flavour of winged bean product might be obtained by soaking or disrupting the seeds in an acidic or alkali media. Recently, De Lumen & Salamat (1980) reported the beneficial effect of soaking winged bean seeds in aqueous alkaline solutions on the inactivation of trypsin inhibitor activity.

Lipoxygenase in the crude extract was highly stable at 60 °C for more than 1 h. It lost half of its activity in 25 min at 65 °C and in 1 min at 70 °C (Fig. 6). The purified enzymes, FI and FII, were more sensitive to heat treatment, losing half of their activities in less than 10 min at 65 °C. However, FII showed a slower rate of inactivation after 10 min at 65 °C. FII still had 25 % of its original activity after 40 min at 65 °C although FI was completely inactivated under these conditions.

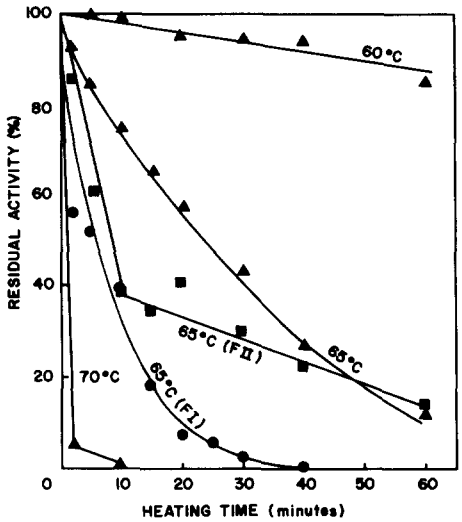


Fig. 6. Thermal stability of winged bean lipoxygenase. ▲—▲, crude extract; ●—●, FI; ■—■, FII.

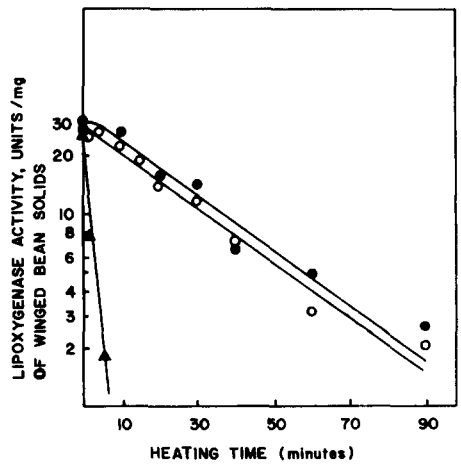


Fig. 7. Effect of heating time on the lipoxygenase levels of whole winged bean seeds; ●—●, dry heat at 100 °C, unsoaked seeds; ○—○ and ▲—▲ steaming at 100 °C, unsoaked and soaked seeds, respectively.

In situ, the enzyme in dry seeds (8 % moisture) was very stable even at 100 °C of dry heat and steam (Fig. 7). Dry seeds of winged bean were heated in an oven for various periods of time and lipoxygenase activity was determined. The inactivation exhibited was a first order type. Only 50 % of the enzyme activity of the dry winged bean seeds was lost by either steaming or dry heating at 100 °C for 20 min. On the other hand, steaming the whole soybean seeds of six varieties (8 % moisture) which possess lipoxygenase activity similar to that of winged bean seeds (average of 22 units v. 26 units per mg solid for soybean and winged bean, respectively) resulted in a negligible lipoxygenase activity after 3 min (Rice, 1972). This discrepancy may be due to the difference in the ultrastructure of the winged bean and soybean seeds which affects the heat penetration.

Soaking the winged bean seeds for 16 h markedly decreased the thermal stability of lipoxygenase as indicated in Fig. 7. Similar observations have been reported in whole soybeans (Yoshida, 1970) and wheat concentrates (Wallace & Wheeler,

1972). These results indicate that soaking of seeds before processing could be useful in preventing further lipoxygenase action on natural substances.

REFERENCES

BEAUX, Y. & DRAPRON, R. (1974). Isolement et quelques caracteristiques physico-chemiques de la lipoxygenase de la feve (*Vicia faba* L.), *Ann. Technol. Agric.*, **23**, 309–21.

BEN-AZIZ, A., GROSSMAN, S. & BUDOWSKI, P. (1970). Linoleate oxidation induced by lipoxygenase and haem protein: A direct spectrophotometric assay, *Anal. Biochem.*, **34**, 88–99.

BONNET, J. L. & CROUZET, J. (1977). Lipoxygenase from tomato fruits: Partial purification and study of some properties, *J. Food Sci.*, **42**, 625–8.

CHRISTOPHER, J. P., PISTORIUS, E. K. & AXELROD, B. (1972). Isolation of a third isoenzyme of soybean lipoxygenase, *Biochim. Biophys. Acta*, **284**, 54–62.

DE LUMEN, B. O. (1978). Formation of volatile flavor compounds in green beans from linoleic and linolenic acids, *J. Food Sci.*, **43**, 698–702.

DE LUMEN, B. O. & SALAMAT, L. A. (1980). Trypsin inhibitor activity in winged bean (*Psophocarpus tetragonolobus*) and the possible role of tannin, *J. Agric. Food Chem.*, **28**, 533–6.

DIEL, E. & STAN, H. J. (1978). Purification and characterization of two isoenzymes of lipoxygenase from soybeans, *Planta*, **142**, 321–8.

ELDRIDGE, A. C., WARNER, K. & WOLF, W. J. (1977). Alcohol treatment of soybeans and soybean protein products. *Cereal Chem.*, **54**, 1229–37.

FLICK, G. J. JR, BURNETTE, F. S., AUNG, L. H., ORY, R. L. & ST. ANGELO, A. J. (1978). Chemical composition and biochemical properties of Mirlitons (*Sechium edule*) and purple, green and white eggplants, *J. Agric. Food Chem.*, **26**, 1000–1005.

GARDNER, H. W. (1975). Decomposition of linoleic acid hydroperoxides. Enzymic reaction compared with non-enzymic, *J. Agric. Food Chem.*, **23**, 129–36.

GROSSMAN, S., TROP, M., AVTALION, R. & PINSKY, A. (1972). Eggplant lipoxygenase: Isolation and partial characterization, *Lipids*, **7**, 467–73.

HAYDAR, M., STEELE, M. and HADZIYEV, D. (1975). Oxidation of pea lipids by pea seed lipoxygenase, *J. Food Sci.*, **40**, 808–14.

HOLDEN, M. (1970). Lipoxidase activity of leaves, *Phytochemistry*, **9**, 507–12.

IRVINE, G. N. & ANDERSON, J. A. (1953). Kinetic studies of the lipoxidase system of wheat, *Cereal Chem.*, **30**, 247–55.

KOCH, R. B., STERN, B. & FERRARI, C. F. (1958). Linoleic acid and trilinolein as substrates for soybean lipoxidase(s), *Arch. Biochem. Biophys.*, **78**, 165–79.

LOWRY, O. H., ROSEBROUGH, N. I., FARR, L. & RANDALL, R. J. (1951). Protein measurements with the Folin-phenol reagent, *J. Biol. Chem.*, **193**, 265–75.

LULAI, E. C. and BAKER, C. W. (1976). Physicochemical characterization of barley lipoxygenase, *Cereal Chem.*, **53**, 775–86.

MITSUDA, H., YASUMOTO, K. & YAMAMOTO, H. (1967). Inhibition of lipoxygenase by saturated monohydric alcohols through hydrophobic bondings, *Arch. Biochem. Biophys.*, **118**, 664–9.

NICOLAS, J. & DRAPRON, R. (1977). Some physicochemical characteristics of horse-bean lipoxygenase (*Vicia faba* L.), *Ann. Technol. Agric.*, **26**, 199–232.

PALLA, J. C. & VERRIER, J. (1974). Inhibition of bean lipoxygenase by hydroquinone derivatives, *Ann. Technol. Agric.*, **23**, 367–73.

PISTORIUS, E. K. & AXELROD, B. (1974). Iron an essential component of lipoxygenase, *J. Biol. Chem.*, **249**, 3183–6.

PRIVETT, O. S., NICKELL, C. & LUNDBERG, W. O. (1955). Products of the lipoxidase-catalyzed oxidation of sodium linoleate, *J. Am. Oil Chem. Soc.*, **32**, 505.

RICE, R. D. (1972). Ph.D. dissertation, University of Illinois, Urbana.

SANDERS, T. J., PATTEE, H. F. & SINGLETON, J. E. (1975). Lipoxygenase isoenzymes of peanut, *Lipids*, **10**, 681–5.

SVENSON, S. G. & ERICKSON, C. E. (1972). Thermal inactivation of lipoxygenase from pea (*Pisum sativum* L.) I. Time–temperature relationships and pH dependence, *Lebensm. Wiss. Technol.*, **5**, 118–23.

TRUONG, VAN DEN. (1980). Lipoxygenase isoenzymes of winged bean, *Psophocarpus tetragonolobus* (L.) DC. Ph.D. dissertation, University of the Philippines at Los Baños College, Laguna, Philippines.

TRUONG, VAN DEN, RAYMUNDO, L. C. & MENDOZA, E. M. T. (1979). Cowpea lipoxygenase: Part I. Physicochemical characterizations, *Bull. Philippine Biochem. Soc.*, **2**, 1–12.

TRUONG, VAN DEN, RAYMUNDO, L. C. & MENDOZA, E. M. T. (1982). Winged bean lipoxygenase—Part 1: Isolation and purification, *Food Chem.*, **8**, 187–201.

VERHUE, W. M. & FRANCKE, A. (1972). The heterogeneity of soybean lipoxygenase, *Biochim. Biophys. Acta.*, **284**, 43–53.

WALLACE, J. M. & WHEELER, E. L. (1975). Lipoxygenase inactivation in wheat protein concentrate by heat moisture treatments, *Cereal Chem.*, **49**, 92–8.

WEBER, K. & OSBORNE, M. (1969). The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis, *J. Biol. Chem.*, **244**, 4406–12.

YOON, S. & KLEIN, B. P. (1979). Some properties of pea lipoxygenase isoenzymes, *J. Agric. Food Chem.*, **27**, 955–62.

YOSHIDA, K. (1970). Lipoxidase activity of soybean and other foods, *J. Japan Soc. Food and Nutrition*, **23**, 422, *Food Sci. Technol. Abstr.*, **3**, 10J1298.

ZIMMERMAN, D. C. & SNYDER, H. G. (1974). Role of calcium in activating soybean lipoxygenase-2, *J. Agric. Food Chem.*, **22**, 802–5.

Food Chemistry 8 (1982) 291–297

CHEMICAL COMPOSITION OF SOME TISSUES OF THE ANTARCTIC FISH *NOTOTHENIA ROSSII MARMORATA*, FISCHER 1885*

J. OEHLENSCHLÄGER & H. REHBEIN

Federal Research Board of Fisheries,
Institute for Biochemistry and Technology,
Palmaille 9, 2000 Hamburg 50, Federal Republic of Germany

(Received: 22 April, 1981)

ABSTRACT

Muscle, liver, stomach, intestinal tract and pyloric caeca of the Antarctic fish Notothenia rossii marmorata, *Fischer 1885, were analysed for dry matter, crude protein, carbohydrate, total lipids, ash, chloride, phosphorus and fluoride. It was found that the levels of these classes of substance varied considerably within ten fishes of a pre-spawning community.*

The highest level of protein was found in the stomach (80·8% mean value on a dry weight basis) and the lowest in the liver (39·6%); for total lipids in the liver and stomach the situation was reversed with the relevant figures being 40·8% and 11·27%, respectively. The pyloric caeca showed a maximum of ash content (6·34%), whilst only 3·92% was found in the liver. The liver was extremely rich in carbohydrates (8·22%) whilst, at 1·64%, the muscle contained least. Although N. rossii marmorata *mainly feeds on the fluoride-containing krill* (Euphausia superba Dana), *the tissues investigated showed only a low level of fluoride (10–40 ppm).*

INTRODUCTION

Notothenia rossii marmorata, Fischer 1885 (also called South Georgian cod or antarctic marble perch) is one of the commercially most valuable fishes of the Antarctic (Kock, 1975). It is well adapted to cold waters and lives exclusively in Antarctic waters, particularly off South Georgia, the South Orkneys and the South Shetlands (Freytag, 1980). *N. rossii marmorata* feeds mainly on krill (*Euphausia superba Dana*) but also on crustaceans, polychaets and salps.

* Results of the Second Antarctic Expedition, 1977/1978, of the Federal Republic of Germany.

Food Chemistry 0308-8146/82/0008-0291/$02·75

Due to its high quality taste and texture (Körner & Schreiber, 1980), the fish flesh is well suited to human consumption. Since 1970 this valuable food fish has been caught commercially in Antarctic waters by Soviet, Polish and other trawlers, and yearly catches of 200–300,000 (1970 for USSR) (Gulland, 1971) and 100,000 (1979 for Poland) (Anon, 1980) are reported.

Although there is a great deal of biological information on this important finfish, data on its chemical composition are limited and refer mainly to the fillet or the edible portion. Numerical data on the chemical composition of organs or tissues of *N. rossii marmorata* are very few (e.g. Rzhavskaya *et al.*, 1977; Korrengk, 1980). The values reported for the fillet range from 25·8 % to 34 % for dry matter, from 54·1 % to 57·8 % for crude protein, from 36·9 % to 41·5 % for total lipids and from 3·9 % to 5·0 % for ash content (all on a dry matter basis). Single values for NaCl (1·5 %) and phosphorus (0·57 %) have also been reported (Koval'cuk, 1970; Sahrhage *et al.*, 1978; Roschke & Schreiber, 1977; Podsevalov & Perova, 1973; Dubrovskaya & Makarov, 1969; Roschke, 1976).

This investigation describes the chemical composition of different tissues such as muscle, liver, stomach, intestinal tract and pyloric caeca, for dry matter, crude protein, total lipids, ash, carbohydrate, chloride, phosphorus and fluoride.

MATERIALS AND METHODS

N. rossii marmorata *specimen*

The fish investigated were caught by the commercial trawler *Julius Fock* (1568 GRT, 2260 hp) during the third stage (23 February–10 April, 1978) of the Second Antarctic Expedition (1977/1978) of the Federal Republic of Germany.

Pre-spawning *N. rossii marmorata* were caught on the 1st of March by bottom trawling (depth, 262–269 m) west of the South Shetland Islands (60° 52′ S, 55° 29′ W).

Directly after hauling of the commercial size catch (60 t), the *N. rossii* specimens used in this investigation were taken at random and killed by cutting their throats. After 10 min bleeding the fish were measured and the organs were carefully removed. The muscle tissue was freed from adhering lipid layers and belly flaps were removed. After emptying the stomach and the intestinal tract, all organs and the contents of the stomach and intestinal tract were sealed separately into polyethylene bags, immediately frozen to −30 °C and held at a storage temperature of −25 °C to −36 °C until analysis after landing.

A general description of the fish is given in Table 1.

Chemical analysis

All samples were weighed, thoroughly homogenised in the frozen state with distilled water 1:1 (w/v) and re-frozen to −30 °C. The frozen homogenates were

TABLE 1
GENERAL DATA ON THE FISH INVESTIGATED

Fish No.	*Total length (cm)*	*Sex*	*Content of stomach*
1	54	Male	Some liquefied krill
2	42	Female	A lot of krill
3	46	Male	Some krill
4	47	Male	Very little krill
5	64	Male	Some liquid
6	51	Male	A lot of liquid
7	48	Female	Sea anemone
8	57	Male	None
9	44	Male	Krill
10	57	Male	Some krill

freeze-dried and re-weighed. The resulting freeze-dried powder had a water content of less than 1 %, determined by the Karl-Fischer method. Prior to chemical analysis all samples were ground in a small mill.

Dry matter

The dry matter portion of the samples was calculated from the difference between the fresh weight and the weight of the freeze-dried powder.

Ash

Ash content was determined by heating the samples in a muffle furnace at 550 °C overnight.

Crude protein

To determine the crude protein content (N × 6·25) the samples were digested with H_2O_2/H_2SO_4/selenium catalyst at 300 °C for 2 h or until the reaction mixture was clear. After subsequent dilution and pH-adjustment to 7·6, the solution was titrated according to the method of Tegge & Bolling (1959).

Total lipids

Total lipid content was evaluated using the procedure of Bligh & Dyer (1959).

Carbohydrate

Carbohydrate was determined by means of a modification of the method of Dubois *et al.* (1956). Dextrose was used as a standard. The following recoveries were obtained after the addition of dextrose to extracts of the samples: for liver, 93·2 %; for stomach, 83·2 %; for intestinal tract, 88·4 %; for pyloric caeca, 88·0 %, and for muscle, 85·5 %, respectively.

The extraction of carbohydrate was undertaken by shaking 50 mg of the freeze-dried sample with 200 ml of 6 % perchloric acid in a water bath maintained at 70 °C

TABLE 2

MEAN VALUES OF CHEMICAL ANALYSES OF TEN FISH OF *N. rossii marmorata*

		On a dry weight basis						
		Dry matter (%)	*Crude protein* ($N \times 6{\cdot}25$) (%)	*Total lipids* (%)	*Ash* (%)	*Carbohydrates* (%)	*Chloride as NaCl* (%)	*Phosphorus* (%)
Muscle	$\bar{x}$	21·7	74·1	18·0	5·14	1·64	0·44	1·81
	s	1·77	9·29	7·7	0·48	0·48	0·13	0·28
	x_{min}–x_{max}	19·5–24·8	57·4–83·9	8·8–32·0	4·43–5·93	1·05–2·66	0·25–0·61	1·59–2·20
	v	8·14%	12·5%	42·8%	9·34%	29·3%	29·6%	15·5%
Liver	$\bar{x}$	37·0	39·6	40·9	3·92	8·22	0·89	1·78
	s	6·38	11·1	11·3	0·91	2·93	0·35	0·31
	x_{min}–x_{max}	28·5–46·7	20·5–53·7	27·7–61·3	3·01–5·97	5·48–15·0	0·45–1·68	1·33–2·24
	v	17·3%	28%	27·8%	23·2%	35·7%	39·3%	17·4%
Stomach	$\bar{x}$	19·5	80·8	11·3	6·26	2·26	2·56	2·15
	s	1·16	2·62	2·40	0·29	0·39	0·48	0·40
	x_{min}–x_{max}	17·8–21·8	73·9–83·0	9·40–16·9	5·80–6·70	1·65–2·70	1·86–3·37	1·39–2·63
	v	5·95%	3·24%	21·3%	4·63%	17·3%	18·8%	18·6%
Pyloric caeca	$\bar{x}$	24·8	63·2	26·9	6·34	2·14	1·36	2·36
	s	2·56	5·54	6·45	0·70	0·39	0·48	0·22
	x_{min}–x_{max}	22·3–30·2	57·1–71·7	18·4–36·9	5·80–7·27	1·66–2·80	1·10–1·78	2·02–2·56
	v	10·34%	8·77%	24·01%	11·4%	18·22%	35·29%	9·23%
Intestinal tract	$\bar{x}$	25·4	56·4	32·9	5·57	2·18	1·35	2·44
	s	3·4	9·05	9·20	1·02	0·30	0·27	0·48
	x_{min}–x_{max}	20·8–30·0	44·5–68·8	19·6–48·3	3·84–6·83	1·79–2·69	1·10–1·96	1·69–3·21
	v	13·4%	16·1%	28·0%	18·3%	13·8%	20·0%	19·6%

$\bar{x}$ = average value; s = standard error; v = coefficient of variation; x_{min}–x_{max} = range of the averages to compute the mean and error of the mean.

for 10 min. After cooling to ambient temperature and filtration, aliquots (2 ml) of the extracts were mixed with 0·1 ml 80 % phenol solution in water, followed by the addition of 5 ml concentrated sulphuric acid as quickly as possible by an automatic pipette. The hot solution was shaken immediately, allowed to stand for 20 min and re-mixed. After 10 min, the extinction was read at a wavelength of 490 nm against a reagent blank.

Phosphorus

Phosphorus was determined using the micro-method of Veerkamp & Broekhuyse (1976).

Chloride

For chloride determination, titration against silver nitrate according to the Mohr method, as modified by Antonacopoulos (1973) was used. Table 2 gives chloride as sodium chloride.

Fluoride

After ashing in a muffle furnace at 550 °C overnight and fusing with sodium hydroxide, the sample was acidified with hydrochloric acid. Fluoride was measured using a fluoride-specific electrode in a buffered system.

pH measurements

pH values were measured on board after dilution and shaking of the squeezed out contents of the stomach and intestinal tract with an equal amount of distilled water. The frozen samples were measured immediately after thawing.

RESULTS AND DISCUSSION

The chemical compositions of different tissues of *N. rossii marmorata* are given in Table 2. The values indicate that the chemical composition within a closely related community like the pre-spawning community varies remarkably. The greatest variations are found in the lipid content of muscle, which ranges from 8·8 % to 32·0 %, and in the carbohydrate and chloride contents of the liver, which ranges from 5·48 % to 15·0 % and from 0·45 % to 1·68 %, respectively. The tissues of organs which participate directly in the digestion of food,—the stomach, the pyloric caeca and the intestinal tract—exhibit a more constant chemical composition (e.g. protein content from 73·9 % to 83·0 % for the stomach). In these tissues a high level of chloride and phosphorus and, necessarily, ash, is found. In particular, the chloride level in the stomach is high due to the hydrochloric acid production in the stomach

wall. The hydrochloric acid produced is also evident in the low pH values of the stomach's contents.

Liver, pyloric caeca and intestinal tract show a low content of crude protein, while their lipid content is rather high. This verifies the fact that *N. rossii marmorata* stores a large part of its depot fat in the viscera (Kock, 1975). The stomach and muscle tissue of the edible part exhibit the highest protein content and the lowest lipid content. The liver is rich in carbohydrate and produces an unusually high portion of dry matter (37·0 %). These findings are in contrast to the values reported by Korrengk (1980): (25·2–27·7 % dry matter, $\sum$ crude protein + crude lipids + water + ash = 99·6 %).

The pH values of the stomach contents (2·71 ± 1·30) and the contents of the intestinal tract (7·42 ± 0·16) show a difference of *ca.* pH 5. Although the pH of the thawed samples with 2·85 ± 1·28 and 8·14 ± 0·26, respectively, is different from that of the fresh samples, the phenomenon is the same. No conclusions can be drawn from the pH value as to the state of feeding.

Generally, the chemical composition of the muscle and liver tissues is more non-homogeneous compared with the other tissues. This seems to be a function of the first two tissues to store available energy in the form of carbohydrates and lipids, while the other tissues, with the more constant lipid and carbohydrate levels, are more involved in the food digestion processes. In the fish investigated the energy stock is supposed to be nearly unaffected because of the short hauling time (< 30 min).

Compared with values given by some other authors, the muscle tissue of *N. rossii marmorata* was lean. The mean value of 18 % on a dry matter basis is lower than values previously reported which ranged from 36·9 % to 41·5 %. The difference originates from the fact that other investigators mainly examine the fish fillets (not the pure muscles) made on commercial filleting machines from whole fish. The largest part of these fillets is covered by a lipid layer, 2–4 mm thick, and they contain the belly flaps, giving rise to very high fat values (Papajewski & Schreiber, 1979). In this investigation, however, pure muscle tissue without the lipid layer was used. The lipid values found are derived from lipid forming an integral part of the muscle. The same relations are applicable to the high protein contents of the muscle tissues investigated, which are around 20 % higher than those reported by other authors.

The fluoride levels found in the organ tissues of *N. rossii marmorata* are low, amounting to 2·5–10 ppm for muscle, around 10 ppm for the liver, 20 ppm for the intestinal tract and the pyloric caeca and 40 ppm for the stomach (all values are on a dry weight basis). In contrast to these results are the fluoride levels for scales (*ca.* 700 ppm) and bones (*ca.* 1400 ppm on a wet weight basis). The fluoride taken up by *N. rossii marmorata* when feeding on the euphasiid krill, which has a high fluoride content of 2400 ppm in fat-free dry matter (Soevik & Braekkan, 1979), is either not incorporated at all, or only poorly incorporated, in the organ tissues of the fish. It is, however, stored in the bones and scales.

ACKNOWLEDGEMENTS

The skilful technical assistance of Miss R. Faust, Mrs V. Bose, Mr W. Krüger and Mr J. Brandt is greatly appreciated. The work was supported by the Federal Ministry of Food, Agriculture and Forestry, and the Federal Ministry of Research and Technology.

REFERENCES

ANON. (1980). Polen mit neuen antarktischen Erkenntnissen. *Allg. Fischw. Ztg.*, (14 January), 14.

ANTONACOPOULOS, N. (1973). Bestimmung des Kochsalzgehaltes. In: *Fische und Fischerzeugnisse* (2nd edn). Ludorff, W. & Meyer, V. (eds), Parey, Hamburg, p. 222.

BLIGH, E. G. & DYER, W. J. (1959). A rapid method of total lipid extraction and purification, *Can. J. Biochem. Physiol.*, **37**, 911–17.

DUBOIS, M., GILLES, K. A., HAMILTON, J. K., REBERS, P. A. & SMITH, F. (1956). Colorimetric method for determination of sugars and selected substances. *Anal. Chem.*, **28**, 350–6.

DUBROVSKAYA, T. A. & MAKAROV, Q. E. (1969). Technochemical characteristics of fish from the Scotia Sea and their use as food. *Trudy Vses. Naucho-Issled. Inst. Morsk. Ryb. Khoz. Okeanogr. (VNIRO)*, **66**, 311–17.

FREYTAG, G. (1980). Length, age and growth of *Notothenia rossii marmorata*, Fischer 1885, in the West Antarctic waters. *Arch. Fisch Wiss.*, **30**, 39–66.

GULLAND, J. A. (Ed.) (1971). *The fish resources of the oceans.* FAO Publications, Fishing News Ltd, London, 225 pp.

KOCK, K.-H. (1975). Verbeitung und Biologie der wichtigsten Nutzfischarten der Antarktis. *Mitteilungen Inst. Seefischerei*, **16**, 24–30.

KÖRNER, W. & SCHREIBER, W. (1980). Die Haltbarkeit einiger antarktischer Fische bei Frostlagerung. *Lebensmittel-Wissenschaft und Technologie.*, **13**, 326–9.

KORRENGK, R. (1980). Erfahrungen bei der Be- und Verarbeitung einiger antarktischer Fischarten. *Seewirtschaft*, **12**(6) 300–3.

KOVAL'CUK, G. K. (1970). Chemotechnical characterization of fish from the Antarctic part of the Indian ocean. *Rybnoe chozjajstvo*, **46**(10) 69–72.

MANTHEY, M. (1980). Fluorid-Gehalte in antarktischen Fischen. *Inf. Fischw.*, **27**, 261–2.

PAPAJEWSKI, H. & SCHREIBER, W. (1979). Fettverteilung beim antarktischen Marmorbarsch. *Fette, Seifen, Anstrichmittel*, **81**, 166–8.

PODSEVALOV, V. N. & PEROVA, L. I. (1973). Technochemical characteristics of some Atlantic species of fishes. *Tr. Atl. Naucho-Issled. Inst. Ryb. Khoz. Okeanogr.*, **52**, 146–64.

ROSCHKE, N. (1976). Chemische Analysen von Krill- und Fisch-produkten der deutschen Antarktis Expedition 1975/76: Methoden und erste Ergebnisse. *Inf. Fischw.*, **23**, 136–9.

ROSCHKE, N. & SCHREIBER, W. (1977). Analytik von Krill, Krillprodukten und antarktischen Fischen. *Arch. Fisch Wiss.*, **28**, 135–41.

RZHAVSKAYA, F. M., MAKAROVA, A. M. & PRAVDINA, L. V. (1977). Nutritional value of lipids in the liver of various fish. *Rybnoe chozjajstvo*, **53**, (12), 58–61.

SAHRHAGE, D., SCHREIBER, W., STEINBERG, R. & HEMPEL, G. (1978). Antarctic Expedition, 1975/76, of the Federal Republic of Germany. *Arch. Fisch Wiss.*, **29**, (1), 90.

SOEVIK, T. & BRAEKKAN, O. R. (1979). Fluoride in Antarctic krill (*Euphausia superba*) and Atlantic krill (*Meganyctiphanes norvegica*). *J. Fish. Res. Board Can.*, **36**, 1414–16.

TEGGE, G. & BOLLING, H. (1959). Die Anwendung der Formoltitration auf die Eiweibbestimmung, *Stärke*, **11**, 162.

VEERKAMP, J. H. & BROEKHUYSE, R. M. (1976). Techniques for the analysis in membrane lipids. In: *Biochemical analysis of membranes.* (Maddy, A. H. (Ed.)) Chapman & Hall, London, p. 275.

Food Chemistry 8 (1982) 299–305

GAPING STUDIES IN BLUE WHITING (*MICROMESISTIUS POUTASSOU*)—THE EFFECT OF PRE-FREEZING TREATMENTS

O. A. AFOLABI & O. L. OKE

Department of Chemistry, University of Ife, Ile-Ife, Nigeria

&

J. LAVÉTY

Torry Research Station, P.O. Box 101, Victoria Rd, Torry, Aberdeen, Great Britain

(Received: 13 July, 1981)

ABSTRACT

Blue whiting caught in January and April were stored in ice, iced sea water and at ambient temperature for varying lengths of time before freezing on board. Thawed fish samples were scored for gaping. The results indicate that storage in iced sea water before freezing reduced both gaping and the seasonal variability of the degree of gaping in the blue whiting.

INTRODUCTION

In recent years attention has turned to some unconventional sources of fish as food in North Atlantic waters, in view of dwindling stocks of traditional fish species and the imposed 200 nautical miles international fishing limit. One of these 'new' fish species is the blue whiting (*Micromesistius poutassou*), a gadoid. Biologists estimate at least 8–10 million metric tons (tonnes) of blue whiting exist in the North Atlantic, allowing annual catches of 1–2 million tonnes (Dagbjartsson, 1975).

Although the gross chemical composition of blue whiting is similar to cod (Dagbjartsson, 1975) research workers in Scotland (Torry Research Station, Aberdeen) and elsewhere, Ström (1978), have been surprised by the much greater degree of seasonal variability of blue whiting than cod and other gadoids.

Blue whiting is always trawled on voyages lasting days if not weeks. After catch, therefore, the storage of fish creates spoilage problems. At ambient temperature fish

Food Chemistry 0308-8146/82/0008-0299/$02·75

spoil by a number of processes, the most important of these being enzymatic, microbiological and to a lesser extent auto-oxidative.

Freezing can reduce or even stop enzymatic and bacterial activities. Present day freezing practice requires prefreezing treatment (viz. chilling in ice, iced sea water, brine, etc.), to yield improved fish and fish products on thawing. Sometimes, on thawing the fish, holes and slits appear on the fillet and this may be so bad that the fillet cannot be sold even though taste and texture after cooking may remain satisfactory. This phenomenon in industry is known as gaping.

The purpose of the present study, therefore, is to look into the effect of prefreezing treatment on gaping characteristics of blue whiting and its seasonal variation.

MATERIALS AND METHODS

All fish used in this work were caught between North East Faroes (62° 16′N, 03° 52′W—January) and St Kilda (57° 50′N, 09° 30′W—April). Blue whiting were caught during daylight hours by small pelagic trawl usually at depths between 280 and 450 m. During each cruise whole fish samples (about 1·5–2·5 tonnes) were subjected to delays of 4, 8, 10, 12, 16, 20, 24, 30, 36, 40, 50, 60, 80 and 100 h either at ambient temperature (20 °C) or boxed in ice or iced sea water before freezing in a vertical plate freezer on board in 100 mm thick blocks (50 kg). The blocks were then stored at −30 °C until required for gaping assessment.

Gaping assessment

Two blocks of each delay and trip were assessed for gaping. The blocks were taken out of the cold store (−30 °C) and thawed at room temperature until they felt soft when gently squeezed. Fish was cut into single fillets and gaping was visually assessed on a scale of 0–5 (0 = smooth fillet; 5 = extensive break through the thickness of the fillet) (Love *et al.*, 1969). All gaping assessment was carried out in the chillroom at 1 °C. Each figure is a mean based on two samples (i.e. both fillets of fish) assessed by three panel members.

pH Determination

The pH was measured after homogenising 10 g of muscle from an anterior portion of blue whiting fillet (obtained from the same batch as above) for 15 s with 20 ml of water in an Ultra-Turrax homogeniser (Type TP 18/2N, Jackee and Kunkel KG/Stanfor I., Brunswick, Germany) using a Corning pH meter (Model 30, Tall pH Dept., Corning Glass Works, Medfield, Massachusetts 02052, USA).

Water content

Water contents of samples used above were calculated from the weight loss after 4 g of muscle had been maintained in an open vessel at 100 °C for 1 week.

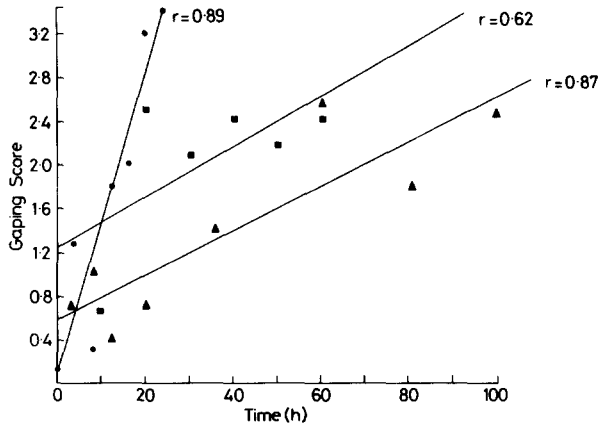


Fig. 1. Relationship between gaping score and prefreezing treatment in blue whiting caught in January. ● ambient, ▲ iced sea water, ■ ice.

RESULTS AND DISCUSSION

The results of these studies show that in general there is an increase in gaping scores with increased delay time (Figs 1 and 2). A comparison of results for the blue whiting caught in January and April (Tables 1 and 2) revealed that the spawning fish (April fish) gapes less than the good condition fish caught in January (treatment not withstanding). Figures 1 and 2 also reveal that the gaping scores vary with treatment according to the rule:

Ambient > Ice > Iced sea water

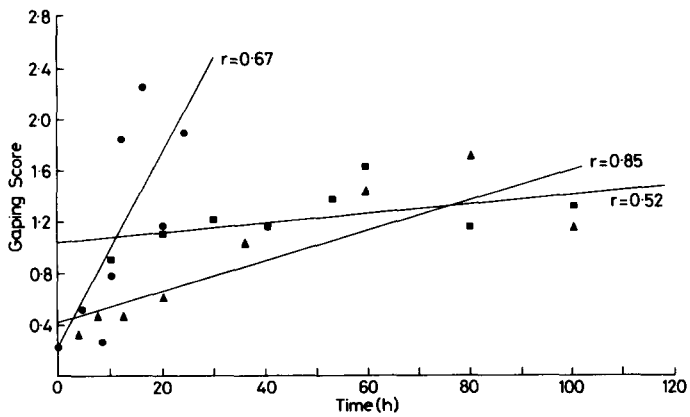


Fig. 2. Relationship between gaping score and prefreezing treatment in blue whiting caught in April. ● ambient, ■ ice, ▲ iced sea water.

TABLE 1

GAPING SCORES, pH AND WATER CONTENT OF BLUE WHITING CAUGHT IN JANUARY AND KEPT IN ICED SEA WATER AND AT AMBIENT TEMPERATURE (20 °C) FOR VARYING DELAY TIMES BEFORE FREEZING[a]

Treatment	*Delay time (h)*	*Gaping score*	*% H_2O*	*pH*
Control	0	0·09 (0·26)	80·7 (0·84)	6·62 (0·14)
	4	1·27 (0·98)	80·7 (0·77)	6·78 (0·10)
	8	0·31 (0·53)	80·9 (0·36)	6·72 (0·09)
	10			
Ambient	12	1·80 (1·05)	80·4 (1·00)	6·73 (0·12)
	16	2·02 (1·84)	80·4 (0·30)	6·78 (0·10)
	20	3·21 (1·03)	80·4 (0·67)	6·62 (0·09)
	24	3·43 (1·20)	80·6 (0·89)	6·79 (0·11)
	10	0·68 (1·29)	80·9 (0·46)	6·77 (0·09)
	20	2·51 (1·04)	80·8 (0·44)	6·67 (0·04)
	30	2·11 (1·04)	80·2 (0·70)	6·73 (0·07)
Ice	40	2·44 (1·13)	80·9 (0·63)	6·62 (0·10)
	50	2·21 (1·19)	81·0 (0·84)	6·78 (0·13)
	60	2·43 (1·03)	80·7 (0·91)	6·72 (0·08)
	80			
	100			
	4	0·69 (0·83)	80·1 (0·54)	6·69 (0·11)
	8	1·02 (0·85)	80·5 (0·98)	6·65 (0·09)
	12	0·41 (0·80)	80·4 (1·04)	6·67 (0·11)
Iced sea water	20	0·71 (0·84)	80·5 (0·62)	6·48 (0·09)
	36	1·42 (1·01)	80·9 (0·43)	6·78 (0·07)
	60	2·61 (2·53)	80·8 (0·49)	6·78 (0·12)
	80	1·82 (1·08)	81·2 (0·84)	6·78 (0·07)
	100	2·52 (2·73)	80·8 (0·30)	6·73 (0·06)

[a] Values expressed are means of 240 scores; values in parentheses are the standard deviations.

In order to monitor the nutritional status of the fish used in these studies, using pH and % water content as indicators (Love & Robertson, 1968) a good correlation was observed between pH and % water content of the fish, (correlation coefficient = 0·96, $P < 0·01$). This observation, according to Love & Robertson (1968) means that the good condition fish has a lower pH value and lower % water content than the spawning fish (Fig. 3).

The main structures of a fish fillet are the contractile cells which run in a roughly anterior–posterior direction, and sheets of connective tissue which cut across them (Love, 1970). The connective tissue sheet (myocommata) do not run vertically from the cut surface to the skin surface, but curve in a complex manner inside the thickness of the fillet (Nursall, 1956). Usually, all the tissues hold together so that a cut surface of the fillet is smooth and continuous, but occasionally the connective tissue structures break down. When this occurs the muscle blocks (myotomes) become separated from the myocommata and holes or slits appear in the fillet.

After death the temperature of all tissues rises. The heat liberated is much larger than that generated by the living body. This is because soon after death an intensive

TABLE 2

GAPING SCORES, pH AND WATER CONTENT OF BLUE WHITING CAUGHT IN APRIL AND KEPT IN ICED SEA WATER AND AT AMBIENT TEMPERATURE (20°C) FOR VARYING DELAY TIMES BEFORE FREEZING[a]

Treatment	*Delay time (h)*	*Gaping score*	*% H_2O*	*pH*
Control	0	0·22 (0·37)	81·5 (0·44)	6·78 (0·08)
	4	0·58 (0·73)	82·7 (0·38)	6·80 (0·16)
	8	0·25 (0·52)	82·9 (1·10)	6·98 (0·14)
	10	0·76 (0·86)	82·2 (0·58)	6·89 (0·11)
Ambient	12	1·86 (0·96)	82·6 (0·60)	6·71 (0·16)
	16	2·25 (2·04)	82·8 (0·18)	6·66 (0·18)
	20	1·16 (0·88)	82·3 (0·69)	7·05 (0·12)
	24	1·79 (1·22)	82·1 (0·77)	6·84 (0·07)
	10	0·98 (0·88)	82·2 (0·51)	6·89 (0·11)
	20	1·12 (0·87)	82·0 (0·57)	6·89 (0·05)
	30	1·22 (0·86)	84·5 (0·66)	6·97 (0·10)
Ice	40	1·16 (0·86)	83·0 (0·80)	6·96 (0·12)
	50	1·38 (0·89)	83·3 (0·86)	6·95 (0·07)
	60	1·63 (1·01)	83·3 (1·50)	6·70 (0·10)
	80	1·15 (0·98)	82·7 (0·78)	7·00 (0·11)
	100	1·32 (0·73)	82·6 (0·83)	7·02 (0·08)
	4	0·33 (0·66)	82·8 (0·89)	7·19 (0·96)
	8	0·48 (0·65)	82·7 (0·86)	6·98 (0·11)
	12	0·46 (0·81)	82·9 (1·01)	6·79 (0·14)
Iced sea water	20	0·62 (0·67)	81·8 (0·93)	6·71 (0·06)
	36	1·03 (0·94)	82·4 (0·63)	6·97 (0·14)
	60	1·09 (0·81)	83·2 (1·75)	6·89 (0·14)
	80	2·04 (1·20)	83·0 (1·69)	6·82 (0·12)
	100	1·15 (0·74)	84·4 (1·67)	6·93 (0·08)

[a] Values expressed are means of 240 scores; values in parentheses are the standard deviation.

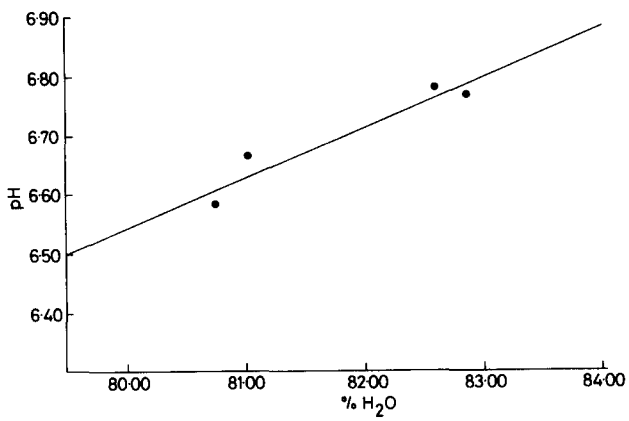


Fig. 3. Relationship between water content and pH of blue whiting used for gaping studies. $r = 0·96$.

development occurs of processes connected with the breakdown of compounds in the muscular tissue (carbohydrates and phosphate esters). The energy liberated during these processes is released in the form of heat, since it can no longer be used for physiological body processes. The more quickly heat is removed, the faster the fish will cool, and the more drastically will biochemical processes be arrested.

The chilling time of fish depends on the properties of the coolant and the conditions under which the process takes place (cross sectional area of fish, thermal efficiency, temperature of coolant, type of motion and velocity of coolant, humidity, heat transfer coefficient from product to coolant). Other workers (Zaitsev, 1969) using sardine as the test material, have found that fish can be chilled more rapidly in liquid coolant than in ice or air. The method used here is to immerse the fish or cover the fish with ice. Sea water has a freezing point below 0 °C and can be kept at a temperature between -1 and $-1{\cdot}5$ °C throughout the chilling process. Chilling by immersion in a liquid is regarded as a process using a homogeneous coolant, in which the fish is placed in uniform surroundings and heat transfer takes place over the entire external area, as reflected in the result obtained for gaping scores: iced sea water < ice < ambient.

It could also be speculated that the lower gaping scores observed for the frozen fish prechilled in iced sea water may be enhanced by the greater osmotic pressure in iced sea water. The myocommata of the fish, post mortem, have been shown by Love & Lavéty (1972) to become hydrated during and following the period of rigor mortis. The fluid absorbed is found to originate partly from the cell exudate and the medium of storage. Love and Lavéty (1972) in their studies with cod, wrapped fish in polythene thus preventing water from diffusing into the fish from the environment after death. This leaves the myocommata less hydrated and the fish gape less after freezing and thawing. They also observed that cod stored over melting water from ice that cannot drain away, exhibit increased gaping.

The lower extent of gaping observed in the poor condition, spawning fish may be explained as being related to the muscular pH, which in turn derives from the nutritional conditions. When a fish swims, the energy comes from glycogen stored in the muscle; when this is depleted more is transported (as glucose) from the liver by the blood to take its place and the store in the liver is replenished from the food consumed by the fish (Love, 1973). In the fish, post mortem, glucose is broken down during the first 24 h after death to lactic acid, thus making the fish muscle slightly acid. The degree of acidity of the fish muscle, is dependent on the nutritional state of the fish. A starving fish has little glycogen in the muscle, so hardly any lactic acid is formed when it dies and the pH is high (MacCallum *et al.*, 1968). Love & Robertson (1968) have established a correlation between the split observed between the myotomes of the fish on filleting and the nutritional status, among other factors. They showed that during deterioration of condition in cod, as measured by the rise in muscle water contents and pH, the gaping seen in the thawed fillets of cod became progressively less. They postulated that the effect of pH on the myotome–

myocommata junction was largely responsible for this observation (Love & Lavéty, 1972). Gaping probably results from the rupture of the fine collagenous process which surrounds the muscle cells and which merges with the myocommata (Love *et al.*, 1969).

Because of the importance of gaping in thawed fillets in commercial practice, a practical implication of the findings in this work is that blue whiting storage life could be prolonged by prechilling in sea water. A further advantage of chilled sea water is that the seasonal variation of gaping in blue whiting is less pronounced.

ACKNOWLEDGEMENTS

The authors wish to thank Dr R. M. Love, Mrs Joyce Crolla, Mrs K. Gun and Mr Bob Anderson for their assistance in this study.

REFERENCES

DAGBJARTSSON, B. (1975). Utilization of blue whiting (*Micromesistius poutassou*) for human consumption, *J. Fish. Res. Board Can.*, **32**, 747.

LOVE, R. M. & ROBERTSON, I. (1968). The connective tissue of fish. I—The influence of biological condition in cod on gaping in frozen-thawed muscle, *J. Food Technol.*, **3**, 215.

LOVE, R. M. (1973). In *Gaping of Fillets*, Torry Advisory Note No. 61, Ministry of Agriculture, Fisheries and Food, Torry Research Station, Aberdeen, UK.

LOVE, R. M., LAVETY, J. & STEEL, P. J. (1969). The connective tissue of fish. II—Gaping in commercial species of frozen fish in relation to rigor mortis, *J. Food Technol.*, **4**, 39.

LOVE, R. M. (1970). In *The Chemical Biology of Fishes*, p. 1, Academic Press Inc., London.

LOVE, R. M. & LAVETY, J. (1972). The connective tissue of fish. VII—Post-mortem hydration and ice crystal formation in myocommata and their influence on gaping, *J. Food Technol.*, **7**, 431.

MACCALLUM, W. A., JAFFARAY, J. I., CHURCHILL, D. N. & IDLER, D. R. (1968). Condition of Newfoundland trap-caught cod and its influence on quality after single and double freezing, *J. Fish Res. Board Can.*, **25**, 733.

NURSALL, J. R. (1956). *Proc. Zool. Soc. London*, **126**, 127.

STRÖM, T. (1978). Processing of blue whiting, survey of raw material handling, production and products Paper No. 5, *Nor Fishing 78, 16th Nordic Fishery Conference*, August 1978.

ZAITSEV, V. (1969). In *Fish Curing and Processing*, Ch. 11, MIR Publishers, Moscow.

Food Chemistry 8 (1982) 307–312

SOME PRODUCTS OF WINGED BEAN LIPOXYGENASE-CATALYSED REACTION: A NOTE

TRUONG VAN DEN,† LEONCIO C. RAYMUNDO & EVELYN MAE T. MENDOZA‡

University of the Philippines at Los Baños (UPLB), Laguna, Philippines 3720

(Received: 27 February, 1981)

ABSTRACT

Purified isoenzymes of lipoxygenase from winged bean were shown to catalyse the formation of hydroperoxides as well as carbonyl compounds. Preliminary experiments indicate formation of two volatile compounds by lipoxygenase in a model purified lipoxygenase–linoleic acid system which are similar to two of the volatile components of raw winged bean homogenate.

INTRODUCTION

Lipoxygenase (linoleate: oxygen oxidoreductase EC 1.13.11.12) has previously been shown to be primarily responsible for the flavour volatile compounds in soybean (Wolf, 1975) and peanuts (St Angelo *et al.*, 1979). Some of these compounds produce the off- and beany flavour which limits the use of legume grains in processed foods.

Because of its high protein content, readily adaptable agronomic qualities, and high yielding capacity, winged bean (*Psophocarpus tetragonolobus* (L.) DC) has become a potentially good source of protein for human consumption. Products of winged bean seeds which are normally produced from soybean have been developed, e.g. milk, curd, tempeh and miso. However, these products have not been well accepted because of their strong beany flavour (Wong, 1978).

The primary objective of our studies is to determine if the development of the beany flavour and rancidity in winged bean seeds is, at least in part, due to oxidation of polyunsaturated fatty acids by the enzyme lipoxygenase. Earlier we reported the

† Present address: National Institutes of Biotechnology and Applied Microbiology (BIOTECH), University of the Philippines at Los Baños College, Laguna, Philippines 3720.
‡ To whom correspondence should be addressed.

Food Chemistry 0308-8146/82/0008-0307/$02·75

purification and characterisation of lipoxygenase isoenzymes FI and FII from winged bean (Truong *et al.*, 1982*a*,*b*). Reported herein are results of preliminary studies on the products of the winged bean lipoxygenase catalysed reaction using the purified isoenzymes.

EXPERIMENTAL

Preparation of purified lipoxygenase

Lipoxygenase FI and FII were prepared from winged bean seeds of Batangas medium line following the procedure described by Truong *et al.* (1982*a*). The procedure consisted of ammonium sulphate fractionations, gel filtration on Sephadex G-150, ion exchange and hydroxyapatite chromatography.

Primary products of lipoxygenase reaction

Purified isoenzymes FI and FII were added to a reaction mixture consisting of 1·6 mM linoleic acid, 0·075% Tween 20, 0·1 M phosphate buffer pH 5·8. The ultraviolet spectra of the reaction products were monitored in a Gilford model 250 recording spectrophotometer. The hydroperoxides and carbonyl products were measured at 234 and 280 nm, respectively (Holman & Burr, 1945; Vioque and Holman, 1962). The reaction was performed at 30 °C.

Determination of winged bean volatiles

Dried seeds of the winged bean line, Batangas medium, were ground to pass through a 40 mesh screen. Freshly prepared meal (15 g) was mixed with 150 ml of 0·1 M phosphate buffer (pH 5·8) in an airtight Erlenmeyer flask fitted with a serum stopper. The vessel was incubated for 1 h at room temperature and then heated to 75 °C for 5 min. The gas (10 ml) was withdrawn and injected into a Pye Unicam gas chromatograph model 24. The sample was run isothermally at 110 °C using a stainless steel column (4·7 mm o.d. × 270 cm) packed with 10% polyethylene glycol 20 M on 100–200 mesh diatomite and a flame ionisation detector. Gas flow rates were 60 ml min^{-1} for nitrogen, 50 ml min^{-1} for hydrogen and 100 ml min^{-1} for air.

In a model system, 0·2 ml of purified winged bean lipoxygenase FII was incubated with 0·6 ml of linoleic acid (1·6 mM) and 5·2 ml of 0·1 M phosphate buffer pH 5·8 in a vessel fitted with a serum stopper, for about 2 h at room temperature. A 5 ml gas sample was withdrawn and injected into the gas chromatograph as described above.

RESULTS AND DISCUSSION

Formation of hydroperoxides and carbonyl compounds

It is known that under certain reaction conditions lipoxygenase produces not only hydroperoxides but also carbonyl compounds (Holman & Burr, 1945; Vioque &

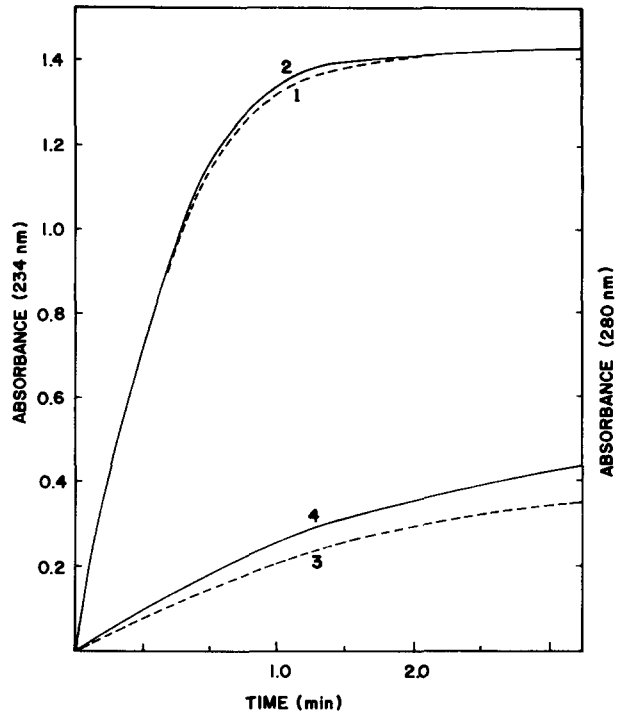


Fig. 1. Carbonyl-producing activity of winged bean lipoxygenase. 1 and 2, formation of conjugated dienes; 3 and 4, formation of carbonyl compounds; ---- FI; —— FII.

Holman, 1962; Garssen *et al.*, 1971; Pistorius, 1974). Winged bean lipoxygenase also possesses the carbonyl-producing ability. With the same activity units (measured as the increase in conjugated dienes absorbing at 234 nm) and linoleic acid concentration (1·6 mM) both FI and FII lipoxygenase isoenzymes had similar carbonyl producing activity (Fig. 1). The ultraviolet spectra of the reaction products generated during the peroxidation of linoleic acid by winged bean FI and FII lipoxygenases both show a major peak around 233 nm and a smaller peak at 280 nm. The peak at 233 nm corresponds to that of linoleic acid hydroperoxide and the peak at 280 nm is attributed to carbonyl compounds (Holman & Burr, 1945; Vioque & Holman, 1962).

The formation of carbonyl compounds is not a general property of all lipoxygenase isoenzymes (Pistorius, 1974). Pea lipoxygenase isoenzymes can be distinguished on the basis of their carbonyl producing ability (Yoon & Klein, 1979). Yoon & Klein (1979) suggested that the production of carbonyl compounds may be due to the breakdown of hydroperoxides or decomposition of intermediates without prior hydroperoxide formation since the increase in absorbance at 280 nm was

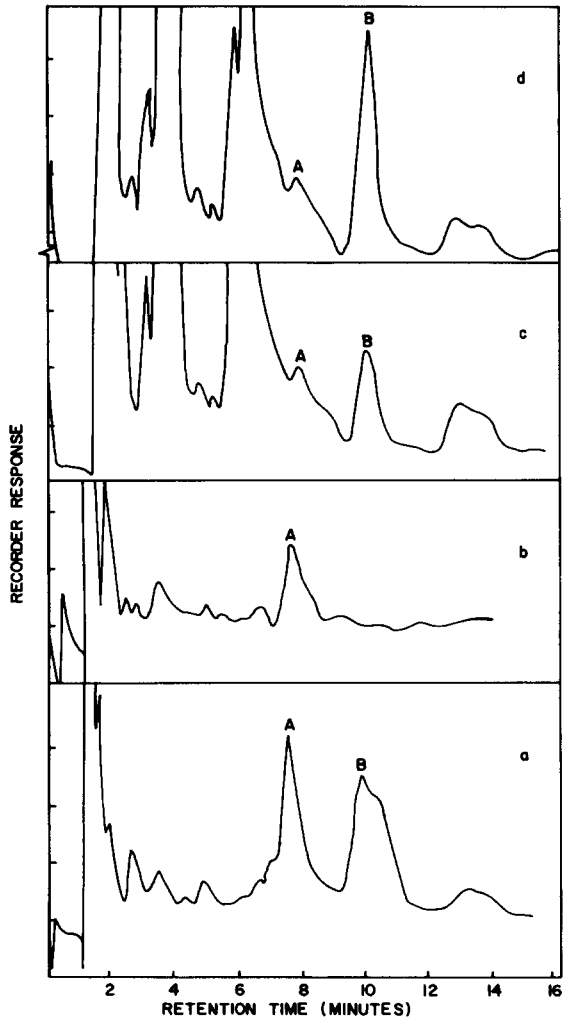


Fig. 2. Chromatogram of volatiles produced by (a) purified winged bean lipoxygenase–linoleate model system; (b) heat-inactivated winged bean lipoxygenase–linoleate model system; (c) raw winged bean homogenate; and (d) raw winged bean homogenate with the addition of linoleic acid.

accompanied by a decrease in conjugated diene. The linear fashion of carbonyl-producing activity of winged bean lipoxygenase was not accompanied by a decrease in conjugated dienes (Fig. 1) suggesting that the above hypothesis regarding carbonyl formation may not be applicable in the case of winged bean lipoxygenase.

Formation of flavour volatile compounds

Preliminary experiments indicate the involvement of lipoxygenase in the

formation of flavour compounds in winged bean. The chromatogram of the volatile components produced in the model lipoxygenase FII–linoleic acid mixture shows two major peaks, A and B (Fig. 2(a)). When heat-inactivated isoenzyme preparation was used, component B disappeared but component A was retained (Fig. 2(b)). Both peaks A and B were present in the volatiles of the raw winged bean homogenate (Fig. 2(c)). Addition of 0·1 ml of linoleic acid to the incubation mixture resulted in an increase in component B but not in A (Fig. 2(d)).

These results indicate that winged bean lipoxygenase is primarily responsible for the production of compound B in the flavour volatiles of raw winged bean seeds. Perhaps, more components are produced during the reaction which could not be detected or separated by the system used. Pattee *et al.* (1974) reported that peanut lipoxygenase was primarily responsible for the production of pentane, an abundant component in the flavour volatiles of raw peanut homogenate. St Angelo *et al.* (1972) found pentane and hexanal to be the principal secondary reaction products formed by the enzymatic oxidation of linoleic acid by soybean lipoxygenase. Using gas chromatography–mass spectrometry, St Angelo *et al.* (1980) were able to identify pentane, hexanal, 2-pentylfuran, *trans*-2, *cis*-4,decadienal and *trans*-2, *trans*-4,decadienal as predominant compounds which are believed to originate from decomposition of either the C-9 or C-13 hydroperoxides generated by peanut lipoxygenase. These compounds contribute to the off- and beany flavours of many legume products (Sessa, 1979).

Further work in identifying the volatile components produced by lipoxygenase in winged bean is in progress to firmly establish the role of lipoxygenase in the production of the off- and beany flavours in winged bean seeds.

REFERENCES

GARSSEN, G., VLIEGENTHART, J. G. G. & BOLDINGH, J. (1971). An anaerobic reaction between lipoxygenase, linoleic acid and its hydroperoxides, *Biochem. J.*, **122**, 327–32.

HOLMAN, R. T. & BURR, G. O. (1945). Spectrophotometric studies of the oxidation of fats. IV. Ultraviolet absorption spectra of lipoxidase-oxidized fats, *Arch. Biochim. Biophys.*, **171**, 459–66.

PATTEE, H. F., SINGLETON, J. A. & JOHNS, E. B. (1974). Pentane production by peanut lipoxygenase, *Lipids*, **9**, 302–6.

PISTORIUS, E. K. (1974). Ph.D. dissertation, Purdue University.

SESSA, D. J. (1979). Biochemical aspects of lipid-derived flavors in legumes, *J. Agric. Food Chem.*, **271**, 234–9.

ST. ANGELO, A. J., DUPUY, H. P. & ORY, R. L. (1972). A simplified gas chromatographic procedure for analysis of lipoxygenase reaction products, *Lipids*, **7**, 793–5.

ST. ANGELO, A. J., KUCK, J. C. & ORY, R. L. (1979). Role of lipoxygenase and lipid oxidation in quality of oil seeds, *J. Agric. Food Chem.*, **27**, 229–34.

ST. ANGELO, A. J., LEGENDRE, M. G. & DUPUY, H. P. (1980). Identification of lipoxygenase–linoleate decomposition products by direct gas chromatography mass spectrometry, *Lipids*, **15**, 45–9.

TRUONG, VAN DEN, RAYMUNDO, L. C. & MENDOZA, E. M. T. (1982*a*). Winged bean lipoxygenase—Part 1: Isolation and purification, *Food Chem.*, **8**, 187–201.

TRUONG, VAN DEN, RAYMUNDO, L. C. & MENDOZA, E. M. T. (1982*b*). Winged bean lipoxygenase—Part 2: Physicochemical properties, *Food Chem.*, **8**, 307–12.

VIOQUE, E. & HOLMAN, R. T. (1962). Characterization of the ketodienes formed in the oxidation of linoleate by lipoxidase, *Arch. Biochem. Biophys.*, **99**, 522–8.

WOLF, W. J. (1975). Lipoxygenase and flavor of soybean protein products, *J. Agric. Food Chem.*, **23**, 136–41.

WONG, K. C. (1978). Uses and current status of winged bean research in Malaysia. *Public Forum on Prospects and Potential Uses of the Winged Bean and the Plans for Its Development*, Manila, Philippines, January 1978.

YOON, S. & KLEIN, B. P. (1979). Some properties of pea lipoxygenase isoenzymes, *J. Agric. Food Chem.*, **27**, 955–62.

Food Chemistry 8 (1982) 313–315

BOOK REVIEWS

Fish and Krill Protein Processing Technology. By Taneko Suzuki, Applied Science Publishers Ltd, London, 1981, 260 pp. Price: £19·50.

The Japanese have a longstanding tradition regarding fish processing and consumption and this book shows the wide range of products under investigation in Japan. It is apparent that it is not only traditional Japanese fish products which are being studied; fish from all over the world are being considered as potential sources of nutrients.

Chapter 1 deals comprehensively with fish meat and protein and changes occurring during processing and storage. Chapter 2 deals with a traditional fish cake—Kamaboko and Chapter 3 with frozen minced meat. Chapter 4 describes the production of food grade fish protein concentrate as an attempt to relieve world protein shortages. A final chapter on Krill utilisation completes the book.

Fish have long been neglected as a major food reserve and it is only in recent years that publications have reflected the importance of fish to us as a protein source and books such as this have been produced.

The book provides useful information to anyone concerned with the subject and will be a valuable addition to any library.

MALCOLM W. KEARSLEY

Handbook of Legumes of World Economic Importance. By J. A. Duke, Plenum Press, New York and London, 345 pp. Price: $45·00.

This book provides a wealth of information on almost 150 legumes of world importance. The bulk of the text deals with each legume under a series of headings

Food Chemistry (8) (1982)—
Printed in Great Britain

Food Chemistry 8 (1982) 313–315

BOOK REVIEWS

Fish and Krill Protein Processing Technology. By Taneko Suzuki, Applied Science Publishers Ltd, London, 1981, 260 pp. Price: £19·50.

The Japanese have a longstanding tradition regarding fish processing and consumption and this book shows the wide range of products under investigation in Japan. It is apparent that it is not only traditional Japanese fish products which are being studied; fish from all over the world are being considered as potential sources of nutrients.

Chapter 1 deals comprehensively with fish meat and protein and changes occurring during processing and storage. Chapter 2 deals with a traditional fish cake—Kamaboko and Chapter 3 with frozen minced meat. Chapter 4 describes the production of food grade fish protein concentrate as an attempt to relieve world protein shortages. A final chapter on Krill utilisation completes the book.

Fish have long been neglected as a major food reserve and it is only in recent years that publications have reflected the importance of fish to us as a protein source and books such as this have been produced.

The book provides useful information to anyone concerned with the subject and will be a valuable addition to any library.

MALCOLM W. KEARSLEY

Handbook of Legumes of World Economic Importance. By J. A. Duke, Plenum Press, New York and London, 345 pp. Price: $45·00.

This book provides a wealth of information on almost 150 legumes of world importance. The bulk of the text deals with each legume under a series of headings

Food Chemistry (8) (1982)—
Printed in Great Britain

where information is available: scientific name; common name(s); synonym(s); uses—in both food and general applications; folk medicine—traditional uses of the product, mainly for medicinal purposes; chemistry—analysis of the plant and seeds for major and minor components—fat, carbohydrate, protein, toxins. Description—of plant, flowers and seeds on a physical basis; germplasm; distribution—throughout the world; ecology—growth requirements; cultivation; harvesting; yields and economics; biotic factors; references.

The many abbreviations (over 100) found in the text tend to be confusing at first and not all are familiar.

Twenty eight pages form a useful appendix at the end of the book, providing more useful information on the legumes in a more assimilable form. Legume toxins, toxicity and generic distribution are given in Table 1 with the data rearranged in Table 2 providing a useful cross reference. Further tables present growth requirements, tolerances and, perhaps most useful, a cross reference for common names and scientific names, a nutritional analysis of each legume and the amino acid distribution. General references complete the book.

Whilst perhaps not a book to be found on everyone's shelves the book would be a most useful addition to many libraries.

MALCOLM W. KEARSLEY

Ultrafiltration Membranes and Applications. Edited by A. R. Cooper. Plenum Press, New York, 1980, xiv + 707 pp. ISBN 0-306-40548-2.

This book is a record of the proceedings of a symposium 'Ultrafiltration Membranes and Applications', held at Washington, DC in September 1979. It is probably the most extensive collection of papers on ultrafiltration, gathered together in one volume. As such it presents a good account of the advances made up to 1979, and the state of ultrafiltration development at that time. There are over 50 contributors, from all the continents, covering all aspects of the subject. There are articles to stimulate a wide audience, ranging from the theoretical physicist, who wants to predict performance data of novel membranes, through research, development and medical scientists, to the entrepreneur, wanting to convert potentially waste material into marketable commodities.

The book is divided into five sections, but the main subject areas are: membrane fundamentals; membrane production techniques, including characterisation and concentration polarisation; and membrane applications, under the headings, industrial, medical and environmental. Food materials feature prominently in the industrial and environmental sections; milk and vegetable proteins also appear in both these sections, viewed both as important industrial raw materials and sources of industrial waste. Some of the papers are original research papers, some are review articles and others are of a general nature.

where information is available: scientific name; common name(s); synonym(s); uses—in both food and general applications; folk medicine—traditional uses of the product, mainly for medicinal purposes; chemistry—analysis of the plant and seeds for major and minor components—fat, carbohydrate, protein, toxins. Description—of plant, flowers and seeds on a physical basis; germplasm; distribution—throughout the world; ecology—growth requirements; cultivation; harvesting; yields and economics; biotic factors; references.

The many abbreviations (over 100) found in the text tend to be confusing at first and not all are familiar.

Twenty eight pages form a useful appendix at the end of the book, providing more useful information on the legumes in a more assimilable form. Legume toxins, toxicity and generic distribution are given in Table 1 with the data rearranged in Table 2 providing a useful cross reference. Further tables present growth requirements, tolerances and, perhaps most useful, a cross reference for common names and scientific names, a nutritional analysis of each legume and the amino acid distribution. General references complete the book.

Whilst perhaps not a book to be found on everyone's shelves the book would be a most useful addition to many libraries.

MALCOLM W. KEARSLEY

Ultrafiltration Membranes and Applications. Edited by A. R. Cooper. Plenum Press, New York, 1980, xiv + 707 pp. ISBN 0-306-40548-2.

This book is a record of the proceedings of a symposium 'Ultrafiltration Membranes and Applications', held at Washington, DC in September 1979. It is probably the most extensive collection of papers on ultrafiltration, gathered together in one volume. As such it presents a good account of the advances made up to 1979, and the state of ultrafiltration development at that time. There are over 50 contributors, from all the continents, covering all aspects of the subject. There are articles to stimulate a wide audience, ranging from the theoretical physicist, who wants to predict performance data of novel membranes, through research, development and medical scientists, to the entrepreneur, wanting to convert potentially waste material into marketable commodities.

The book is divided into five sections, but the main subject areas are: membrane fundamentals; membrane production techniques, including characterisation and concentration polarisation; and membrane applications, under the headings, industrial, medical and environmental. Food materials feature prominently in the industrial and environmental sections; milk and vegetable proteins also appear in both these sections, viewed both as important industrial raw materials and sources of industrial waste. Some of the papers are original research papers, some are review articles and others are of a general nature.

The presentation of the book is typical of those where the most important criterion is to get the proceedings published as quickly as possible. Very little editing appears to have been done and the papers have been collected together as received. Many different typefaces are used and there is no uniformity in the layout of the chapters; for example, abstracts sometimes appear at the beginning of the article, sometimes at the end, sometimes not at all and, on one occasion, only the abstract appears; no consistent referencing system is used. However, a limited index has been included, probably drawn from keywords in the paper titles.

Anyone with a general interest in separation and extraction techniques, as well as in ultrafiltration, should be aware of the existence of this volume.

M. J. LEWIS

Food Science & Technology. By Magnus Pyke, Fourth edition, revised and enlarged by Lelio Parducci. John Murray, London, 304 pp. Price: £7·50.

The purpose of this book is to describe in simple terms the principles of food science and technology and how they may be applied in the various methods of food manufacture and preservation. After two preliminary chapters dealing with the elements of chemistry and traditional methods of processing food, cereals, meat, fish and poultry, milk, cheese and eggs, fats and oils, sugar, syrup and soft drinks, fruit and vegetables, and modern technological processes are systematically discussed. These seven chapters form the main part of the book. Finally, there are two chapters dealing with the present scope of food science and technology, and food technology in the next 20 years. Two appendices give some useful nutritional data.

The book is very readable and achieves its objective. There are some minor inaccuracies, and some items of information are not quite up to date; but, bearing in mind that the book is written for the non-professional reader, these are not significant. No one can be expected to have a detailed and up to date knowledge of all branches of such a vast subject. The book is well illustrated and is eminently suitable both for the general public and those young people contemplating a career in the food industry. Some references to standard works on specific foods and particular applications would be useful.

J. G. DAVIS

The presentation of the book is typical of those where the most important criterion is to get the proceedings published as quickly as possible. Very little editing appears to have been done and the papers have been collected together as received. Many different typefaces are used and there is no uniformity in the layout of the chapters; for example, abstracts sometimes appear at the beginning of the article, sometimes at the end, sometimes not at all and, on one occasion, only the abstract appears; no consistent referencing system is used. However, a limited index has been included, probably drawn from keywords in the paper titles.

Anyone with a general interest in separation and extraction techniques, as well as in ultrafiltration, should be aware of the existence of this volume.

M. J. LEWIS

Food Science & Technology. By Magnus Pyke, Fourth edition, revised and enlarged by Lelio Parducci. John Murray, London, 304 pp. Price: £7·50.

The purpose of this book is to describe in simple terms the principles of food science and technology and how they may be applied in the various methods of food manufacture and preservation. After two preliminary chapters dealing with the elements of chemistry and traditional methods of processing food, cereals, meat, fish and poultry, milk, cheese and eggs, fats and oils, sugar, syrup and soft drinks, fruit and vegetables, and modern technological processes are systematically discussed. These seven chapters form the main part of the book. Finally, there are two chapters dealing with the present scope of food science and technology, and food technology in the next 20 years. Two appendices give some useful nutritional data.

The book is very readable and achieves its objective. There are some minor inaccuracies, and some items of information are not quite up to date; but, bearing in mind that the book is written for the non-professional reader, these are not significant. No one can be expected to have a detailed and up to date knowledge of all branches of such a vast subject. The book is well illustrated and is eminently suitable both for the general public and those young people contemplating a career in the food industry. Some references to standard works on specific foods and particular applications would be useful.

J. G. DAVIS

Food Chemistry 8 (1982) 317–319

CONTENTS OF VOLUME 8

Number 1, January 1982

Number 2, February 1982

Number 3, April 1982

Number 4, June 1982

FOOD CHEMISTRY

An International Journal

This journal is concerned mainly with the chemistry and biochemistry of foods and chemical and biochemical changes occurring in foods. It is also concerned with sensory and nutritional properties of food within a chemical or biochemical framework. The journal provides a vehicle for the widest possible dissemination of knowledge in food chemistry, mainly as research papers and reviews.

Research papers must be factual and original and must not have been published elsewhere, though papers whose biological or technological aspects have already been published may be accepted if previously unpublished chemical information and conclusions are now included. The following subjects are among those open for discussion: pure and applied food chemistry; structural changes in molecules during the processing and storage of foods; chemical aspects of nutrition, physiology and health; chemical quality control in food chemical engineering and technology; direct effects on food of the use of agrochemicals; chemistry relating to sensory aspects; chemical additives; chemical toxins; chemical analysis of food; food biochemistry; and chemistry relating to microbiological aspects.

Type of contributions Research papers; Book reviews; Conferences and meetings; Review articles; Short communications; Letters to the Editor

Submission of papers Papers should be sent to the Editors (Dr G. G. Birch and Mr L. F. Green, c/o National College of Food Technology, St. Georges Avenue, Weybridge, Surrey, England), to members of the Editorial Board for submission to the Editors, or to the publishers.

Special regulations for readers in the USA This journal has been registered with the Copyright Clearance Center, Inc., 21 Congress Street, Salem, MA 01970, USA. Consent is given for copying of articles for personal or internal use, or for the personal or internal use of specific clients. This consent is given on the condition that the copier pay through the Center the per-copy fee stated in the code on the first page of each article for copying beyond that permitted by Sections 107 or 108 of the US Copyright Law. If no code appears in an article, the author has not given broad consent to copy and permission to copy must be obtained directly from the author. All articles published prior to 1979 may be copied for a per-copy fee of US$2.75, also payable through the Center. This consent does not extend to other kinds of copying, such as for general distribution, resale, advertising and promotion purposes, or for creating new collective works.

Subscriptions Four issues per volume—two volumes per year. Subscription price for 1982 (Volumes 8 and 9): UK, £92.00; USA and Canada, £101.00; elsewhere, £106.00. All prices include postage and packing. Subscriptions for delivery to USA, Canada, Japan and Australia are air-speeded at no extra charge. Subscription orders should be addressed to:

APPLIED SCIENCE PUBLISHERS LTD, Ripple Road, Barking, Essex, England

LIST OF MEMBERS OF EDITORIAL BOARD