ENZYME HYDROLYSIS OF MALT GLUCANS USING A MOLLUSC CARBOHYDRASE PREPARATION

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ABSTRACT

In the production of beer, non-fermentable high molecular weight (1→3)-β-D-*glucans from malt are waste materials which cause considerable filtration problems, increasing filtration times and blocking filter cakes. The solubilisation of the majority of these entrapped polysaccharides of the filter mat is described by utilising the digestive enzymes of the surf clam,* Spisula solidissima. *Laboratory studies indicate that this system has considerable potential for full-scale application.*

INTRODUCTION

The majority of the digestive carbohydrase enzymes of the surf clam, *Spisula solidissima,* are located in the crystalline style, an anatomical structure found in the stomach. As the style grinds particulate diatomaceous and algal food matter, the carbohydrase enzymes are released, coating the fragmented material and initiating hydrolysis of polysaccharides to oligo- and monosaccharides. Cellulase and amylase enzymic activities are demonstrable properties of the crystalline style, the latter activity being chloride dependent, but the major style carbohydrase activity seems to be that of laminarinase (E.C. 3.2.1.6), a (1→3)-β-D-glucan hydrolase (Shallenberger *et al.*, 1974).

In the surf clam processing industry the stomach and intestine are waste materials. However, the carbohydrase enzymes of the digestive system have potential uses in the food processing industry, as well as in scientific and technological areas. One food processing area where this enzyme system has potential application is in the brewing industry.

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In the brewing process, the conversion of barley to malt results in an increase in the amount of β-glucan consisting of (1→4)-β- and (1→3)-β-linked glucopyranose residues in the proportion 3:1 (Igarashi & Sakurai, 1965). The (1→3)-β-glucans are not fermented by yeast and are carbohydrate waste. In addition to being waste, these glucans impede filtration in the Lauter tubs and in primary beer filter operations where they eventually clog the filters as they are removed from the beer. β-Glucanase enzymes have been used to reduce wort viscosity, enabling easier filtration (Wieg, 1970) and, on a pilot scale, saving filtration time (Enkenlund, 1972).

This paper describes the hydrolysis and solubilisation of primary filter clogging carbohydrates by an acetone-precipitated powder of the crystalline style of the surf clam.

MATERIALS AND METHODS

Carbohydrase enzymes of the crystalline style

Frozen styles (10 g), supplied by the Shelter Island Oyster Company, Long Island, New York, were triturated with a minimum volume of water and centrifuged. To the supernatant was added an equal volume of cold acetone. The precipitated material was separated by centrifugation and dried by repeated washing with acetone to yield a friable, white powder (1·0 g; 10% yield) which contained the carbohydrase enzymes.

Filter cake

Spent filter cake from the 'primary filter' of the brewing process was obtained from the Genesee Brewing Company, Rochester, New York. Analysis showed that the diatomaceous earth filter mat contained 4% carbohydrate.

Enzymatic digestion of filter cake carbohydrates

To the filter cake (5 g) in water (100 ml) was added the acetone precipitated powder in varying amounts such that the enzyme–substrate ratio ranged from 0·01 to 0·50. The mixture was stirred at room temperature for 16 h, filtered, and the filtrate analysed colorimetrically for total reducing sugars produced, and by paper chromatography for their distribution. The percentage enzyme hydrolysis is expressed as the total amount of reducing substances relative to that of the reducing substances produced by an acid hydrolysis of the carbohydrate material of the filter cake. Duplicate experiments were carried out with sodium chloride solution (0·02M) replacing water.

Analysis of digest products

(i) *Paper chromatography:* Reaction mixtures were analysed by descending

irrigation (20 h) on Whatman No. 1 paper in the solvent system butan-1-ol-pyridine-water-benzene (5:3:3:1, by volume) at 22°C. Chromatograms were visualised with alkaline silver nitrate.

(ii) *Reducing sugars:* Reaction mixture reducing sugars were determined by the method of Somogyi (1945) after acidic hydrolysis of solubilised saccharides to glucose.

RESULTS AND DISCUSSION

The percentage of carbohydrate solubilised at different enzyme–substrate ratios is shown graphically in Fig. 1. The results of the paper chromatographic analysis are shown in Table 1.

It is clear from Fig. 1 that at high enzyme concentrations most of the carbohydrate material of the filter cake is solubilised. The effect of sodium chloride at high enzyme concentrations is to enhance the action of the amylase component which presumably hydrolyses traces of unfermented amylose. The identification of maltose by paper chromatography substantiates this. At the lowest enzyme–substrate ratio (0·01) in water, there is only a small increase in the amount of

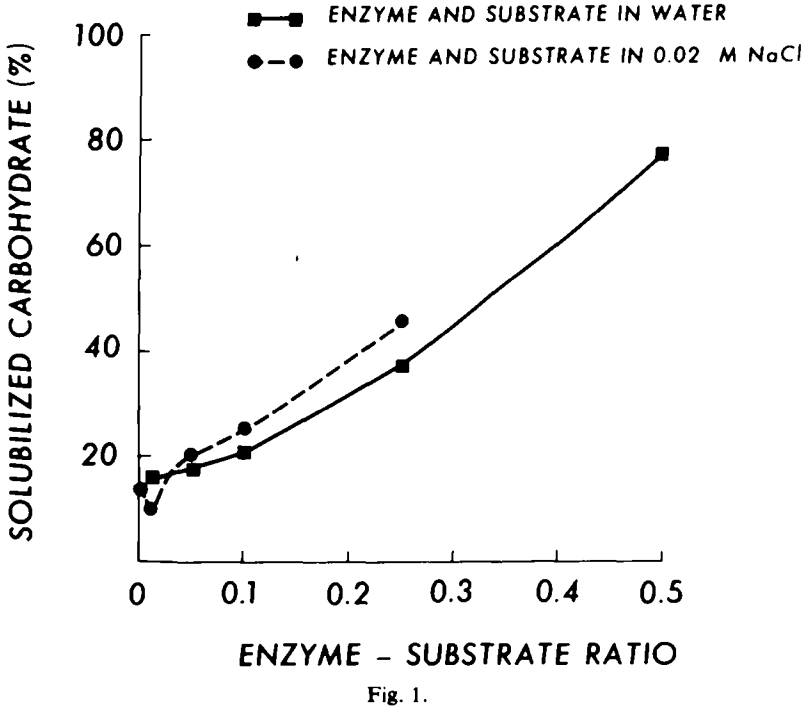

Fig. 1.

TABLE 1

PAPER CHROMATOGRAPHIC ANALYSIS OF SOLUBILISED CARBOHYDRATES

Enzyme–Substrate ratio	*Saccharides in solution*				
	Mono-	*Di-*	*Tri-*	*Tetra-*	*Higher*
0	—	—	—	—	—
0·01 (H_2O)	+	—	—	—	—
0·01 (0·02M NaCl)	tr	tr*	—	—	—
0·1 (H_2O)	tr	—	+	+	+
0·1 (0·02M NaCl)	tr	tr*	+	+	+
0·25 (H_2O)	+	—	+ +	+ +	+
0·25 (0·02M NaCl)	+	tr*	+ +	+ +	+

* Identified as maltose: R_G 0·56.
+, + +, + + +: Indicates relative amounts of saccharides in solution.
— —: Indicates absence of saccharides in solution.
tr: Indicates trace amount detected.

carbohydrate solubilised, shown by paper chromatography to be predominantly glucose. However, in 0·02M NaCl, at the same enzyme–substrate ratio, there is a reduction in the amount of carbohydrate solubilised. There are two possible reasons for this phenomenon. Firstly, the sodium chloride could be inhibiting enzymic hydrolysis. This is unlikely as at higher enzyme–substrate ratios sodium chloride enhances hydrolysis. The alternative possibility is transglycosylation. Transferase enzymes catalyse the transfer of radicals such as phosphoryl, glycosyl and methyl moieties. They are, for example, responsible for the synthesis of polysaccharides, proteins and co-enzymes (Sumner & Somers, 1953). In order for transglycosylation to be responsible for the reduction in soluble carbohydrates, transglycosylase enzyme(s) must convert soluble saccharides to high molecular weight insoluble oligosaccharides, a reaction which cannot at this time be confirmed. If, as seems likely, transglycosylation is taking place, the sodium chloride either acts as a co-factor for specific transglycosylases or the amylase component also acts as a transglycosylase stimulated by sodium chloride.

CONCLUSIONS

The hydrolysis and solubilisation of the filter clogging carbohydrates of the brewing process have been accomplished with the carbohydrase enzymes of the surf clam. However, in these studies the time allowed for enzymic hydrolysis could not practically be applied to a full-scale operation, nor could the incorporation of sodium chloride in such quantities. The enzymes can, however, be immobilised on inert substrates such as glass and the incorporation of the enzymes in this form at some point in the filtration operation is feasible. Thus a lengthy contact time with the substrate would be allowed with no contamination of the final product. The use of waste materials from one food processing operation to minimise waste in a different processing area makes such a system potentially valuable and desirable.

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REFERENCES

ENKENLUND, J. (1972). Externally added beta-glucanase, *Process Biochem.*, 7, 27–9.

IGARASHI, O. & SAKURAI, Y. (1965). Non starchy polysaccharides of the endosperm of naked barley. I. Preparation of the water soluble β-glucans from naked barley endosperm and their properties, *Agric. Biol. Chem.*, **29**, 678–86.

SHALLENBERGER, R. S., SEARLES, C. & LEWIS, B. A. (1974). Laminarinase activity in the crystalline style of the surf clam (*Spisula solidissima*), *Experientia*, **30**, 597–8.

SOMOGYI, M. (1945). A new reagent for the determination of sugars, *J. Biol. Chem.*, **160**, 61–8.

SUMNER, J. B. & SOMERS, G. F. (1953). *Chemistry and methods of enzymes*, New York, Academic Press Inc.

WIEG, A. J. (1970). The technology of processing barley in breweries, *Naarden News*, **21**, 4–7.

UREA-DISC-ELECTROPHORESIS OF ACTIN, TROPOMYOSIN, TROPONIN, α-ACTININ, β-ACTININ AND THE EXTRA PROTEIN FRACTION FROM STRIATED MUSCLE

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ABSTRACT

Purified actin, prepared from both beef and rabbit myofibrils, on being subjected to electrophoresis on gels containing 7M *urea produced a diffuse, heterogeneous and lightly-staining band over a range of* R_m = 0·26 *to* 0·33. *After protecting against oxidation by the addition of DTT (dithiothreitol), the actin band concentrated sharply at* R_m = 0·26 *and exhibited little contamination. Tropomyosin prepared from both rabbit and beef muscle and chromatographed on DEAE-cellulose produced heterogeneous electrophoretic patterns with bands always being present at* R_m = 0·24 *and* 0·43. *Treatment with DTT or sulphite prior to electrophoresis always produced a single band of* R_m = 0·43, *which was concluded to be SH-reduced tropomyosin. Rabbit muscle troponin sulphydryl groups were not protected by DTT or sulphite, but evidence suggested that troponin was localised at* R_m = 0·88 *upon electrophoresis. Both α-actinin and β-actinin were prepared from rabbit muscle. Each sedimented as a single symmetrical peak on ultracentrifugation. Upon urea-disc-gel-electrophoresis, α-actinin produced two bands at* R_m = 0·02 *and* 0·07 *while β-actinin exhibited an inconsistent heterogeneous pattern. Extra protein prepared from rabbit myofibrils by washing* 8–10 *times and chromatographing on DEAE cellulose was eluted from the column into five fractions (I, I-A, II, III and IV) by increasing salt concentrations.*

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Fractions I and IV were shown to be sarcoplasmic- and nucleo-proteins, respectively. Although fractions II and III were not identified, evidence suggests that they were not of myofibrillar origin. Fraction I-A appeared to be identical to troponin. Thus, results suggest that extra protein is an artifact of preparation.

INTRODUCTION

A number of investigators have used gel electrophoresis to separate the subunits and fragments of myosin (Small *et al.*, 1961; Florini & Brivio, 1969; Parsons *et al.*, 1969), of actin (Carsten & Katz, 1964; Rees & Young, 1967; Tsuboi, 1968), of tropomyosin (Woods, 1967; Bodwell, 1967; Arai & Watanabe, 1968; Yasui *et al.*, 1968) and of troponin (Arai & Watanabe, 1968; Yasui *et al.*, 1968). Since the development of SDS (sodium dodecyl sulphate) polyacrylamide gel electrophoresis by Weber & Osborn (1969), this procedure has largely replaced conventional gel electrophoresis for separating the myofibrillar proteins. Work from our laboratory has already reported on the separation of myosin (Rampton *et al.*, in press) and actomyosin and Weber-Edsall extract from skeletal muscle (Rampton *et al.*, 1971).

The present study describes standardised procedures for separating purified actin and tropomyosin with and without DTT (dithiothreitol) and sulphite treatment for protection of SH-groups. In addition, urea-disc-gel-electrophoresis for separating and characterising troponin, α- and β-actinin and the 'extra protein' fraction is presented and discussed.

METHODS

Preparation of myofibrillar proteins

F-actin was isolated directly from beef and rabbit myofibrils, as described by Hama *et al.* (1965). Tropomyosin was prepared from both beef and rabbit muscle, as outlined by Bailey (1948) with the following modifications suggested by Woods (1967): (1) ethanol was glass-distilled before use; (2) all work was carried out at 3°C except for denaturation with the organic solvents and (3) all working solutions contained 0·01M EDTA (pH 7·0) except for the saturated ammonium sulphate. The latter was prepared by saturating 0·2M EDTA (pH 8·0) with solid ammonium sulphate. Tropomyosin prepared in this manner was then chromatographed on DEAE-cellulose according to the procedure of Davey & Gilbert (1968).

Rabbit muscle was used as the starting material for preparation of troponin, α-actinin and β-actinin. Troponin was prepared from acetone-extracted dried muscle powder according to the procedure of Katz (1966) or from crude actin as described by Azuma & Watanabe (1966). Both preparations were subjected to urea-disc-gel-electrophoresis and compared. The method of Seraydarian *et al.* (1967) was utilised for preparing α-actinin, with precipitation by 3·3M KCl, all

subsequent steps being repeated to improve its purity. The procedure of Maruyama (1965) was utilised for the preparation of β-actinin. The extra protein fraction was prepared according to the procedure of Perry & Zydowo (1959*a*).

All work was carried out at 3°C unless otherwise indicated. Reagent grade chemicals were used throughout. Urea was used as a 10M stock solution, which was deionised by passing over a bed of Amberlite MB-3 resin.

Electrophoresis

Disc electrophoresis was carried out by the method of Davis (1964) with the modifications outlined below. The running gel (0·6 × 4·5 cm) contained 5·0% polyacrylamide with 3·0% crosslinkage from methylene bisacrylamide. The spacer gel (0·6 × 0·5 cm) contained 3·0% polyacrylamide with 5·0% of crosslinkage. All gels contained 7M urea. Gels were made up from three stock solutions as described earlier by Jolley *et al.* (1967), which were tightly covered and could be stored for up to 3 weeks at 3°C without apparent change. The tracking dye (bromophenol blue) was added to the sample and it was layered directly under the reservoir buffer on to the acrylamide gel. Disc electrophoresis was carried out at room temperature, since cooling was found to increase the tailing of the bands, whilst the higher temperature appeared to have no adverse effects upon the separation. A current of 2 mA per tube was maintained throughout electrophoresis, and the dye marker was allowed to run to the end of the tube.

Electrophoretic patterns were checked for artifacts due to persulphate oxidation by comparing the electrophoretic properties of gels polymerised by persulphate with those polymerised by riboflavin. Identical patterns with and without persulphate indicated that persulphate oxidation was not a problem.

On completion of electrophoresis, the gels were stained for at least 2 h with Amido Black. De-staining was accomplished in a Canalco 'Quick Gel De-stainer'. Both reservoirs were filled with 7% acetic acid, and 0·5 ml of the Amido Black stain was added to the cathode reservoir in order to prevent stripping of loosely-bound dye from the protein zones. After de-staining, the gels were immersed in a test tube containing 7% acetic acid for 48 h, during which time the staining pattern was intensified and sharpened. The residual background stain was removed by immersing in two to three changes of fresh acetic acid. Band intensity was read on a Photovolt Densicord recording densitometer.

To measure the relative mobility (R_m) of a given band, the position of the trailing edge was compared with the position of the dye marker. A series of runs using different sample sizes showed that the trailing edge of a band remained more constant than the leading edge.

Protection of sulphydryl groups

For protection of sulphydryl groups, actin and tropomyosin samples were reduced and subjected to electrophoresis, using dithiothreitol (DTT). Samples in

8M urea were incubated for 2 h at room temperature in an excess (usually 0·02M) of DTT under a blanket of N_2. The upper buffer reservoir was adjusted to 0·002M DTT, and electrophoresis was performed as in the absence of DTT. Samples without DTT were compared with those protected by its use.

Sulphite reduction was also used on some of the actin and tropomyosin samples to protect the sulphydryl groups by using the procedure of Bailey (1967).

Analytical ultracentrifugation

Analytical ultracentrifugation was carried out in a Beckman Model E analytical ultracentrifuge.

Protein determination

Protein was determined by the biuret method calibrated with serum albumin, as outlined by Gornall *et al.* (1949).

RESULTS AND DISCUSSION

Actin

Actin was prepared three times from rabbit and once from beef myofibrils. G-actin from both sources produced a diffuse, lightly-staining band over the range of R_m = 0·26 to 0·33. The protein bands exhibited varying amounts of heterogeneity. A typical separation is shown in Fig. 1(A). Tsuboi (1968) has also reported

Fig. 1. Disc electrophoresis of G-actin. A = 0·05 ml of beef actin preparation containing 2·0 mg protein/ml. B = 0·04 ml of DTT-reduced beef actin preparation containing 1·8 mg protein/ml.

heterogeneity during acrylamide-gel-electrophoresis of actin in the presence of urea. In the present study, G-actin reduced with DTT always exhibited a relatively sharp band at $R_m = 0{\cdot}26$; sometimes small amounts of contaminating proteins were observed. A typical pattern from DTT-reduced actin is seen in Fig. 1(B). These results are in agreement with the report of Rees & Young (1967) showing that SH-reduced and alkylated, chromatographically purified actin produced a single band during acrylamide gel electrophoresis in 8M urea. Thus, reduced G-actin was assigned an R_m value of 0·26.

F-actin is known to depolymerise in the presence of 5M urea (Poglazov, 1966). The present study substantiates this fact, as F-actin, previously dialysed against 8M urea, entered the acrylamide gel completely and produced a diffuse band at $R_m = 0{\cdot}27$–$0{\cdot}33$ similar to unreduced G-actin, or a well-defined band at $R_m = 0{\cdot}26$ for SH-reduced samples.

Tropomyosin

Tropomyosin was prepared twice from rabbit and once from beef muscle. After chromatography on DEAE-cellulose (Davey & Gilbert, 1968), rabbit and beef tropomyosin each produced heterogeneous electrophoretic patterns typical of the one shown in Fig. 2(A). The bands at $R_m = 0{\cdot}24$ and 0·43 were always present in

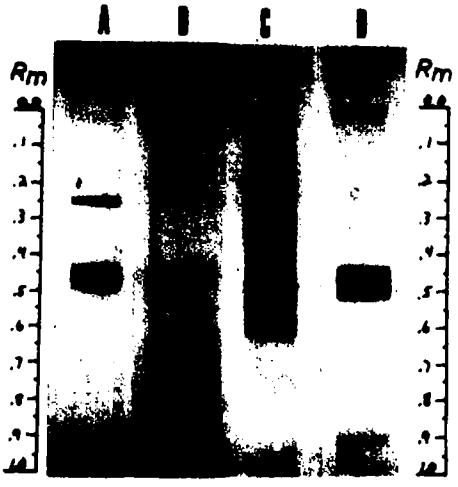

Fig. 2. Disc electrophoresis of tropomyosin. A = 0·02 ml of beef tropomyosin preparation containing 2·4 mg protein/ml. B = 0·02 ml of DTT-reduced beef tropomyosin preparation containing 2·1 mg protein/ml. C = 0·10 ml of beef tropomyosin preparation containing 2·4 mg protein/ml after 2 weeks at 3°. D = 0·02 ml of beef tropomyosin from sample C (above) after DTT reduction.

unreduced tropomyosin samples; however, the relative intensity of the bands varied for different preparations. Sulphydryl reduction with DTT, followed by electrophoresis in the presence of DTT, produced a single band at R_m = 0·43 (Fig. 2(B)). In some cases there was a faint band at R_m = 0·88 (Fig. 2(B)), which later was shown to be due to contamination with troponin.

Storage of tropomyosin samples for 3 weeks in 0·5M KCl containing 0·02M Tris-HCl (pH 8·2) or contamination of the samples with heavy metals resulted in the formation of many new electrophoretic species (Fig. 2(C)). However, the stored samples, after reduction and electrophoresis in the presence of DTT, produced a single band at R_m = 0·43, which is shown in Fig. 2(D). Similar results were reported by Woods (1967) and Yasui *et al.* (1968). Reduction and oxidation by sulphite, followed by electrophoresis without DTT, produced results identical with those in Fig. 2(D). Thus, a single band at R_m = 0·43 in the electrophoretic system utilised in this study was observed for tropomyosin preparations in which the sulphydryl groups were protected.

Troponin, α-actinin and β-actinin

Attempts to isolate troponin free from contaminating proteins were unsuccessful. Perry (1967) stated that similar difficulties were encountered in several laboratories. Troponin prepared by the method of Katz (1966) produced an electrophoretic pattern similar to the one already shown in Fig. 2(A) for unreduced tropomyosin. Thus, the preparation contained large amounts of unreduced tropomyosin and

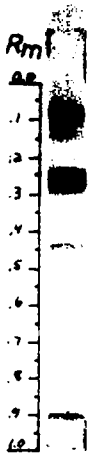

Fig. 3. Disc electrophoresis of troponin prepared by the method of Azuma & Watanabe (1966). Sample consisted of 0·01 ml of effluent collected during chromatography on Sephadex G-200.

lesser quantities of another protein—probably troponin. A second preparation of troponin was prepared from crude actin and purified as described by Azuma & Watanabe (1966). Disc electrophoresis of the latter preparation yielded several different electrophoretic species, having R_m values of 0·07, 0·24, 0·43 and 0·88 (Fig. 3). Yasui *et al.* (1968) isolated a troponin preparation which migrated as a single band in urea-acrylamide-gel-electrophoresis. They then showed that, on dissociation of the troponin-tropomyosin complex, troponin migrates faster than tropomyosin during urea-acrylamide-gel-electrophoresis, and that reduction with DTT does not affect the R_m value of troponin. Thus, our results suggested

Fig. 4. Disc electrophoresis of the actinins. A = 0·06 ml of α-actinin prepared from rabbit muscle. Sample contained 0·3 mg protein/ml. B = 0·10 ml of β-actinin preparation from rabbit muscle. Sample contained 0·3 mg protein/ml.

that troponin was localised at R_m = 0·88 in the present electrophoretic system, since the band at 0·88 is the only one meeting the above requirements. The identification of troponin has since been confirmed by Hapchuk (1974) who found it to be localised on urea-disc-gel electrophoresis at R_m = 0·83–0·85.

One preparation of α-actinin and one of β-actinin were isolated from rabbit muscle. Each actinin preparation sedimented as a single, symmetrical peak in the ultracentrifuge. As shown in Fig. 4(A), upon disc electrophoresis, α-actinin produced two bands with relative mobilities of 0·02 and 0·07. These two bands appear to be due to α-actinin, but better methods for its preparation are now avail-

able (Masaki & Takaiti, 1969). β-actinin exhibited a heterogeneous pattern (Fig. 4(B)), showing no clearly characteristic pattern.

Extra protein

Two preparations of extra protein were isolated from well-washed rabbit myofibrils and chromatographed on DEAE-cellulose, as described by Davey & Gilbert (1968). A typical pattern obtained from the chromatography of extra protein is shown in Fig. 5. The peaks were identified as fractions I, II, III and IV on the basis of the salt concentration required for their elution from the column. Perry & Zydowo (1959*a*) observed a small peak following fraction I which did not decrease during washing of the fibrils. It has been labelled as fraction IA in Fig. 5.

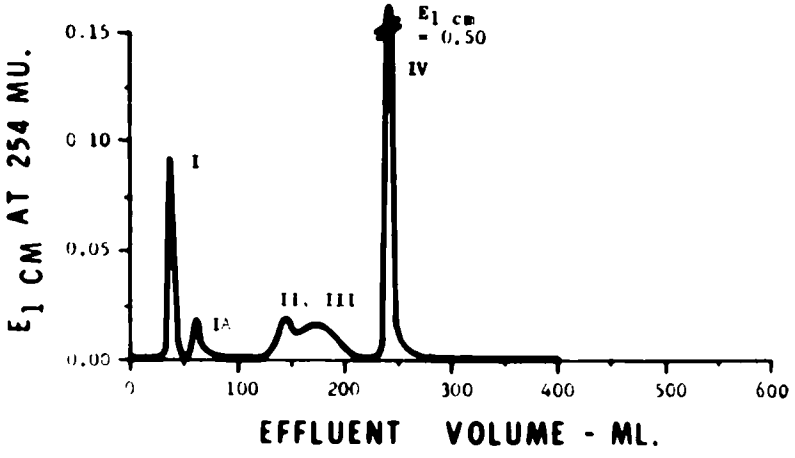

Fig. 5. Chromatography of extra protein from rabbit myofibrils on DEAE-cellulose by the method of Perry & Zydowo (1959*a*) using a 2·5 × 12 cm column. A ten millilitre sample of extra protein preparation containing 1·7 mg protein/ml was applied at 0 ml effluent volume. Elution was carried out with a linear gradient of 100 ml of limit buffer (0·35M KCl containing 0·02M Tris, pH 7·6). At 200 ml a step was made to 2·0M KCl containing 0·02M Tris, pH 7·6.

Serial disc electrophoretic analyses were performed on the extra protein fractions, and the results are shown in Fig. 6. Fraction I produced a very broad band with an R_m value of 0·14. Fraction IA contained a single electrophoretic component with an R_m value of 0·88. The bands produced by fractions II and III were extremely faint, even before photographing the gels. Fraction IV contained two electrophoretic bands with R_m values of 0·22 and 0·44.

Perry & Zydowo (1959*a*) showed that sarcoplasmic protein exhibited a chromatographic behaviour identical to fraction I of the extra protein group. They concluded that fraction I consisted mainly of sarcoplasmic material, which had not been removed from the fibrils prior to extraction of extra protein. In the present study,

a similar test was performed in which sarcoplasmic proteins of rabbit muscle were subjected to electrophoresis and the resulting pattern was compared with that of fraction I. The pattern obtained from the sarcoplasmic preparation (Fig. 7) appeared to contain several diffuse bands with R_m values between 0·00 and 0·14. These results substantiate the conclusion of Perry & Zydowo (1959*a*) that fraction I consists mainly of sarcoplasmic material.

Perry & Zydowo (1959*a*) also reported that extra protein fractions I, II and III are leached out during the washings employed during preparation of the myofibrils. In the present investigation, the myofibrils were washed 8–10 times prior to extraction of the extra protein fraction. This probably accounts for the relatively low levels of fractions I, II and III as shown in Fig. 5.

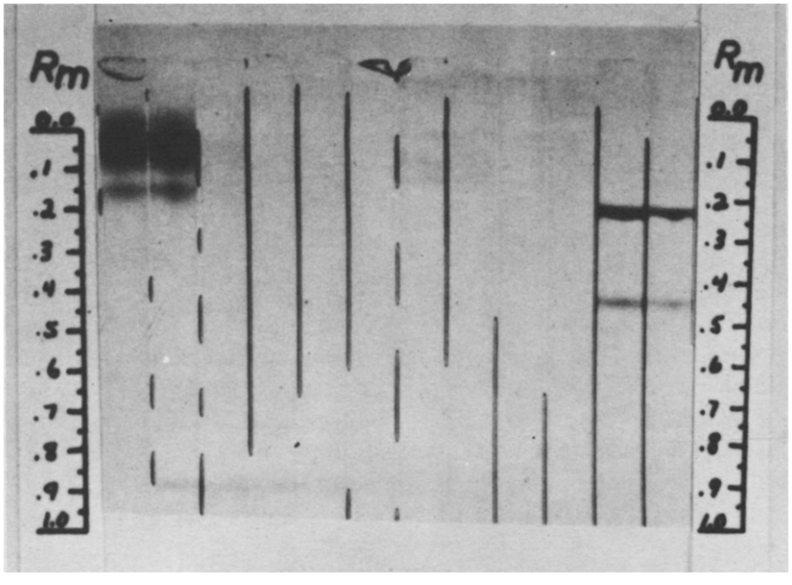

Fig. 6. Serial disc electrophoretic analyses of the extra protein fractions eluted as shown in Fig. 5. From left to right, 0·12 ml samples taken at effluent volumes of 30, 40, 50, 60, 70, 80, 140, 150, 160, 170, 240 and 250 ml are shown.

As shown in Fig. 6, extra protein fraction IA localised at $R_m = 0{\cdot}88$ during disc electrophoresis. We have observed the band at $R_m = 0{\cdot}88$ in most Weber-Edsall extracts, many actomyosin preparations, many tropomyosin preparations and troponin samples prepared according to Azuma & Watanabe (1966). These results suggest that extra protein fraction IA is actually troponin.

Perry & Zydowo (1959*a*,*b*) identified fraction IV as a ribonucleoprotein. In the present study, ultraviolet absorption spectra of fraction IV and tropomyosin showed absorption maxima at 258 and 278 nm, respectively. By using the data of

Fig. 7. Disc electrophoresis of 0·01 ml of sarcoplasmic extract from rabbit skeletal muscle. Sample contained 17·0 mg protein/ml.

Warburg & Christian (1941) and the E_{280}·E_{260} ratio of each preparation, it was estimated that fraction IV contained 60% nucleic acids, whereas tropomyosin contained none. This is in good agreement with the value of 50% ribonucleic acid in fraction IV, which was reported earlier by Perry & Zydowo (1959*b*). Tropomyosin is often isolated as a complex containing ribonucleic acid (Hamoir, 1951; Needham & Williams, 1963; Carsten, 1968). Nucleotropomyosin contained 10–15% ribonucleic acid, suggesting that nucleotropomyosin is not identical with extra protein fraction IV. Thus, these results suggest that extra protein may be an artifact of preparation.

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REFERENCES

ARAI, K. & WATANABE, S. (1968). A study of troponin, a myofibrillar protein from rabbit skeletal muscle, *J. Biol. Chem.*, **243**, 5670–8.

AZUMA, N. & WATANABE, S. (1966). The minor component of metin from rabbit skeletal muscle, *J. Biol. Chem.*, **240**, 3852–7.

BAILEY, J. L. (1967). *Techniques in protein chemistry*, 2nd ed., p. 126. London, Elsevier Publ. Co.

BAILEY, K. (1948). Tropomyosin: A new asymmetric protein component of the muscle fibril, *Biochem. J.*, **43**, 271–9.

BODWELL, C. E. (1967). Two subunits of tropomyosin B, *Arch. Biochem. Biophys.*, **122**, 246–61.

CARSTEN, M. E. (1968). Tropomyosin from smooth muscle of the uterus, *Biochemistry*, **7**, 960–70.

CARSTEN, M. E. & KATZ, A. M. (1964). Actin: A comparative study, *Biochim. Biophys. Acta*, **90**, 534–41.

DAVEY, C. L. & GILBERT, K. V. (1968). Studies in meat tenderness. 6. The nature of myofibrillar proteins extracted from meat during ageing, *J. Food Sci.*, **33**, 343–8.

DAVIS, B. J. (1964). Disc electrophoresis. II. Method and application to human serum proteins, *Ann. N.Y. Acad. Sci.*, **121**, 404–27.

FLORINI, J. R. & BRIVIO, R. P. (1969). Disc electrophoresis of myosin and myosin derivatives in dilute polyacrylamide gels, *Anal. Biochem.*, **30**, 358–67.

GORNALL, A. G., BARDAWILL, C. J. & DAVID, M. M. (1949). Determination of serum proteins by means of the biuret reaction, *J. Biol. Chem.*, **177**, 751–66.

HAMA, H., MARUYAMA, K. & NODA, H. (1965). Natural F-actin. I. Direct isolation of F-actin from myofibrils and its physico-chemical properties, *Biochim. Biophys. Acta*, **102**, 249–60.

HAMOIR, G. (1951). Fish tropomyosin and fish nucleotropomyosin, *Biochem. J.*, **48**, 146–51.

HAPCHUK, L. T. (1974). Proteolysis of porcine muscle by *Clostridium perfringens*, Ph.D. Thesis. Michigan State University, East Lansing, Michigan.

JOLLEY, W. B., ALLEN, H. W. & GRIFFITH, O. M. (1967). Ultracentrifugation using polyacrylamide gel, *Anal. Biochem.*, **21**, 454–61.

KATZ, A. M. (1966). Purification and properties of a tropomyosin-containing protein fraction that sensitises reconstituted actomyosin to calcium-binding agents, *J. Biol. Chem.*, **241**, 1522–9.

MASAKI, T. & TAKAITI, O. (1969). Some properties of chicken α-actinin, *J. Biochem. Japan*, **66**, 637–43.

MARUYAMA, K. (1965). Some physico-chemical properties of β-actinin, 'actin-factor', isolated from striated muscle, *Biochim. Biophys. Acta*, **102**, 542–8.

NEEDHAM, D. M. & WILLIAMS, J. M. (1963). Proteins of the uterine contractile mechanism, *Biochem. J.*, **89**, 552–61.

PARSONS, A. L., PARSONS, J. L., BLANSHARD, J. M. V. & LAWRIE, R. A. (1969). Electrophoretic differentiation of myofibrillar proteins in the pig, *Biochem. J.*, **112**, 673–8.

PERRY, S. V. (1967). The structure and interactions of myosin. In *Progress in biophysics and molecular biology*, eds. J. A. V. Butler & H. E. Huxley, **17**, 325–42, Oxford, Pergamon Press.

PERRY, S. V. & ZYDOWO, M. (1959*a*). The nature of the extra protein fraction from myofibrils of striated muscle, *Biochem. J.*, **71**, 220–8.

PERRY, S. V. & ZYDOWO, M. (1959*b*). A ribonucleoprotein of skeletal muscle and its relation to the myofibril, *Biochem. J.*, **72**, 682–90.

POGLAZOV, B. F. (1966). *Structure and function of contractile proteins*, 56. New York, Academic Press.

PORZIO, M. A. & PEARSON, A. M. (1975). Sodium dodecyl sulfate polyacrylamide gel electrophoresis: Improved resolution of myofibrillar proteins, *Anal. Biochem.*, **68**, Submitted.

RAMPTON, J. H., PEARSON, A. M., WALKER, J. E. & KAPSALIS, J. G. (1971). Disc electrophoresis of Weber-Edsall extract and actomyosin from skeletal muscle, *J. Agr. Food Chem.*, **19**, 238–40.

RAMPTON, J. H., PEARSON, A. M., BECHTEL, P. J., WALKER, J. E. & KAPSALIS, J. G. (1976). Urea disc-gel electrophoresis of rabbit and bovine myosin, *Fd. Chem.*, **1**, 49–55.

REES, M. K. & YOUNG, M. (1967). Studies on the isolation and molecular properties of homogeneous globular actin, *J. Biol. Chem.*, **242**, 4449–58.

SCOPES, R. K. & PENNY, I. F. (1971). Subunit sizes of muscle proteins as determined by sodium dodecyl sulfate gel electrophoresis, *Biochim. Biophys. Acta*, **236**, 409–15.

SENDER, P. M. (1971). Muscle fibrils: Solubilisation and gel electrophoresis, *FEBS Letters*, **17**, 106–10.

SERAYDARIAN, K., BRISKEY, E. J. & MOMMAERTS, W. F. H. M. (1967). The modification of actomyosin by α-actinin. I. A survey of experimental conditions, *Biochim. Biophys. Acta*, **133**, 399–411.

SMALL, P. A., HARRINGTON, W. F. & KIELLEY, W. W. (1961). The electrophoretic homogeneity of the myosin subunits, *Biochim. Biophys. Acta*, **49**, 462–70.

TSUBOI, K. K. (1968). Actin and bound-nucleotide stoichiometry, *Biochim. Biophys. Acta*, **160**, 420–34.

WAKABAYASHI, T. & EBASHI, S. (1968). Reversible change in physical state of troponin induced by calcium ion, *J. Biochem. Japan*, **64**, 731–2.

WARBURG, O. & CHRISTIAN, W. (1941). Isolierung and Kristallisation des Gärungs-ferments Enolase, *Biochem. Z.*, **310**, 384–421.
WEBER, K. & OSBORN, M. (1969). The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis, *J. Biol. Chem.*, **244**, 4406–12.
WOODS, E. F. (1967). Molecular weight and subunit structure of tropomyosin B, *J. Biol. Chem.*, **242**, 2859–71.
YASUI, B., FUCHS, F. & BRIGGS, F. N. (1968). The role of the sulfhydryl groups of tropomyosin and troponin in the calcium control of actomyosin contractility, *J. Biol. Chem.*, **243**, 735–42.

COMPOSITION OF WEST INDIAN BAY OILS

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ABSTRACT

Bay oil is obtained by the steam distillation of leaves of Pimenta racemosa (*Mill.*) *J. W. Moore. In Dominica, now the only major producer of bay oil, two other varieties of bay exist which, although similar in appearance to* P. racemosa, *yield oils with aroma characteristics very different from those of bay oil. These trees are generally described as anise- or lemon-scented, according to the aroma of their oils. Any contamination of bay oil with these oils is considered to be highly undesirable. Using a variety of chromatographic and spectroscopic techniques, a detailed study has now been made of the constituents of the three types of oil. The results show that oils from* P. racemosa *and the anise-scented variety have similar overall compositions but differ in the functional state of the major phenolic constituents. Eugenol and chavicol are the phenolic constituents of the former but in the anise-scented variety these phenols are mainly present as their methyl ethers. The lemon-scented variety resembles bay oil only in the minor constituents and contains no phenols or phenolic ethers. Citral comprises more than* 80% *of this oil.*

INTRODUCTION

Bay oil is obtained by the steam distillation of leaves of *Pimenta racemosa* (Mill.) J. W. Moore. It has a distinctive spicy aroma and is a useful ingredient for the formulation of seasonings and sauces. In Dominica, now the only major producer of bay oil, two other varieties of bay exist which, although similar in appearance to *P. racemosa*, yield oils with aroma characteristics very different from those of bay oil. These trees are generally described as anise-scented and lemon-scented, from the aroma of their oils. Any contamination of bay oil with these oils is considered to be highly undesirable. Botanically, the lemon-scented variety has been known as *Pimenta acris*, var. *citrifolia*. The anise-scented variety does not appear to have been classified. The West Indian bays are botanically quite distinct from the sweet bay, *Lauris nobilis L.*, which grows in Europe and other temperate zones.

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Markoe (1877) recognised that the heavy fraction of bay oil consisted mainly of eugenol and Power & Kleber (1895) established the occurrence of olefinic terpenes in nature when they found myrcene in bay oil. Compounds that have been reported as constituents of bay oil are given in Table 1.

There has been some controversy over the occurrence of methyl eugenol and methyl chavicol in bay oil. Palkin & Wells (1933) failed, in their detailed studies on bay oil, to confirm the presence of these two phenolic ethers which had been reported previously as being present at significant levels (Power & Kleber, 1895). It was suggested that the bay leaves used for the earlier studies had been contaminated with appreciable quantities of the anise-scented leaves.

TABLE 1
COMPOUNDS REPORTED IN BAY OIL

α-Pinene[a]
β-Pinene[b]
α-Phellandrene[a]
Myrcene[c]
(−)-Limonene[a]
β-Phellandrene[b]
p-Cymene[b]
Ocimene[b]
1,8-Cineole[a]
(−)-Linalool[d]
(±)-Terpin-1-en-4-ol[d]
(−)-α-Terpineol[d]
(±)-Octan-3-ol[d]
(±)-Oct-1-en-3-ol[d]
Citral[c]
Eugenol[c]
Chavicol[c]
Methyl eugenol[c]
Methyl chavicol[c]

[a] Palkin & Wells (1933).
[b] Ikeda *et al.* (1962).
[c] Power & Kleber (1895).
[d] Lamparsky (1963).
[e] Markoe (1877).

Whilst there have been fairly comprehensive studies on the composition of true bay oil, little work has been done on the oils from the other two varieties. Watts & Tempany (1909) described a lemon-scented bay oil which contained 44% of citral and 10% of phenols. They also recorded that the citral content could be as high as 65%. According to Warneford (1927), the anise-scented oil contains 15% of methyl chavicol and 13% of methyl eugenol in addition to myrcene, chavicol and eugenol. Warneford concluded that the oil contained 25% of phenols and 40% of tar.

Recently, Ames *et al.* (1971) have published gas chromatograms which clearly distinguish the three varieties of bay oil. These workers did not comment on the absence of citral isomers from the gas chromatogram of bay oil, a fact that conflicts with the earlier reports that bay oil contains citral (Power & Kleber, 1895; Palkin &

Wells, 1933). The uncertainty attached to the results of the earlier work, coupled with a lack of knowledge of the minor constituents of the lemon-scented and anise-scented varieties, prompted us to re-examine the compositions of the three bay oils.

MATERIALS AND METHODS

Oils

Leaves from the three bay varieties were obtained from Dominica and processed two months after harvesting. They were cut into 0·5 cm strips and an aliquot (150 g) was mixed with water (2 litres) and conventionally steam distilled for 3 h. The distillate was extracted with diethyl ether (3 × 100 ml). The combined extract was dried and the solvent removed at atmospheric pressure on a waterbath kept below 50°C. The yields were 3·9, 4·7 and 4·1% for bay, anise-scented and lemon-scented oil, respectively. Small samples of oil were also produced from leaf (20 g) by Clevenger distillation and isolated without solvent extraction.

Column chromatography

The oil (1·5 g) was adsorbed on a column (25 × 2 cm) of silica gel (Hopkin & Williams MFC, 100 to 200 mesh) prepared from a slurry with light petroleum, boiling point below 40°C. The oil was fractionated by elution with aliquots (200 ml) of light petroleum containing 0, 2, 3, 4, 5, 6, 8 and 10% of diethyl ether and finally with diethyl ether. After evaporation of the solvent, the resulting fractions were analysed by GC-MS. Recoveries from the column were in excess of 95%.

Instrumental methods

Oil analyses were carried out on a Perkin-Elmer 990 gas chromatograph fitted with a flame ionisation detector and coupled to a Hitachi RMU-6L mass spectrometer via a Watson-Biemann molecular separator. A 4 m × 2·2 mm I.D. column filled with Carbowax 20M on 80–100 mesh Chromosorb W (10:90 w/w) and temperature programmed at the rate of 6°/min from 100–195°C was used. The temperature was 230°C at the injector port and detector and 210°C at the interface. The IR spectra were recorded as liquid films on a Hilger & Watts H800 spectrophotometer.

RESULTS AND DISCUSSION

Because of the difficulty in simulating the commercial distillation on a small scale in the laboratory, the oils were obtained by conventional steam distillation and diethyl ether extraction of the distillate. Small samples of oil were also produced

for comparison purposes by Clevenger distillation and separated without solvent extraction. It was thought that this method of distillation would approximate to the cohobation procedure used in the commercial production of bay oil, and yield oils of similar composition. The oil yields from the conventional steam distillations were considerably in excess of those obtained during commercial distillations where they can vary between 1 and 2%. Although extraction of the aqueous distillate almost certainly contributed to the higher yield, it is also possible that the leaves were dryer than usual when distilled. It has been reported (Ames *et al.*, 1971) that oils obtained from relatively dry leaves tend to have higher density and phenol content than those from fresh leaves, possibly due to some differential evaporation or polymerisation of the lower density compounds during the storage or drying period. Since the Clevenger distilled oil also had an abnormally high phenol content, the indication is that the leaves were dryer than usual.

The conventionally steam distilled oils were subjected to column chromatography on silica gel. Elution with light petroleum containing increasing proportions of diethyl ether separated the oil constituents roughly into chemical classes. The resulting fractions were analysed by gas chromatography and coupled mass spectrometry. The fractionation procedure facilitated the identification of minor constituents of the oils.

Compounds identified in the steam distilled oils are listed in Table 2 in order of ascending GLC retention time. Quantitative data are based on integrated peak area measurements for whole oils.

Of the compounds previously reported in bay oil (Table 1) the presence of all except the citral isomers has been confirmed. However, only trace amounts of methyl chavicol and methyl eugenol were detected, and these in the appropriate fractions from the silica gel column. The more abundant methyl eugenol was estimated to be present at a level of 0·05% in the whole oil. The retention data of the ocimene corresponds to that of the *trans*-β-isomer.

In addition, a number of other compounds have been detected, but not all have been identified. Compounds positively identified include terpinolene, octan-3-one, acetyl chavicol and oct-1-en-3-yl acetate. The hydrocarbon fraction eluted from the column by light petroleum contained at least seven sesquiterpenes of molecular weight 204. The sesquiterpene fraction was about 1% of the whole oil. Insufficient data were available to identify these compounds unequivocally. Besides cineole, octanone and the esters, the fraction eluted from the silica gel column by 2% diethyl ether in light petroleum contained three minor constituents. These have not yet been identified.

Methyl eugenol and methyl chavicol were the major constituents of the anise-scented oil. They were present at a level considerably in excess of that reported by the earlier workers and comprised about 75% of the oil. Methyl eugenol was the main constituent and accounted for 43% of the oil. Minor amounts of two other phenolic ethers were detected. One was present, together with methyl chavicol, in

the fraction eluted from the silica gel column by 3% diethyl ether in light petroleum. It was identified as anethole from the retention data and mass spectrum. The second ether was more polar and was eluted in the chavicol fraction. It was

TABLE 2
COMPOSITION OF THE THREE VARIETIES OF BAY OIL

Compound	*Area, %*			*Identification*
	Bay	*Anise*	*Lemon*	
α-Pinene	0·1	0·1	0·1	GC
β-Pinene	–	+	+	GC
Myrcene	13·9	12·0	3·0	GC, MS, IR
α-Phellandrene	0·4	0·2	–	GC, MS
Limonene	1·4	0·8	1·9	GC, MS
1,8-Cineole	0·2	–	–	GC, MS
β-Phellandrene	0·1	0·1	–	GC, MS
trans-β-Ocimene	0·4	0·3	0·1	GC, MS
Octan-3-one	1·1	0·1	–	GC, MS
p-Cymene	0·1	0·1	0·2	GC, MS
Terpinolene	0·1	0·1	–	GC, MS
6-Methylhept-5-en-2-one	–	–	0·2	GC, MS
Oct-1-en-3-yl acetate	+	+	–	GC, MS
Octan-3-ol	0·6	0·1	0·1	GC, MS
Oct-1-en-3-ol	1·0	1·3	0·7	GC, MS, IR
Terpene ester	+	0·1	–	GC, MS
Linalool	1·7	1·6	3·0	GC, MS, IR
$C_{15}H_{24}$	+	0·1	0·1	GC, MS
Terpin-1-en-4-ol	0·3	0·3	0·3	GC, MS
$C_{15}H_{24}$	+	0·1	+	GC, MS
Menthadienol	–	–	–	GC, MS
Methyl chavicol	+	31·6	–	GC, MS, IR
Neral	–	–	32·6	GC, MS
α-Terpineol	0·1	+	–	GC, MS
$C_{15}H_{24}$	+	–	+	GC, MS
Geranial	–	–	53·2	GC, MS
Menthadienol	–	–	+	GC, MS
$C_{15}H_{24}$	–	+	+	GC, MS
$C_{15}H_{24}$	0·2	0·3	–	GC, MS
Geranyl acetate	–	–	+	GC, MS
$C_{15}H_{24}$	+	–	–	GC, MS
$C_{15}H_{24}$	0·2	0·1	+	GC, MS
Geraniol	–	–	2·8	GC, MS, IR
Anethole	–	+	–	GC, MS
p-Cymenol	–	–	+	GC, MS
$C_{15}H_{22}$	+	+	–	GC, MS
Acetyl chavicol	–	+	–	GC, MS
Methyl eugenol	+	43·1	–	GC, MS, IR
Eugenol	56·2	4·3	–	GC, MS, IR
Trimethoxyphenylpropene	–	+	–	GC, MS
Chavicol	21·6	2·0	–	GC, MS, IR
$C_{15}H_{26}O$	–	–	+	GC, MS

\+ detected after fractionation on silica gel.
– not detected.

identified as a trimethoxyphenylpropene from its mass spectrum. Both eugenol and chavicol were present and the total phenol content was about 7% of the oil. The ratio of eugenol to chavicol was similar to the ratio of their ethers.

The hydrocarbon fraction of the oil contained the same constituents as bay oil with myrcene the major component, and the gas chromatographic pattern of the sesquiterpenes was similar. The oil also contained a number of the minor oxygen-containing constituents found in bay oil.

Geranial and neral accounted for 75–80% of the volatile part of the lemon-scented variety. The hydrocarbon content was less than in the other oils and comprised about 5% of the oil with myrcene and limonene the major constituents. The monoterpene hydrocarbon pattern resembled that of the other two oils, but the sesquiterpene pattern was different.

In addition to some of the oxygen-containing constituents present in the bay and anise-scented oils, the lemon-scented oil contained geraniol, geranyl acetate and 6-methylhept-5-en-2-one. There were also two menthadienols present and a number of alcohols which were not identified. No α-terpineol or cineole was detected. Since citral is chemically a very active material and one susceptible to many acid cyclisation reactions (Baines *et al.*, 1970), some of the minor alcohols found in the lemon-scented oil may originate during the steam distillation process.

TABLE 3
COMPARISON OF BAY OILS

Compound	*Composition, %*		
	Extracted	*Clevenger*	*Commercial*
Myrcene	13·9	18·4	29·1
Limonene	1·4	2·5	2·6
Octanone : ocimene	1·5	1·7	1·9
Octenol	1·0	0·9	1·0
Linalool	1·7	2·0	2·1
Eugenol	56·2	56·9	45·5
Chavicol	21·6	12·2	12·0

It is apparent from Table 2 that the bay and the anise-scented variety are qualitatively similar, but differ in the functional state of the major phenolic constituents. The lemon-scented variety resembles the other two oils only in certain of the minor constituents.

The most striking feature of the composition of the bay oil sample obtained by steam distillation and extraction is the phenol content. This is considerably in excess of that of any commercial oil. The composition of the bay oil prepared by Clevenger distillation is closer to that obtained in practice. In Table 3, the two laboratory produced oils are compared with a typical high quality commercial oil. Only principal constituents are considered.

The Clevenger oil has a higher monoterpene content than the extracted oil. A major factor contributing to this is the appreciable water solubility of the phenolic constituents. During the Clevenger distillation, the oil is continuously extracted by water which returns to the distilling flask. More chavicol than eugenol is removed from the oil by this process because the former has a higher water

solubility. It is estimated that, relative to the hydrocarbons, only 66% of the total phenols expected from the leaf was found in the Clevenger oil. The figures for the commercial oil suggest that the loss may be even higher in practice.

Very few of the commercial bay oils that we have examined have been completely free from methyl eugenol and methyl chavicol. The best quality oils contain less than 0·5% of methyl eugenol, but oils have been found that contain up to 12% of total phenolic ether. The odour of such oils is distinctly buttery and not acceptable.

Contamination of bay oil with the lemon-scented variety is still found occasionally but less frequently than contamination with the anise-scented variety, possibly because it is easier to detect the lemon-scented variety at the leaf stage.

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REFERENCES

AMES, G. R., BARROW, M., BORTON, C., CASEY, T. E., MATTHEWS, W. S. & NABNEY, J. (1971). Bay oil distillation in Dominica, *Tropical Science*, **13**, 13.

BAINES, D. A., JONES, R. A., WEBB, T. C. & CAMPION-SMITH, I. H. (1970). The chemistry of terpenes—I. The effect of hydrogen ion concentration upon the acid catalysed cyclisation of citral, *Tetrahedron*, **26**, 4901.

IKEDA, R. M., STANLEY, W. L., VANNIER, S. H. & SPITLER, E. M. (1962). The monoterpene hydrocarbon composition of some essential oils, *J. Food Sci.*, **27**, 455.

LAMPARSKY, D. (1963). Etudes sur les matières végétales volatiles. CLXXXII. Sur les alcools presents dans l'huile essentielle de Bay, *Helv. Chim. Acta*, **46**, 185.

MARKOE, G. F. H. (1877). Volatile oil of leaves of *Myrcia acris*, *Proc. Amer. Pharm. Assoc.*, **25**, 435.

PALKIN, S. & WELLS, P. A. (1933). Composition of the non-phenol portion of bay oil, *J. Amer. Chem. Soc.*, **55**, 1549.

POWER, F. B. & KLEBER, C. (1895). On the constituents of oil of bay, *Pharm. Rundsch, New York*, **13**, 60.

WARNEFORD, F. H. S. (1927). Anise-scented oil of bay, *Trop. Agr. Trinidad.*, **4**, 128.

WATTS, F. & TEMPANY, H. A. (1909). Notes on essential oils, *West Indian Bull.*, **9**, 271.

ACTION OF AQUEOUS SODIUM HYDROXIDE ON GLUCOSE SYRUPS

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ABSTRACT

The effect of alkali concentration on the degradation of glucose syrups is studied and alkali numbers for a series of glucose syrup fractions and commercial glucose syrups are determined.

Volatile acidity produced is shown to be dependent on alkali concentration and volatile acidity values are determined on 28 samples. Again, as DE increases, so does volatile acidity.

Two methods of analysis of glucose syrup are described, the first concerning the determination of the DE of glucose syrups and the second a theoretical example of the analysis of glucose syrup–sucrose mixtures.

INTRODUCTION

The action of aqueous alkali on sugars is very complex and is a subject which has received much attention this century.

For convenience the reactions may be divided into three types:

1. Isomerisations.
2. Fragmentations.
3. Intramolecular oxidations and reductions.

The products from these may combine and produce further compounds (Noller, 1965).

1. *Isomerisations*

These result from the action of dilute alkali at room temperature and occur by way of enediol intermediates. Thus a solution of glucose in aqueous calcium hydroxide yields fructose and mannose, as shown in Fig. 1.

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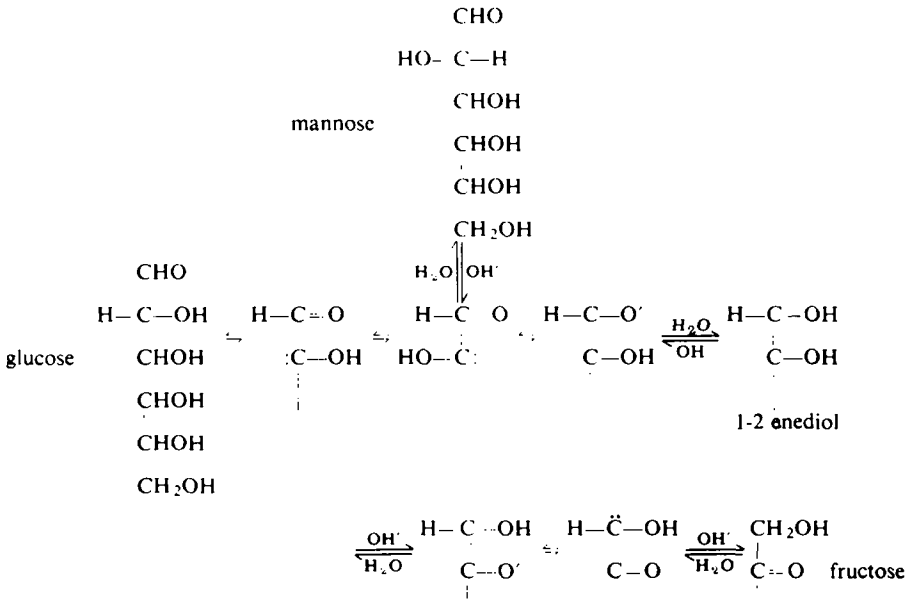

Fig. 1. Degradation reactions of carbohydrates: Isomerisation reactions. (Lobry de Bruyn and Alberda van Ekenstein isomerisations.)

These reactions are commonly known as the Lobry de Bruyn–Alberda van Ekenstein transformations, after their discoverers (Speck, 1958), and although the reactions are reversible, equilibrium is not reached owing to other side reactions. The final composition of such a system varies with the starting sugar, the concentration and nature of the alkali.

2. *Fragmentations*

This process is frequently referred to as degradation and involves, as the name suggests, scission of the parent sugar into smaller 'fragment' compounds such as glyceraldehyde, formic and acetic acids. This may be illustrated by Fig. 2.

3. *Intramolecular oxidations and reductions*

These result in the formation of saccharinic acids, as shown in Fig. 3 (Feather & Harris, 1973). This figure gives a rather simplified version of the action of alkali on sugars which, in reality, is a very complex system of reactions with a vast number of possible compounds being formed. Many workers have devoted time to the subject, in order to ascertain breakdown mechanisms and the effects of reaction conditions. Evans *et al.* (1926) studied the effect of different alkali (KOH) concentrations and temperatures on glucose and galactose and found that the

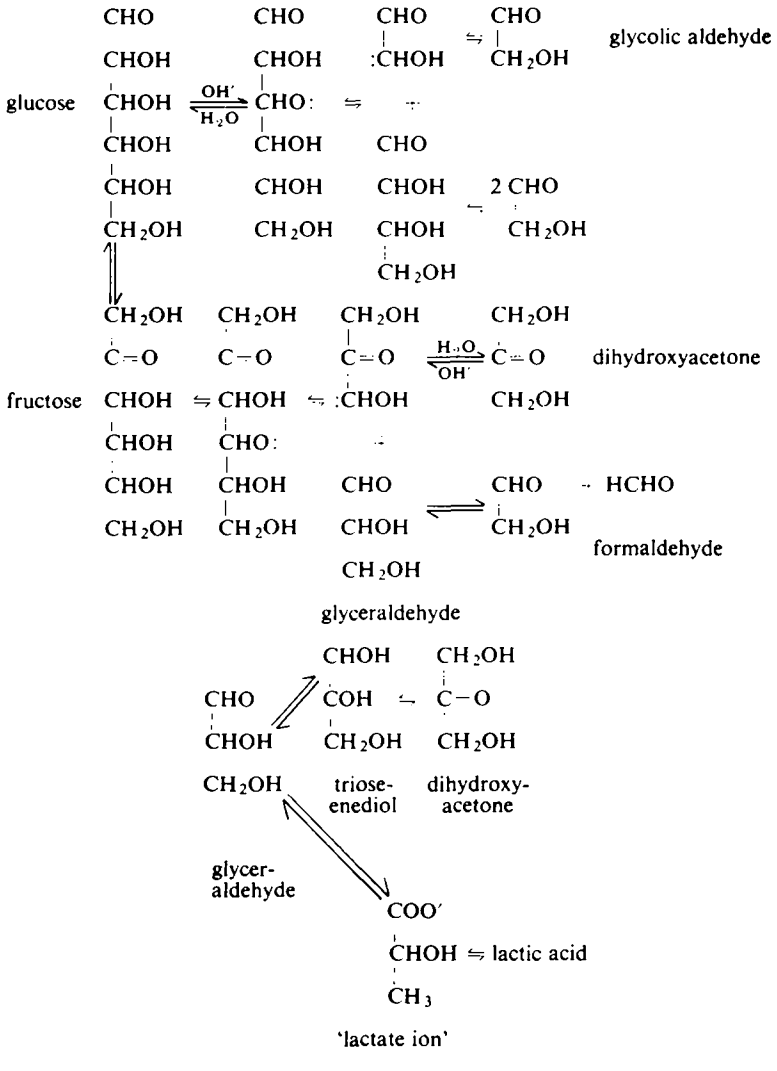

Fig. 2. Degradation reactions of carbohydrates: Fragmentation reactions.

lactic acid produced in the reaction was an index of the extent to which the carbohydrates were converted to 3–4 enediols. Since more was produced from glucose than galactose they concluded that glucose could more easily change the equilibrium of its enediols. They measured the acetic and formic acids produced and found that they reached a peak at 0·6–0·7N alkali concentration whilst lactic acid increased with increasing alkali concentration.

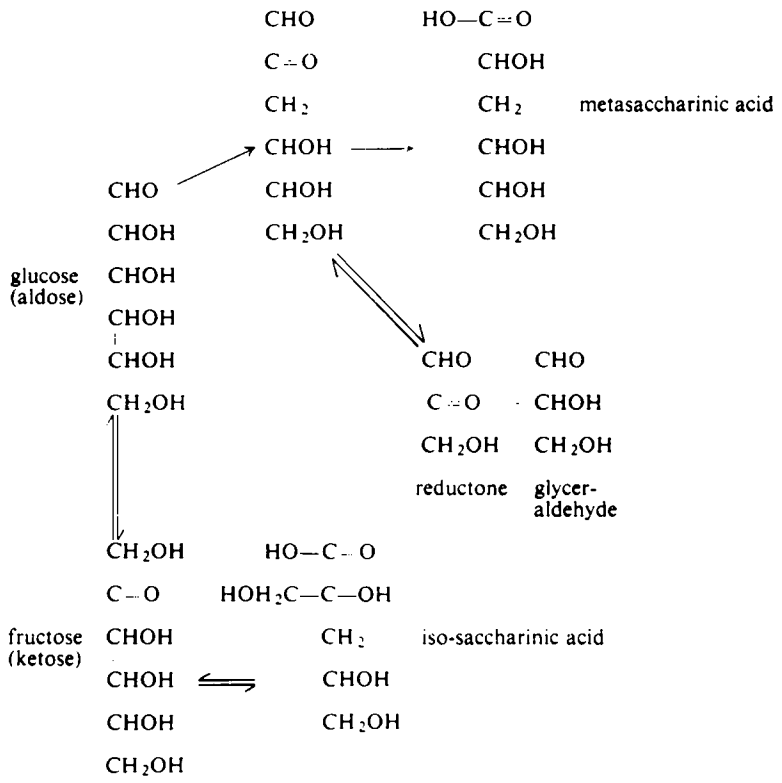

Fig. 3. Degradation reactions of carbohydrates: Saccharinic acid formation.

By isomerisation reactions glucose may be converted into fructose and Evans & Hutchman (1928) established that the action of alkali on fructose gave almost exactly the same end products as alkali on glucose. When mannose, another isomerisation product, was compared to glucose and fructose a similar result was obtained (Evans & O'Donnell, 1928).

Glyceraldehyde and dihydroxyacetone, fragmentation products from fructose, were found to react with alkali, producing lactic, formic and acetic acids (Evans & Hass, 1926; Evans & Cornthwaite, 1928) and formed systems in alkaline solution similar to those observed by Lobry de Bruyn and Alberda van Ekenstein for hexoses in alkaline solution (Evans, 1929).

Although some work was carried out on the effect of alkali on starches, most research was directed to the effects on monosaccharides. Results concerning the effect of alkali on maltose showed that three possible reactions could occur (Evans & Benoy, 1930):

1. The disaccharide may undergo hydrolysis to two glucose units, then the reactions proceed as for glucose.

2. The disaccharide may undergo enolisation without hydrolysis.
3. Enolisation and hydrolysis may occur simultaneously.

Obviously when a mixture of saccharides from glucose to maltohexaose (DP_1–DP_6 inclusive), as found in glucose syrups, is subjected to alkali the number of possible end products is very large.

Maltose was found to yield more formic acid than glucose and less acetic and lactic acids and Evans & Benoy (1930) attributed this to the fact that although maltose forms the 1–2 and 2–3 enediols, the 3–4 enediol is stable owing to a glucose residue on C4.

Taylor & Salzmann (1933) found that the action of alkali on starch was different for unmodified and modified starches and were able to distinguish them by this method and later Taylor *et al.* (1935) developed a method to determine the alkali-labile value of starch and starch products. Stacey *et al.* (1956) showed that the presence of air during the reaction of starch and alkali caused more breakdown of the starch, and Schoch & Jensen (1940) estimated the acidic substances derived from the decomposition of starch by hot alkali. They used this as a measure of the alkali-lability properties of the starch and derived the 'alkali number' as a measure of the rate of decomposition of the starch. (N.B. Alkali number: the volume in millilitres of 0·1N sodium hydroxide consumed by 1 g of starch during digestion in alkali for 1 h at 100°C.)

Sowden & Schaffer (1952*a*) described the effect of alkali on D-Glucose in D_2O and confirmed earlier work concerning isomerisation products and intermediate compounds and described a method for producing labelled fructose for use in other studies. Later (Sowden & Schaffer, 1952*b*) they described the quantitative measurement of these compounds.

More recently, reports have been published showing the effect of alkali on reducing end groups, hydroxyl groups, on different glucose–glucose linkages and on substituted groups in the sugar molecule and the dehydration reactions of carbohydrates in alkaline solutions (Whistler & BeMiller, 1958).

Birch *et al.* (1971) provided evidence that the alkali attack was not confined to the reducing residue in a maltodextrin and a peeling action occurred along the chain of glucose residues. The fact that reducing sugars, but not non-reducing sugars, are degraded by alkali has been applied to the determination of the component sugars in sweetened condensed milk products (Birch & Mwangelwa, 1974) and since glucose syrups fall into the category of 'reducing sugars' the use of alkaline degradation may be applied to mixtures of glucose syrups and non-reducing sugars in the future to facilitate their analysis.

MATERIALS, METHODS AND RESULTS

Materials

Commercial glucose syrups were obtained from Corn Products Ltd, Manchester,

England, in the form of heavy syrups—75–80% solids. These were dried at 65°C under vacuum to constant weight (0% moisture) to produce solid samples.

Glucose syrup fractions produced in a pilot scale reverse osmosis unit, using cellulose acetate membranes, as previously described (Birch & Kearsley, 1974) were obtained in solid form by a similar process after concentration to about 80% solids by evaporation under vacuum.

Selected fractions were demineralised by an ion exchange technique (Kearsley & Birch, 1975) for use in the study.

Analar D-glucose from BDH Chemicals Ltd, Poole, Dorset, England, was used as the 100 DE sample in the work.

Methods and results

Initial work was directed to the effect of concentration of alkali on three different glucose syrup fractions to quantitatively determine the amount of degradation induced in each fraction by the alkali, and to establish the concentration of alkali which produced the maximum degradation. The DE's of the three fractions were 16, 43 and 62 and the alkali used throughout the work was sodium hydroxide (Analar Grade Reagent). The six concentrations used initially were 0·1, 0·5, 1·0, 2·0, 5·0 and 10·0 Normal.

Fifty grammes (±0·01 g) of each fraction were dissolved in 200 ml of the appropriate concentration of alkali and a 0·5 ml sample taken for total carbohydrate analysis by the Phenol/Sulphuric method (Dubois *et al.*, 1956). The remainder was refluxed for 4 h and 0·5 ml samples taken every 30 min for total carbohydrate determination.

The results for the 43 DE fraction at the six alkali concentrations are shown in Fig. 4.

The values shown refer to the percentage carbohydrate remaining at a particular time, calculated as:

$$\left(\frac{\text{total carbohydrate at time } X \text{ min}}{\text{total carbohydrate at the start}} \times 100\right) \%$$

Above an alkali concentration of about 2N only a small further amount of degradation was produced and thus 2N was taken as the optimum concentration for further work where a single alkali concentration was required.

This method had very obvious limitations as a quantitative measure of degradation owing to the delay in taking the initial sample whilst the solid fraction dissolved. During this period some degradation would occur and the subsequent calculations of percentage carbohydrate remaining therefore would be higher than their true value. This effect increases with increasing strength of alkali.

The work was thus repeated using a slightly modified method. Fifty (±0·01) grammes of five glucose syrup fractions (16, 24, 43, 52 and 68 DE) and dextrose (100 DE) were each dissolved in about 150 ml of distilled water in a 250 ml

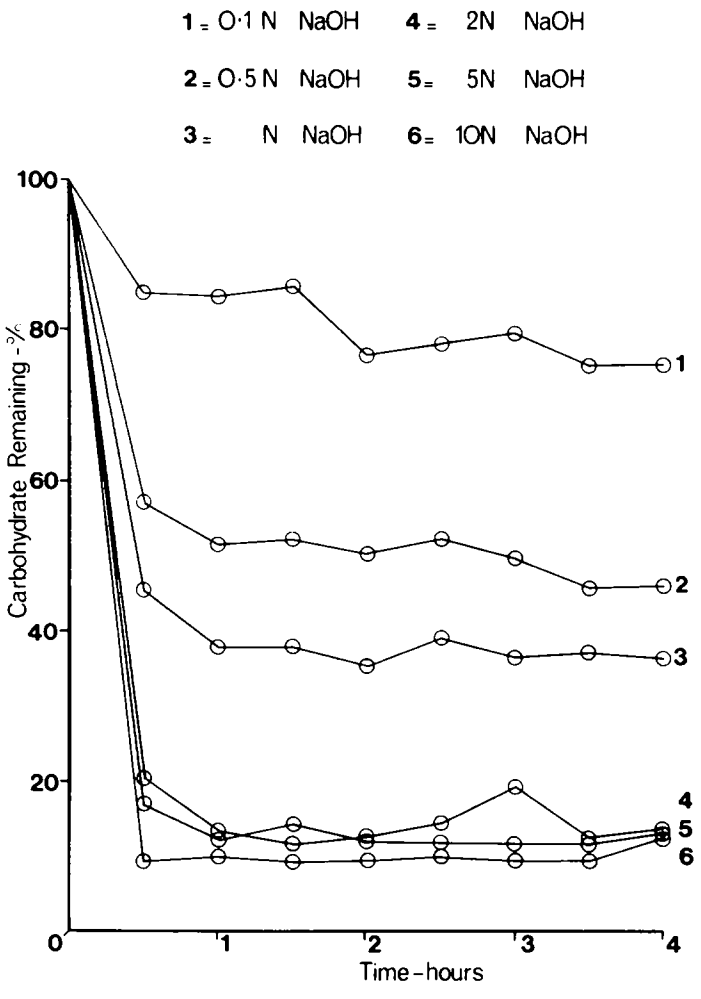

Fig. 4. Carbohydrate remaining (%) versus time for a 43 DE glucose syrup.

volumetric flask. Fifty millilitres of alkali of five times the final required normality were added to bring the final alkali concentration to either 0·1, 0·5, 1·0 or 2·0 Normal, and the contents were made up to 250 ml with distilled water. A 0·5 ml sample was taken for the total carbohydrate analysis and the remainder transferred to a 500 ml flask and refluxed for 3 h.

After cooling the total carbohydrate was redetermined and the percentage remaining calculated for each fraction at each alkali concentration. The results are shown in Table 1.

Each solution, after refluxing for 3 h, was spotted onto a paper chromatogram and these developed for 30 h. After drying the sugars were located using silver nitrate/sodium hydroxide reagents and the results compared for sugars remaining after the degradation.

Figure 5 shows the results for a 24 DE fraction and D-glucose at the four alkali concentrations.

On both photographs L was a 43 DE glucose syrup, not reacted with alkali and used as a standard to locate the other sugars in the degraded samples. The 24 DE derivatives from 0·1 and 0·5N alkali show low molecular weight sugars formed (labelled A), presumably trioses by fragmentation reactions. No great degradation is apparent until the alkali concentration reaches 1·0N, after which only a compound corresponding to a position between maltose (G_2) and maltotriose (G_3) remained. Some splitting of the maltose and maltotriose components seemed to occur at the lower concentrations, as evidenced by 'double spots' for these derivatives, and some breakdown of the glucose was evident.

TABLE 1
% CARBOHYDRATE REMAINING AFTER ALKALI TREATMENT

	Normality of sodium hydroxide			
DE	0·1N	0·5N	1·0N	2·0N
16	95	92	42	29
24	96	93	43	25
43	92	85	36	17
52	87	85	31	10
68	86	80	29	8
D-Glucose (100)	84	71	25	2

The D-glucose (100 DE), on the other hand, showed a more dramatic breakdown even with 0·1N alkali and again with 2N alkali only one compound in the position between maltose and maltotriose was found.

No concentrations above 2N alkali were used, since these, as shown above, did not increase the degradation appreciably. The results showed that as DE increased so the degradation effect increased and this may be attributable to some 'protecting' effect induced by the longer chain oligosaccharides in the lower DE fractions or simply that only the terminal glucose residue is attacked at low alkali concentrations.

When the glucose syrups were degraded by alkali the percentage carbohydrate remaining did not change appreciably after the first 30 min of the reaction and one may assume the reaction was complete after that time. The possibility that this may be due to the alkali being 'used up' rather than the reaction being finished was considered and indeed below an alkali concentration of 2N the final product had a pH of less than 7 (between 5·5 and 7), presumably due to the formation of saccharinic acids, lactic, formic and acetic acids. Above a concentration of 2N excess alkali remained and thus the need arose to determine the amount of alkali

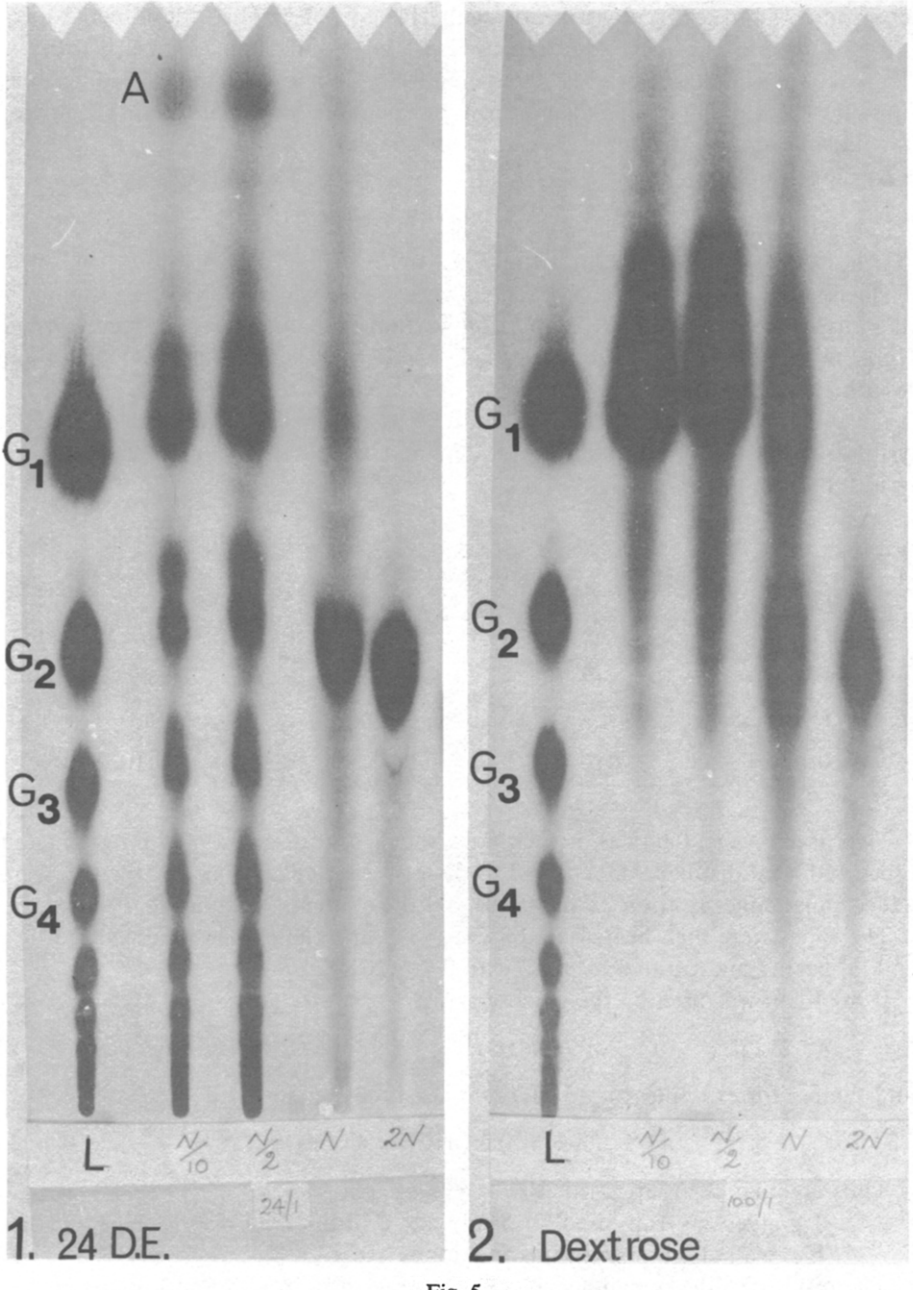
A
G1
G2
G3
G4
L
N/10
N/2
N
2N
24/1
1. 24 D.E.
G1
G2
G3
G4
L
N/10
N/2
N
2N
100/1
2. Dextrose

Fig. 5.

used in the reaction. Knowing the initial concentration of alkali, the final concentration was measured by titration with HCl to pH 7. Three fractions (16, 35 and 52 DE) and D-glucose were prepared as above and refluxed for 3 h with 2N alkali. Twenty millilitre samples were taken every 30 min over this period and the amount of alkali remaining determined by titration with 0·4N HCl. (100-titre) was the amount of alkali used by the 20 ml sample.

At the start:

$$20 \text{ ml } 2\text{N NaOH} = 100 \text{ ml } 0{\cdot}4\text{N HCl}$$

so if no alkali was used the titre would be 100.

The results are given in Table 2 and show that as DE increased so the amount of alkali used increased. Again, the reaction was almost complete after 30 min with a slight increase after that time. The alkali number for starch has previously been described and was applied to glucose syrups for comparison purposes.

Thirty-one samples were used in the determination, 16 demineralised glucose syrup fractions, seven fractions not demineralised, seven commercial glucose syrups and D-glucose.

TABLE 2
ALKALI REMAINING AFTER TREATMENT OF CARBOHYDRATE

	(Time) and Titre (ml 0·4N HCl)						
DE	(0)	(30)	(60)	(90)	(120)	(150)	(180)
16	100	43·4	42·7	42·2	42·2	42·1	41·9
35	100	34·7	34·4	33·9	33·2	32·9	32·9
52	100	32·6	32·1	32·0	31·3	31·2	31·2
D-Glucose (100)	100	24·7	24·3	24·2	23·9	23·6	23·5

Two grammes (±0·001 g) of each were weighed into separate 25 ml graduated tubes and 4 ml distilled water added to dissolve the solids; 4 ml of 5N alkali were added (final concentration 2N in 10 ml) and the contents made up to 10 ml.

The tubes were then heated at 100°C for 1 h and the contents transferred to a 250 ml beaker and titrated to pH 7 with 0·2N HCl.

If no alkali was used by the glucose syrup:

$$10 \text{ ml of } 2\text{N NaOH} = 100 \text{ ml } 0{\cdot}2\text{N HCl}$$

and taking, for example, the 15 DE demineralised fraction:

$$\text{Titre of } 0{\cdot}2\text{N HCl} = 43 \text{ ml}$$

Thus 2 g glucose syrup 'used' 100 − 43 = 57 ml

1 g glucose syrup 'used' 28·5 ml 0·2N HCl ≡ 57 ml of 0·1N HCl and this is the alkali number of this fraction.

The results for the remainder of the glucose syrups are given in Fig. 6.

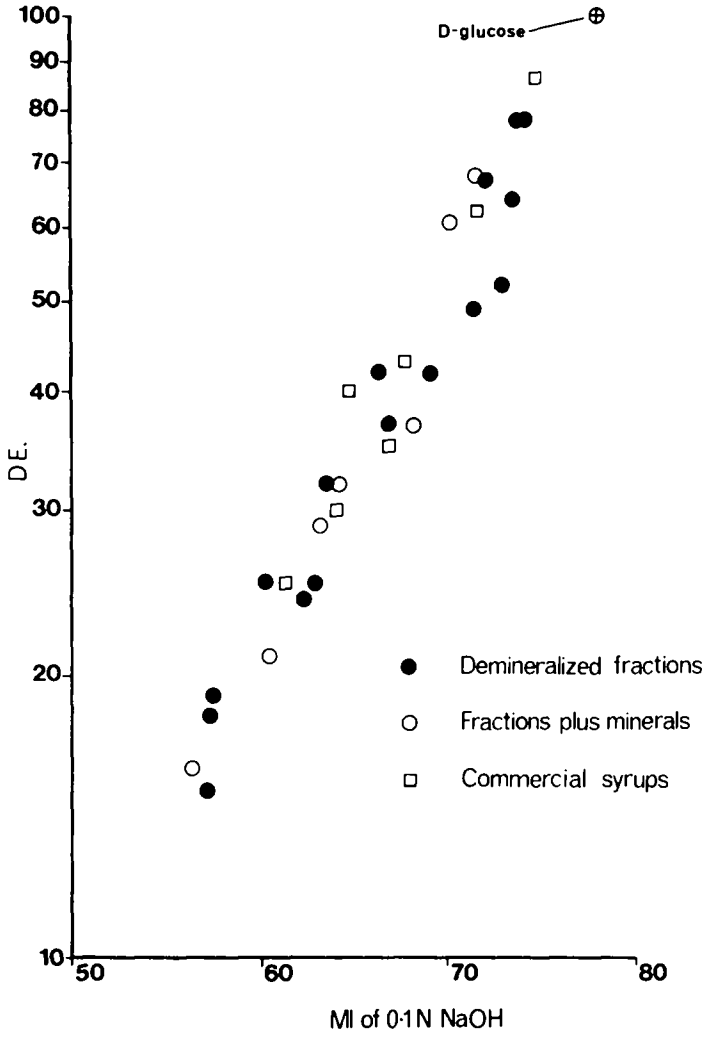

Fig. 6. Alkali number versus DE.

A linear relationship was found between 'alkali number' and log DE overall. Slight differences were apparent in fractions before and after demineralisation and also between demineralised fractions of the same DE due to different materials of manufacture producing slightly different carbohydrate compositions. Schoch & Jensen (1940) provided data on the alkali numbers of a selection of starches and modified starches. The alkali number of corn starch from which the glucose syrup fractions and commercial syrups were derived was between 9·8 and 12·1. As the

starch was hydrolysed so more reducing end units would be produced and thus more would be available to partake in the reaction with the alkali, with a corresponding increase in alkali number for the glucose syrup, as found.

The production of formic, lactic and acetic acids has previously been mentioned and Evans *et al.* (1926) described a method for measuring the volatile acidity of the degraded carbohydrates. The method was modified slightly and applied to glucose syrups. They found the optimum alkali concentration to be about 0·6–0·7N for maximum acetic and formic acid formation and a similar preliminary experiment was carried out to determine the optimum alkali concentration for volatile acid production.

Two fractions, 15 and 43 DE, and D-glucose were used. Initially, 2 g (±0·001 g) were weighed into a graduated 25 ml tube, dissolved in 4 ml of distilled water and 4 ml alkali added to bring the final concentration in 10 ml to either 0·1N, 0·2N, 1·0N, 2·0N or 5·0N. After heating for 2 h at 100°C, the contents of the tube were transferred to a 500 ml flask and water added to give a total volume of 300 ml. The pH was adjusted to 3·0 with concentrated sulphuric acid and the contents distilled.

The first 200 ml of distillate was collected and titrated with 0·05N alkali to pH 7, to give the volatile acidity expressed in terms of ml 0·05N alkali/2 g sample.

The results are given in Table 3 and show the optimum concentration of alkali to be 1·0N for maximum volatile acid production and thus the work was repeated

TABLE 3
VOLATILE ACIDITY VERSUS (NORMALITY)

DE	(0·1N)	(0·2N)	(1·0N)	(2·0N)	(5·0N)
15	4·3	8·0	24·0	17·0	9·9
43	4·4	8·2	27·1	17·1	12·3
D-Glucose (100)	4·5	8·2	30·8	19·6	12·8

using this concentration. Ten glucose syrup fractions not demineralised, 10 demineralised fractions, seven commercial glucose syrups and D-glucose were treated similarly and the volatile acidity determined for each. Figure 7 shows the results obtained.

Again an approximate relationship was found between the volatile acidity (ml of 0·05N NaOH/2 g) and log DE. The higher the DE, the more volatile acids were formed. As stated earlier, Evans & Benoy (1930) found that maltose yielded less acetic acid than glucose and presumably maltotriose would yield less than maltose. Since acetic acid would be one of the major volatile acids in the degradation mixture, as DE decreased so the volatile acidity would decrease, as found.

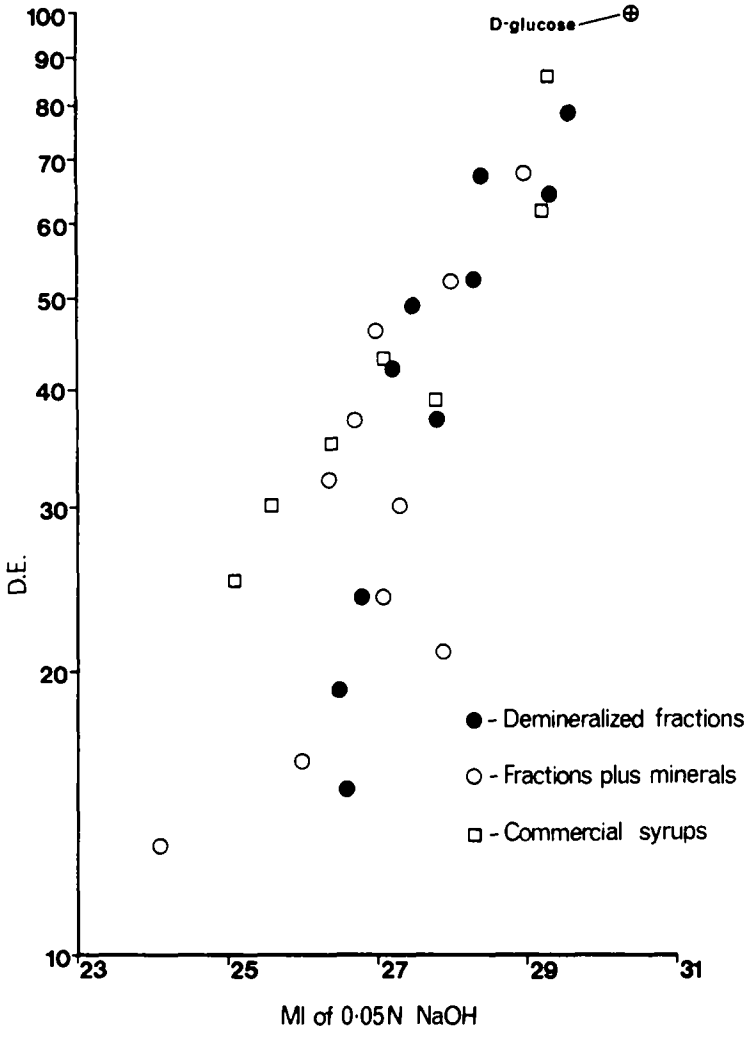

Fig. 7. Volatile acidity versus DE.

DISCUSSION AND CONCLUSION

Glucose syrups are easily degraded by alkali at concentrations of 0·1–2·0N into a wide variety of end products and this fact may be useful for the development of a method to facilitate the analysis of mixtures of glucose syrups and non-reducing sugars. Since the alkali did not totally degrade the syrups some allowance would have to be made for this carbohydrate remaining but if the DE of the added syrup

were known and its carbohydrate content known after degradation, then the percentage of glucose syrup could be determined by a simple calculation. A theoretical example follows.

If in a mixture of a 43 DE glucose syrup and sucrose, a 2 g sample were taken and made up to 10 ml with a final alkali concentration of 2N, say, after heating for 1 h at 100°C the total carbohydrate remaining is 1·5 g.

From previous work we found that 2N alkali reduced a 43 DE syrup to 14% of the original weight after 1 h at 100°C. Therefore the 0·5 g degraded corresponded to 86% of the total weight of the syrup. Thus 100% syrup corresponded to:

$$\frac{0{\cdot}5 \times 100}{86}\ \text{g} = 0{\cdot}58\ \text{g} = 29\%$$

Thus weight of non-reducing sugar was 1·42 g = 71%.

A further use would be in the determination of the DE of glucose syrup samples by the 'alkali number' of the samples. From a standard curve the DE's of unknown samples could be read off directly and, since no 'end points' have to be determined, operator error would be reduced. By suitable rearrangement the method could perhaps be performed on an Auto Analyser, thus reducing errors further.

Whilst the above work has attempted to explore new ground regarding the effect of alkali on glucose syrups, obviously more work is needed to fully understand the complex reactions involved and to develop methods from this work to facilitate the analysis of these substances.

ACKNOWLEDGEMENTS

The author is extremely grateful to Dr G. G. Birch and Professor E. J. Rolfe of the National College of Food Technology for their help and constructive criticisms during the course of this work, and to the Science Research Council and Beechams Products Ltd for a CAPS award in support of this work.

REFERENCES

BIRCH, G. G. & KEARSLEY, M. W. (1974). The fractionation of glucose syrups by reverse osmosis, *Die Starke* (7), 220–4.

BIRCH, G. G., KHEIRI, M. S. A. & HUFTON, D. C. (1971). Dextrose equivalents of maltodextrins and the Lane and Eynon titration, *J. Fd. Technol.*, **6**, 439–41.

BIRCH, G. G. & MWANGELWA, O. M. (1974). Colorimetric determination of sugars in sweetened condensed milk products, *J. Sci. Fd. Agric.*, **25**, 1355–62.

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

EVANS, W. L. (1929). The mechanism of carbohydrate oxidation, *Chem. Rev.*, **6**, 281–315.

EVANS, W. L. & BENOY, M. P. (1930). The mechanism of carbohydrate oxidation XI. The action of potassium hydroxide on maltose, *J. Am. Chem. Soc.*, **52**, 294–307.

EVANS, W. L. & CORNTHWAITE, W. R. (1928). The mechanism of carbohydrate oxidation VII. The action of potassium hydroxide on dihydroxy acetone, *J. Am. Chem. Soc.*, **50**, 486–92.

EVANS, W. L., EDGAR, R. H. & HOFF, G. P. (1926). The mechanism of carbohydrate oxidation IV. The action of potassium hydroxide on D-Glucose and D-Galactose, *J. Am. Chem. Soc.*, **48**, 2665–77.

EVANS, W. L. & HASS, H. B. (1926). The mechanism of carbohydrate oxidation VI. The action of potassium hydroxide on dl glyceric aldehyde, *J. Am. Chem. Soc.*, **48**, 2703–14.

EVANS, W. L. & HUTCHMAN, J. E. (1928). The mechanism of carbohydrate oxidation VIII. The action of potassium hydroxide on fructose, *J. Am. Chem. Soc.*, **50**, 1496–503.

EVANS, W. L. & O'DONNELL, D. C. (1928). The mechanism of carbohydrate oxidation X. The action of potassium hydroxide on mannose, *J. Am. Chem. Soc.*, **50**, 2543–3556.

FEATHER, M. S. & HARRIS, J. F. (1973). Dehydration reactions of carbohydrates. In *Advances in carbohydrate chemistry and biochemistry*, eds. R. S. Tipson & D. Horton, **28**, 161–224, New York, Academic Press.

KEARSLEY, M. W. & BIRCH, G. G. (1975). Selected physical properties of glucose syrup fractions produced by reverse osmosis, *J. Fd. Technol.*, **10**, 613, 625.

NOLLER, C. R. (1965). *Chemistry of organic compounds*, 3rd edn, London, Saunders.

SCHOCH, T. J. & JENSEN, C. C. (1940). A simplified alkali-lability determination for starch products, *Ind. Engng Chem. analyt. Edn.*, **12**, 531–2.

SOWDEN, J. C. & SCHAFFER, R. (1952*a*). The isomerisation of D-Glucose by alkali in D_2O at 25 C, *J. Am. Chem. Soc.*, **74**, 505–7.

SOWDEN, J. C. & SCHAFFER, R. (1952*b*). The reaction of D-Glucose, D-Mannose and D-Fructose in 0·035N sodium hydroxide at 35 C, *J. Am. Chem. Soc.*, **74**, 499–504.

SPECK, J. C. (1958). The Lobry de Bruyn–Alberda van Ekenstein Transformation. In *Advances in carbohydrate chemistry*, ed. M. L. Wolfrom, **13**, 63–103, New York, Academic Press.

STACEY, C. J., FOSTER, J. F. & ERLANDER, S. N. (1956). Light scattering and viscosity studies on some branched starch polymers, *Makromol. Chem.*, **17**, 181–8.

TAYLOR, T. C., FLETCHER, H. H. & ADAMS, M. H. (1935). Determination of the alkali-labile value of starches and starch products, *Ind. Engng. Chem. analyt. Edn.*, **7**, 321–4.

TAYLOR, T. C. & SALZMANN, G. M. (1933). The action of aqueous alkali on starches, amyloses and modified starches, *J. Am. Chem. Soc.*, **55**, 264–75.

WHISTLER, R. L. & BEMILLER, J. N. (1958). Alkaline degradation of polysaccharides. In *Advances in carbohydrate chemistry*, ed. M. L. Wolfrom, **13**, 289–329, New York, Academic Press.

STUDIES ON PARBOILED RICE—PART 3: CHARACTERISTICS OF PARBOILED RICE ON RECOOKING*

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ABSTRACT

The apparent solubility and cookwater loss of samples of parboiled rice, subjected to various steam treatments, were measured after recooking the rice in boiling water. Both parameters were reduced according to the severity of treatment received, the reduction of apparent solubility being the most marked.

The results showed a highly significant, negative linear correlation ($r = 0{\cdot}972$) *between apparent solubility and the relative amount of complexed amylose in the starch. This confirmed the suggestion made in Part* 1 *of this paper that complexed amylose was responsible for the resistance of the starch towards solubilisation.*

Estimation of the amylose and amylopectin released into the cooking water and solubilised inside the kernel revealed that both fractions were insolubilised to a similar extent. It was proposed that amylose was insolubilised by complexing with free fatty acids and the amylopectin insolubilised by interaction with the complexed amylose.

INTRODUCTION

It was shown previously (Priestley, 1974) that parboiled rice is resistant to solubilisation in hot water and it was suggested that this effect is due to the presence of an insoluble amylose complex. Further work revealed that the degree of complex formation depended on the extent of solubilisation achieved during steaming.

The present investigation was carried out to determine the relationship between this insolubilising effect and the presence of complexed amylose and to study the role of each starch fraction in the phenomenon.

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MATERIALS AND METHODS

Materials

Parboiled rice, variety Ribe, was prepared as described earlier (Priestley, 1974).

Methods

Apparent solubility and cookwater loss: This method has been described previously (Priestley, 1974). A standard cooking time of 40 min was used.

Amylose and amylopectin: The method of Wolf *et al.* (1970) was used with slight modifications (Priestley, 1974). After the sample had been dissolved in 90% dimethyl sulphoxide, the starch content was measured by means of an automatic polarimeter and amylose estimated spectrophotometrically using iodine reagent. Amylopectin was obtained by difference.

RESULTS AND DISCUSSION

Figure 1 shows the apparent solubility of the parboiled rice on recooking. It is clear that the samples which had received the most severe steaming treatment during parboiling were the most resistant to solubilisation.

It was demonstrated earlier (Priestley, 1974) that the formation of complexed amylose increased with an increase in the severity of steaming. The highly significant negative linear correlation ($r = 0{\cdot}972$) between apparent solubility after recooking and the relative content of complexed amylose, indicated by the height of the V_1 X-ray diffraction peak, can be seen in Fig. 2. This provides strong confirmatory evidence that the formation of an amylose-lipid complex is responsible for the resistance to solubilisation of parboiled rice.

The results for cookwater loss are presented in Table 1. Increased steaming pressure during parboiling also resulted in a lowering of the solids leached into the cooking water, although the effect was less marked than for apparent solubility. These results are probably more dependent on the starch at the periphery of the kernel which would be expected to solubilise rapidly even at low steaming pressures. Although cookwater loss is a less reliable index than apparent solubility, its measurement was necessary in order to follow the fate of the starch fractions.

The amylose content of both the starch released into the cooking water and the solubilised starch in the cooked kernel was determined. It did not change markedly in either case. The amylose content of the solubilised starch in the cooked kernels which had received no steam treatment was 8·4% and 5·5% for the rice which had received the most severe treatment (60 min at 15 psig). Corresponding results for the starch in the cooking water were 38% and 29%. The high content of amylose in the starch leached out on cooking reflects the greater ease with which the linear fraction can escape from the swollen starch granules. The solubilised starch in

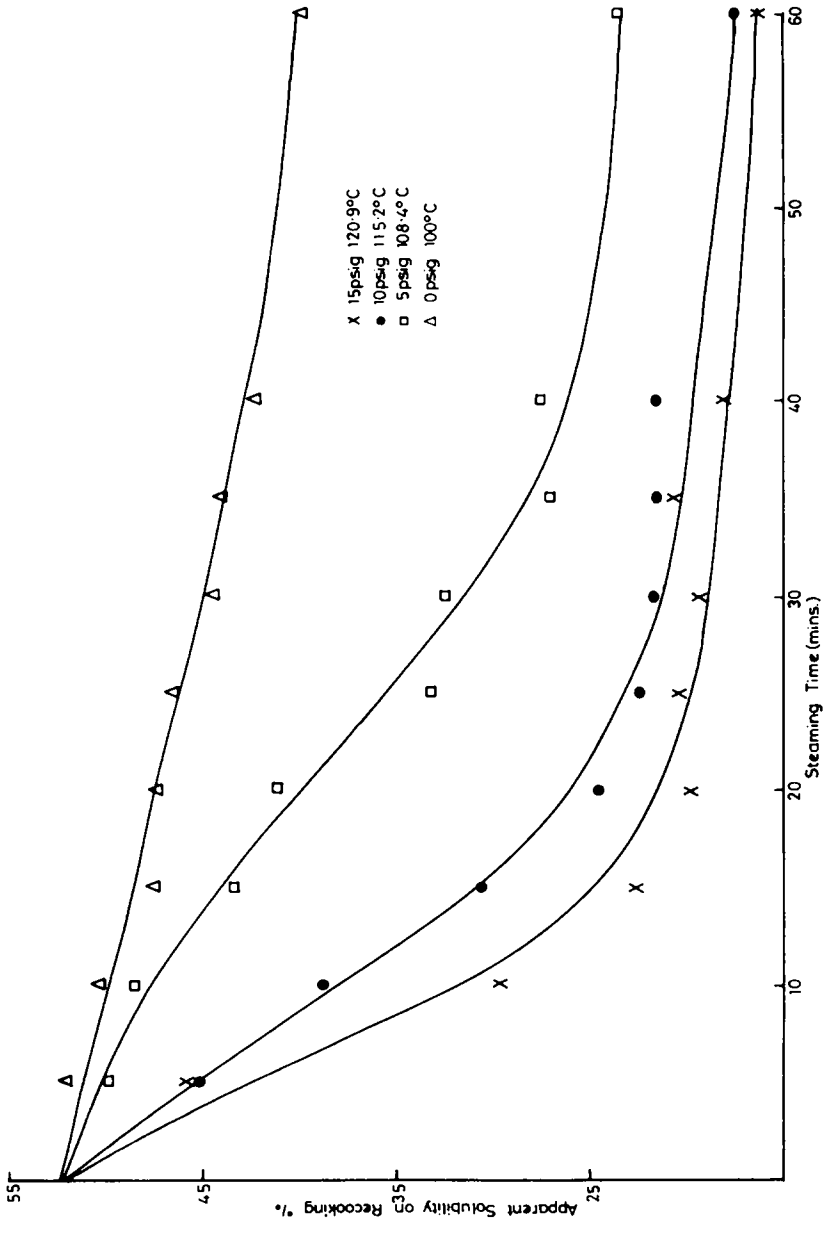

Fig. 1. Apparent solubility of parboiled rice after recooking for 40 min.

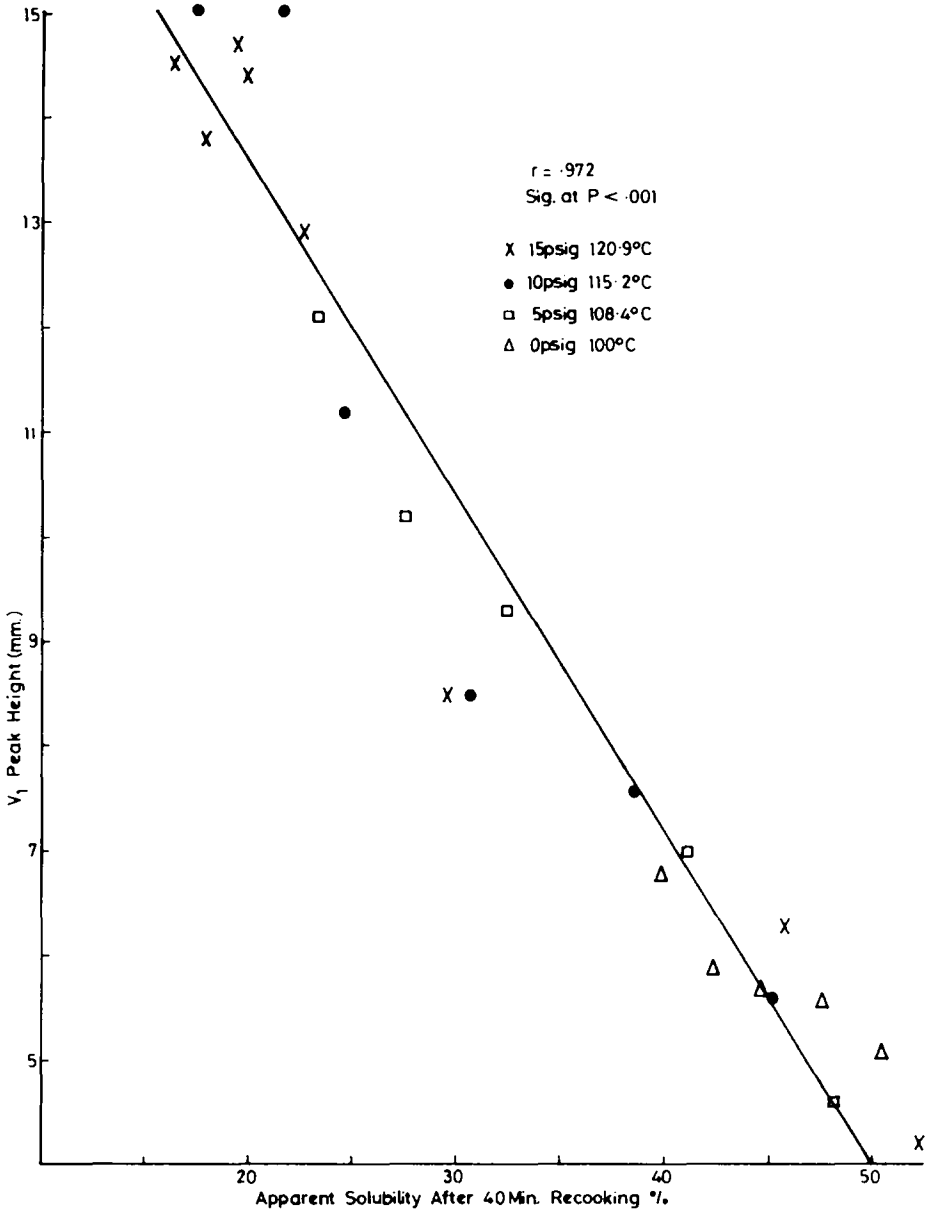

Fig. 2. V_1 peak height versus apparent solubility after recooking.

TABLE 1
COOKWATER LOSS OF PARBOILED RICE AFTER RECOOKING FOR 40 MIN

Steaming time (min)	*Cookwater loss (% of original rice)*			
	0 psig 100°C	5 psig 108·4°C	10 psig 115·2°C	15 psig 120·9°C
0	13·8	13·8	13·8	13·8
5	14·0	11·6	11·0	10·1
10	13·8	9·8	8·6	7·2
15	13·5	8·3	7·3	7·4
20	10·8	8·3	7·3	6·2
25	9·8	7·7	6·6	6·6
30	9·4	7·9	7·2	7·0
35	9·3	7·0	7·1	7·2
40	8·0	7·4	6·9	7·2
60	8·6	6·8	6·9	6·5

the cooked kernels, however, was much lower in amylose content than the starch in the uncooked kernel. This was due to insolubilisation by complex formation and the loss of much of the solubilised amylose into the cooking water.

In order to examine the fate of each starch fraction, the results of the amylose analyses (amylopectin obtained by difference) are reported as a proportion of the total amylose and amylopectin present in the kernels before cooking (Table 2). Approximately equal quantities of solubilised amylose were present in the cooking water and the cooked kernel. The major part of the solubilised amylopectin, however, remained in the cooked kernel. The proportions of total amylose and amylopectin in the starch leached into the cooking water or solubilised in the kernel were both reduced markedly as steaming pressure was increased. Since the amylose:amylopectin ratio did not vary considerably, this reduction was mainly a reflection of the lowered apparent solubility and cookwater loss.

In order to examine this insolubilisation effect more closely, the solubilised proportion of each starch fraction in the cooking water and cooked kernel was subtracted from the total present in the uncooked kernel and recorded as the proportion remaining insolubilised after cooking. This data is given in Table 3. The overall effect of insolubilisation on each starch fraction can then be seen without the need to separately consider the starch in the cooking water and in the cooked kernel.

Insoluble amylose in the cooked kernel rose from 46% of the total for untreated rice to 85% for the most severely steamed rice. This can be attributed to complex formation between amylose and lipid material. Insoluble amylopectin increased by a similar order from 31% to 76%. This cannot be readily explained in the same manner as there has been no evidence to suggest that the branched fraction is capable of forming such complexes. A possible explanation of this phenomenon is that it is caused by an interaction between amylopectin and the insoluble amylose complex. This interaction could arise from either or both of the following mechanisms:

TABLE 2
FATE OF THE SOLUBILISED STARCH FRACTIONS IN RECOOKED PARBOILED RICE

Steaming conditions	*Steaming time (min)*	*Starch in the cooking water*		*Solubilised starch in the cooked kernel*	
		Proportion of total amylose %	*Proportion of total amylopectin %*	*Proportion of total amylose %*	*Proportion of total amylopectin %*
0 psig 100°C	0	29·3	10·4	24·3	58·5
	10	27·4	10·8	21·4	56·8
	20	25·5	7·6	19·2	53·9
	30	23·5	6·3	20·2	50·0
	40	20·0	5·3	15·7	48·2
	60	21·0	5·8	17·8	44·7
5 psig 108·4°C	10	18·5	7·9	23·1	54·2
	20	15·6	6·7	17·8	46·4
	30	17·0	5·9	14·9	36·4
	40	13·8	6·0	10·9	31·3
	60	12·4	5·6	9·5	26·7
10 psig 115·2°C	5	22·1	8·5	13·2	52·3
	10	16·3	6·9	12·4	44·6
	15	12·4	6·2	9·7	35·3
	20	12·9	6·1	7·9	28·3
	30	12·7	6·0	7·2	24·9
	60	11·2	6·0	5·5	20·1
15 psig 120·9°C	5	20·8	7·7	15·2	52·6
	10	13·7	5·8	10·2	34·0
	15	13·0	6·2	5·6	26·4
	20	11·4	5·1	6·2	22·8
	30	12·6	5·8	6·4	22·2
	40	12·4	6·0	5·0	25·1
	60	10·3	5·7	5·0	18·8

(i) Under the conditions of steaming, the starch fractions are not dissociated from each other so that, on the subsequent insolubilisation of amylose, the amylopectin associated with it is also insolubilised.

(ii) After complex formation amylose interacts with amylopectin by a process of molecular entanglement during the slow drying stage.

The insolubilisation of amylopectin noted by Osman-Ismail & Solms (1973), and attributed to complex formation by the branched fraction, can also be accounted for in this manner.

There is ample supporting evidence in the literature of the insolubilisation of amylopectin by amylose or its complexes. It is well known that in order to obtain pure fractions of amylose by selective complex formation the fractions must be well dispersed. Briones *et al.* (1968) found that fractionation of gelatinised rice starch was difficult to achieve, as a high molecular weight amylopectin subfraction was precipitated with the amylose. The moisture content attained during steaming of the rice used in the present study (below 50% wb) would not favour dispersion of the fractions.

TABLE 3
PROPORTION OF EACH STARCH FRACTION IN PARBOILED RICE REMAINING INSOLUBILISED AFTER COOKING

Steaming conditions	*Steaming time (min)*	*Proportion of total amylose insolubilised in the cooked kernel %*	*Proportion of total amylopectin insolubilised in the cooked kernel %*
0 psig 100°C	0	46	31
	10	51	32
	20	55	39
	30	56	44
	40	64	47
	60	61	50
5 psig 108·4°C	10	58	38
	20	67	47
	30	68	58
	40	75	63
	60	78	68
10 psig 115·2°C	5	65	39
	10	71	49
	15	78	59
	20	79	66
	30	80	69
	60	83	74
15 psig 120·9°C	5	64	40
	10	76	60
	15	81	67
	20	82	72
	30	81	72
	40	83	69
	60	85	76

Whistler (1965) reported that, during the course of retrogradation studies, autoclaved tapioca, arrowroot and potato starches deposited insoluble material rich in the linear component. Cereal starches, on the other hand, deposited material of similar composition to the native starch. This, Whistler suggested, was due to incomplete dispersion of the starch fractions, a view shared by Collison (1968). Ott & Hester (1965) measured the recovery of soluble amylose added to amylopectin pastes by hot water extraction. They concluded that incomplete recovery was caused by an interaction between the two fractions.

The extent of solubilisation of the starch in rice would be expected to influence its texture. Further work would be desirable to verify this as texture is the most important organoleptic property of rice and a single test related to this property would be very useful.

Further work would also be desirable to investigate the nature of the amylose complex in more detail. Of the lipid material present in rice, free fatty acids probably play the greatest role as complexing agents. Complex formation between amylose and fatty acids during cooking may also provide an explanation for the 'ageing' effect encountered in rice storage. Freshly harvested rice becomes a pasty

gruel on cooking, but if previously stored for some weeks, the tendency of the kernels to disrupt and stick together is greatly reduced (Desikachar & Subrahmanyan, 1959). During storage the content of free fatty acids increases rapidly (Barber *et al.*, 1967) which would lead to a higher content of complexed amylose in the cooked kernels. Japanese consumers, however, prefer sticky rice and, in order to preserve this characteristic, fresh rice is stored under refrigeration which markedly reduces the rate of free fatty acid formation (Barber, 1969).

CONCLUSIONS

The results obtained confirmed that the apparent solubility of recooked parboiled rice was governed by the formation of an insoluble amylose complex. It is thought that amylopectin, which does not form such complexes, was insolubilised by an interaction with the complexed linear component. There is much scope for further research relating to these phenomena.

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REFERENCES

BARBER, S. (1969). Basic studies on ageing of milled rice and application to discriminating quality factors, ARS Project No. E-25-AMS-(9), USA.

BARBER, S., BENEDITO DE BARBER, C. & TORTOSA, E. (1967). Almacenamiento de arroz elaborabo III Cambios en la composicion de los lipidos segun la localizacion en el grano, *Rev. Agroquim. Tecnol. Alimentos*, **7**, 235–40.

BRIONES, V. P., MAGBANUA, L. G. & JULIANO, B. O. (1968). Changes in physicochemical properties of starch of developing rice grain, *Cereal Chem.*, **45**, 351–7.

COLLISON, R. (1968). In *Starch and its derivatives*, ed. J. A. Radley, 194, London, Chapman and Hall.

DESIKACHAR, H. S. R. & SUBRAHMANYAN, V. (1959). Expansion of new and old rice during cooking, *Cereal Chem.*, **36**, 385–94.

OSMAN-ISMAIL, F. & SOLMS, J. (1973). The formation of inclusion compounds of starches with flavour substances, *Lebensm-Wiss. u. Technol.*, **6**, 147–50.

OTT, M. & HESTER, E. H. (1965). Gel formation as related to concentration of amylose and degree of starch swelling, *Cereal Chem.*, **42**, 476–84.

PRIESTLEY, R. J. (1974). *Physicochemical studies of starch with special reference to the processing of rice*, Ph.D. Thesis, University of Reading.

WHISTLER, R. L. (1965). In *Starch, chemistry and technology*, ed. R. L. Whistler & E. F. Paschall, **1**, 331, New York, Academic Press.

WOLF, M. J., MELVIN, E. H., GARCIA, W. J., DIMLER, R. J. & KWOLEK, W. F. (1970). Amylose determination in dimethyl sulfoxide extracts of maize, *Cereal Chem.*, **47**, 437–46.

VARIATIONS IN THE LOSS OF VITAMIN C IN LEAFY VEGETABLES WITH VARIOUS METHODS OF FOOD PREPARATION

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ABSTRACT

The vitamin C content of twelve common edible leaves, freshly harvested, ranged from 109 *to* 421 *mg per* 100 *g on a dry weight basis. These were lowered by cooking for* 5 *min in* 2, 4, 6, 8 *and* 10 *volumes of water by* 15 *to* 22%, 28 *to* 39%, 54 *to* 60%, 59 *to* 70% *and* 63 *to* 76%, *respectively. Significantly, cooking in the same volumes of water for* 10 *min slightly increased their ascorbate loss. There was greater loss of vitamin C with increased volume of the blanching water, although the rate differed for different leaves. Generally, there was an increase in the rate of ascorbate loss per volume of the blanching water until about* 6 *volumes during the two periods considered.*

INTRODUCTION

The green leafy vegetables furnish a large part of the vitamin C in the diets of urban areas of the world (Caldwell, 1972). In most parts of Africa and Asia, edible leaves are blanched for different periods of time in varying amounts of water: usually, the blanching water is discarded before the cooked leaves are incorporated into stews.

In view of the labile nature of vitamin C which is required by humans in greater amounts than all the other vitamins, especially in the tropics (Ezell & Wilcox, 1959; Hindson, 1968, 1970), information is required as to the extent of losses in the tropical edible leaves due to various methods of food preparation. This could be used as a guide for the evaluation of dietary vitamin C in the compilation of the much-needed food consumption data of the areas concerned.

EXPERIMENTAL

The edible leaves

Twelve common ones were selected from their four-week-old plants grown on the Departmental Experimental Farm.

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They were quickly brought to the laboratory and then washed with water to get rid of sand and other foreign materials. Ascorbic acid content was determined on aliquots of fresh and cooked samples of each vegetable.

Cooking of the leaves

About 20-g portions of fresh leaves were cut into smaller pieces and then cooked at about 98°C for two different periods of 5 and 10 min in 2, 4, 6, 8 and 10 volumes of tap water (pH 6·6–6·8) in covered cooking pots, as is the normal custom.

Determination of vitamin C

About 10-g portions of leaves were ground with sand and a few drops of 4% (w/v) aqueous metaphosphoric acid solution using a large pestle and mortar. The mixture was then filtered into a 50-ml volumetric flask and made up to volume with distilled water. Aliquots of this mixture were analysed colorimetrically in duplicate with 2,6-dichlorophenol-indophenol according to the methods of Hughes (1959), and of Twomey & Goodchild (1970).

RESULTS AND DISCUSSION

The ascorbic acid contents of the fresh leaves are given in Table 1. The lowest ascorbic acid value was found in cassava leaves (*Manihot esculenta*) with 109 mg and the highest in *Celocia argentea* with 421 mg per 100 g dry weight. They all fall within the wide range of 23 to 643 mg reported for some Malaysian edible leaves (Caldwell, 1972).

The effect of cooking these leaves in 2 to 10 volumes of water for 5 and 10 min on their ascorbate loss is shown graphically in Fig. 1.

TABLE 1

THE ASCORBATE CONTENTS OF THE TWELVE FARM-FRESH LEAFY VEGETABLES

Local name	*Botanical name*	% *Dry matter*	*Ascorbate content (mg/100 g dry wt)*
Soko	*Celocia argentea*	19	421
Tete	*Amaranthus hybridus*	15	405
Gbure	*Talinum triangulare*	11	280
Ewedu	*Corchorus olitorius*	21	165
Ewuro	*Vernonia amygdalina*	23	345
Ogunmo	*Solanum nodiflorum*	24	367
Ugu	*Telfairia occidentalis*	21	341
Uziza	*Piper guineensis*	29	220
Ukazi	*Gnetum bucholzianum*	33	278
Ewe paki (Cassava leaves)	*Manihot esculenta*	12	109
Ewe koko	*Xanthosoma sagittifolium*	16	198
Ilasa (Okro leaves)	*Hibiscus esculentus*	20	180

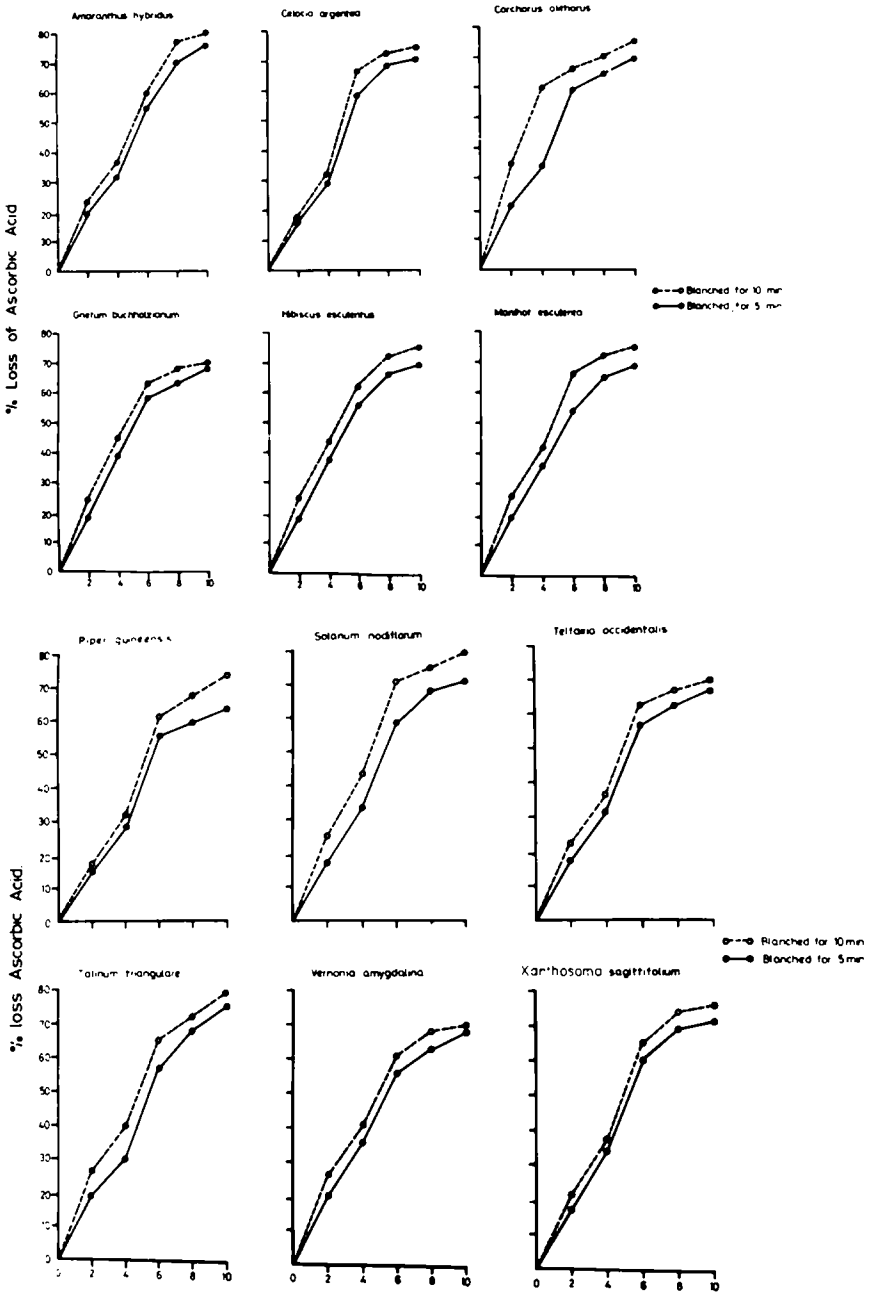

Fig. 1. Loss of ascorbic acid of fresh edible leaves blanched for 5 and 10 min in different volumes of water. Abscissa: volume of blanching water.

TABLE 2
VARIATIONS IN THE % ASCORBATE LOSS BY FRESH LEAVES BLANCHED IN DIFFERENT VOLUMES OF WATER FOR 5 AND 10 MINUTES

Blanching water	*Range of ascorbate loss (%)*	
	Five minutes	*Ten minutes*
* 2 volumes	15 to 22	17 to 26
* 4 volumes	28 to 39	32 to 60
*** 6 volumes	54 to 60	60 to 70
*** 8 volumes	59 to 70	66 to 77
*** 10 volumes	63 to 76	69 to 80

* $P = 0.05$.
*** $P = 0.001$.

Table 2 shows that, for a given volume of blanching water, losses of vitamin C were significantly greater when the leafy vegetables were cooked for 10 min than for 5 min. The differences are significant at the 5% level in 2 and 4 volumes of water and at the 1% level in 6, 8 and 10 volumes.

Greater losses were found with increasing volume of the blanching water, although the rate differed for different leaves (Fig. 1). Ten volumes of water was chosen as the optimum because this was about the largest volume an average housewife would use for cooking any leafy vegetable.

An analysis of variance (Table 3) for 5- and 10-min blanching time did not demonstrate any significant difference among the means of the different vegetables ($P > 0.05$), but for the different volumes of water considered there was a significant difference among the various means ($P < 0.001$).

The twelve leafy vegetables lost between 69 to 80% of their vitamin C when blanched for 10 min in 10 volumes of water (Table 2). This was in close agreement

TABLE 3
ANALYSIS OF VARIANCE

Five minutes

Source of variation	*DF*	*SSQ*	*MSQ*	*F-ratio*
Leafy vegetables	11	188·1	17·1	2·659
Volumes of blanching water	4	22,996·3	5,749·08	894·272
Residual	44	282·9	6·43	
Total	59	23,467·3		

Ten minutes

Source of variation	*DF*	*SSQ*	*MSQ*	*F-ratio*
Leafy vegetables	11	346·9	31·53	1·661
Volumes of blanching water	4	22,461·4	5,615·35	295·757
Residual	44	835·4	18·99	
Total	59	23,643·7		

with the 82% loss obtained by Oliveros & Sumabat (1968) for some vegetables cooked for 9 min under simulated Philippino home-cooking conditions, although the volume of the cooking water was not mentioned. At this extreme level of home cooking, about 20 to 30% of the vitamin C content of the fresh leaves was retained; this could be the 'protected' amount reported to be relatively stable in leaves, especially in spinach (Von Kamienski, 1972). It amounts to between 27 and 81 mg of the vitamin, which is still nutritionally significant if 75 mg is to be taken as the optimum daily allowance (Abt *et al.*, 1963).

The appreciably high levels of vitamin C in the cooked leaves investigated could be a major reason for the very low incidence of scurvy in areas where they are frequently consumed.

REFERENCES

ABT, A. F., VON SCHUCHING, S. & ENNS, T. (1963). *Amer. J. Clin. Nutr.*, **12**, 21.
CALDWELL, M. (1972). *Ecology of food and nutrition*, **1** (4), 313.
EZELL, B. D. & WILCOX, M. S. (1959). *J. Agric. Fd. Chem.*, **7** (7), 509.
HINDSON, T. C. (1968). *Lancet*, **i**, 1347.
HINDSON, T. C. (1970). *Br. J. Nutr.*, **24**, 801.
HUGHES, R. E. (1959). *Biochem. J.*, **64**, 103.
OLIVEROS, M. S. & SUMABAT, L. M. (1968). *Philippine J. Nutr.*, **21** (4), 241.
TWOMEY, D. G. & GOODCHILD, J. (1970). *J. Sci. Fd. Agric.*, **21**, 313.
VON KAMIENSKI, S. (1972). *Scienza deu Alimentazione*, **18** (7), 243 (quoted from *Food Sci. Tech. Abstr.*, **5** (4), No. J540, 1973).

PHYSICAL ASPECTS OF THE IMPROVEMENT OF DOUGH BY FAT*

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ABSTRACT

Shortening containing a fraction which is solid at dough temperatures is an essential ingredient in rapid breadmaking processes, but optional in long fermentation processes. Hypotheses concerning the action of fat are reviewed, and it is concluded that physical mechanisms, rather than those involving lipid oxidation, account for the increased loaf volume on the addition of shortening to the dough. Recent work using 'model' fats has shown that increased loaf volume results when sufficient of the solid component remains 'free' in the dough, and work with stored flour confirmed the importance of the free lipid fraction. A study of carbon dioxide release from doughs during baking showed that fat increases gas retention in the initial stage of rapid expansion. It is suggested that the solid components of the fat facilitate the production of orientated structures in dough, which can persist even when the temperature exceeds the melting point of the fat, and that these structures favour gas retention in the earliest stages of baking.

INTRODUCTION

Breadmaking processes

Commercial bread is produced by methods that consist essentially of five stages: mixing the ingredients, dough development, dividing and moulding, proof, and baking. Three main processes are in current use in Great Britain, differing in the way in which the dough is developed, *i.e.* brought to a state in which it will retain

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gas in the oven. The traditional method (LFP) (abbreviations used: LFP, long fermentation process; CBP, Chorleywood Bread Process; ADD, Activated Dough Development) uses a long period of bulk fermentation, 3 h or more, to achieve this result, and the energy used in mixing the dough is relatively low, of the order of 8–16 kJ/kg. More modern methods employ either intense mechanical working, with an energy input of about 40 kJ kg applied in less than 5 min, as in the Chorleywood Bread Process (CBP: Chamberlain *et al.*, 1962; Axford *et al.*, 1963), or, alternatively, chemical reduction followed by re-oxidation—a process known as 'Activated Dough Development' (ADD: Chamberlain, 1970). In the latter process, slow mixing can be employed, as the energy requirement is only 8–16 kJ/kg, and the need for a long period of fermentation is avoided by the use of a combination of a fast-acting reducing agent and a slow-acting oxidising agent (for example, cysteine hydrochloride and potassium bromate).

All three methods require a final period of fermentation or 'proof' of about 45 min; an additional period of 'intermediate proof' (about 10 min) following dough development is frequently employed. By eliminating bulk fermentation, the two recent methods considerably reduce the total processing time.

Ingredients

The traditional ingredients—flour, water, yeast and salt suffice for the production of good bread by the LFP although, in modern practice, low levels of oxidants are also commonly employed. Additional ingredients are essential with the other processes: higher levels of oxidants are required in the CBP than are used in the LFP, and the combination of reductant and oxidant, mentioned above, is used in ADD. Furthermore, the addition of bakery fat or shortening, optional in the LFP, is essential in both the CBP and ADD if good bread is to be obtained. Nowadays fat is frequently added as part of a 'compound improver' containing other ingredients. The term 'improver' is usually restricted to those agents used to increase loaf volume. Further ingredients, such as yeast foods, malt, or enzyme-active soya, may be included in the dough. Each may impart properties to the bread which are considered desirable by the baker, but their omission does not result in unsaleable bread.

The use of fat in breadmaking

The benefits of adding fat to the dough are not only an increase in loaf volume of up to 25%, but also a soft, relatively even-textured crumb. The amount of fat originally recommended for the CBP was 0·7% by weight of flour if the fat had suitable properties, but this level may be considerably reduced (Chamberlain *et al.*, 1965). The main requirement appeared to be the presence of a certain minimal amount of solid fat in the shortening at the temperature of mixing and final proof.

The effects of the fat added to dough only become apparent in the oven (Baker & Mize, 1939; Elton & Fisher, 1966).

The lipids in dough are derived from two main sources, the bakery fat and the flour lipids, with a very small contribution from the yeast lipids and the optional ingredients. Any hypothesis attempting to explain the improving effect of fat must not only take into account the small amounts required, but also the fact that this fat has been shown by microscopical examination (*e.g.* by Standing, 1973) to be unevenly distributed in the dough. Moreover, there will undoubtedly be interactions between the added fat and the lipids present in the flour.

When flour is made into dough, a change takes place in the association of the lipids with the other dough ingredients, particularly the proteins, resulting in the lipids becoming much less extractable by non-polar solvents (Olcott & Mecham, 1947). Cereal lipids are referred to as 'free' if they are extractable by petrol, or ether, from the dried sample, and 'bound' if polar solvents are required for their extraction. It is generally believed that lipids in the free state in dough are beneficial to baking behaviour and that the effect of shortening may be related to the consequent increase in the amount of free lipid in the dough (Daniels *et al.*, 1969). In doughs mixed anaerobically a greater proportion of the free lipid was derived from the flour when hard fat was included than when a soft fat was used, but this difference was not observed in the presence of air (Daniels *et al.*, 1971). Oxidative reactions involving the enzyme lipoxygenase at lipid-binding sites in the proteins influenced the amount of free lipid in the dough. A dynamic interchange of lipids between the free and bound fractions during doughmixing has been demonstrated (Wood *et al.*, 1974).

The present authors have also obtained evidence which suggests that free—rather than bound—lipid is beneficial in baking. A model fat consisting of a binary mixture of solid and liquid triglycerides was as effective in increasing loaf volume as a good commercial shortening, when used at the same level. Lipid binding studies with this system showed that 86% of the solid component, glyceryl tripalmitate, remained free during doughmixing, and only 14% became bound. The amount of free lipid in dough made from a fresh flour was much greater than in one made from flour which had deteriorated through long-term storage (Bell *et al.*, 1974; Bell & Fisher, to be published). More conclusive evidence was provided by work in which equal amounts of several model fats, containing increasing levels of a solid triglyceride, were added. The level of this constituent was virtually constant in the bound fraction, irrespective of the composition of the model fat, but loaf volume only increased when sufficient of the solid constituent was added for its level in the free lipid to exceed that in the bound lipid of the dough.

The various hypotheses which have been advanced to explain the beneficial effects of lipids in baking may be divided, for convenience, into two main groups—chemical and physical. The chemical hypotheses will only be briefly outlined, as we believe that they are not essentially involved in the mechanism of action of the added fat.

CHEMICAL HYPOTHESES

The chemical hypotheses depend on lipid oxidation, mainly involving lipoxygenase. However, considerable evidence has been accumulated suggesting that the fat added in doughmaking exerts its improving action independently of oxidative reactions. This evidence appears to refute two basic ideas: (a) that unsaturated constituents of the shortening are oxidised and in turn oxidise sulphydryl groups in gluten to give the improving effect and (b) that the effect of shortening is bound up with the oxidation of flour lipids, and the ensuing secondary reactions.

Summary of evidence against the chemical hypotheses

(1) Inorganic oxidants used in breadmaking, such as potassium bromate or (in the United States) potassium iodate, are believed to act by oxidising sulphydryl groups of the flour proteins, thus promoting the development of the dough by the formation of disulphide bridges. If bakery fats were intermediates in the oxidation of the same sulphydryl groups which are affected by the inorganic oxidants, the simultaneous use of fat and oxidant would be equivalent to the use of a higher level of oxidant alone. Thus a curve of loaf volume versus oxidant level in the presence of fat should show a displacement to the left as compared with the corresponding curve for loaves without fat. In reality, however, the main effect of fat was to displace the curve upwards (Fig. 1; Fisher, 1964).

(2) Rheological measurements show that oxidants cause a substantial decrease

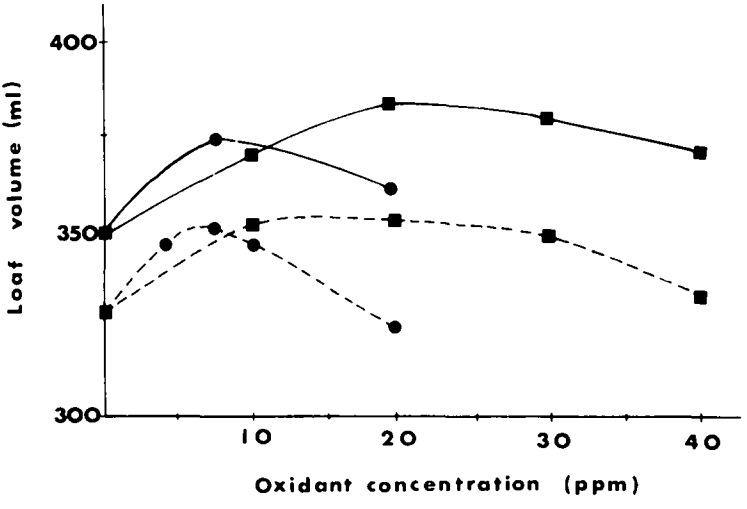

Fig. 1. The effect of fat on loaf volume in the presence of oxidants. Potassium bromate ■. Potassium iodate ●. Without fat · · · . With fat ———.

in the extensibility of the dough and increase its resistance to extension, while fat added in amounts sufficient to increase loaf volume to the same extent causes no visible changes in these properties (Elton & Fisher, 1966; Jelaca & Dodds, 1969).

(3) Certain pure, solid, saturated hydrocarbons give similar effects to fats in baking (Elton & Fisher, 1968). Oxidation of hydrocarbons would be unlikely to occur during doughmaking, and the absence of functional groups excludes other types of chemical interaction.

(4) Oxidation of the unsaturated constituents of shortening itself does not occur to an appreciable extent during doughmixing (Mann & Morrison, 1974). Indeed, it has been shown that unsaturation is not essential to the effectiveness of a shortening

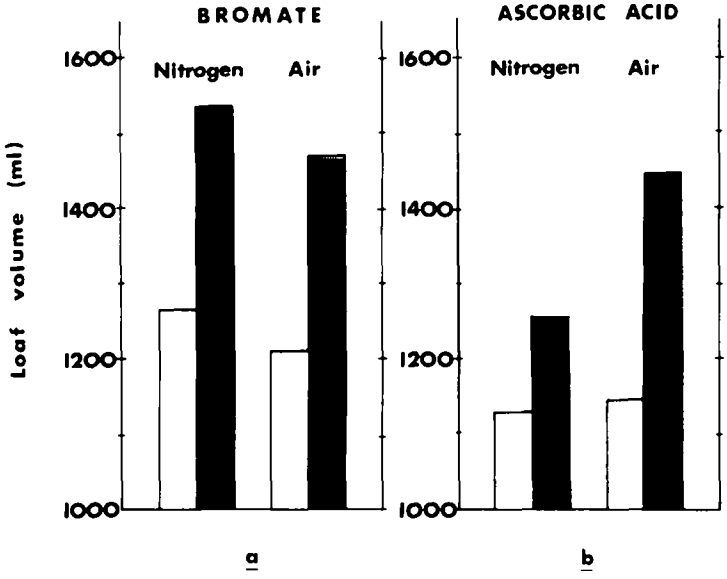

Fig. 2. The effect of fat on loaf volume from doughs mixed in the presence or absence of air. (a) Containing potassium bromate as oxidant. (b) Containing ascorbic acid as oxidant. Without fat: open bars. With fat: hatched bars.

(Baldwin *et al.*, 1963; Chamberlain *et al.*, 1965). In this laboratory it was shown that a model fat containing glyceryl tricaprylate and glyceryl tripalmitate was just as effective as one containing glyceryl trioleate and glyceryl tripalmitate.

(5) The results of baking tests, illustrated in Figs 2(a) and 2(b), show that fat exerts its effects in doughs mixed in nitrogen. The interactions between the effects on loaf volume of mixer atmosphere and fat, evident in doughs containing ascorbic acid (Fig. 2(b)), may involve the lack of the effective oxidant, dehydroascorbic acid, in the doughs mixed under nitrogen. When pre-formed dehydroascorbic acid was incorporated in doughs containing fat, mixed in nitrogen, the same loaf specific

volume was obtained as when ascorbic acid was used in doughs mixed in air (Dodds, unpublished results). It is therefore reasonable to assume that ascorbic dehydrogenase is inactive in nitrogen-mixed doughs, and that lipoxygenase would be inactive in these conditions also. The lipoxygenase inhibitor nordihydroguaiaretic acid (NDGA) also did not prevent the fat response (Fisher & Collins, unpublished results).

(6) In doughs mixed in air, it has been shown that hard and soft fats may be bound to the same extent (Daniels *et al.*, 1971; Bell *et al.*, 1974). However, these types of fat affect loaf volume very differently.

Thus oxidative reactions are not, in the present authors' opinion, essential to the improving action of shortening fat in doughs.

PHYSICAL HYPOTHESES

There are numerous hypotheses which try to explain the effects of lipid improvers on the basis of the physical properties of the lipids and physical interactions with other dough constituents.

Lipoprotein structures in gluten

From their observations on lipid binding during doughmixing, Olcott & Mecham (1947) inferred that gluten has a lipoprotein structure. Confirming the results of earlier X-ray diffraction studies on wheat proteins (Hess *et al.*, 1952, 1954; Traub *et al.*, 1957), Grosskreutz (1960, 1961) proposed that the formation of a relatively small amount of a lipoprotein structure in gluten enabled it to form sheets separated by slip-planes along which large plastic deformations might be facilitated. However, the significance of gluten–lipid complexes had been questioned by Traub *et al.* (1957) on the grounds that extraction from flour of the phospholipids responsible for the prominent 47 Å spacing in gluten did not impair its ability to give well-risen loaves (in the absence of added fat). Nevertheless, such components may still be indirectly involved in the response to fat, since reduced loaf volume generally results when shortening is added to defatted flours (Johnson & Whitcomb, 1931; Cookson & Coppock, 1956).

The effects of flour lipid fractions on the baking of normal and defatted flours, with and without shortening, were examined by Daftary *et al.* (1968). Polar lipids were generally the most beneficial, glycolipids being more effective than phospholipids. Other studies showed that glycolipids may be bound to gliadin by hydrogen bonds, and to glutenin by hydrophobic bonds (Hoseney *et al.*, 1970; Pomeranz *et al.*, 1970). The interactions between dough components and surface-active improvers have recently been described (Chung & Tsen, 1975). However, the saturated triglycerides are much less polar than the surfactants, so it is unlikely that their improving action results from the same mechanism.

The 'lubrication hypothesis'

The 'lubrication hypothesis', according to which fats act by interposing an oily layer between the gluten strands and the starch granules enabling the dough to preserve a continuous gas-retaining structure during expansion, held sway for many years after it was first proposed by Platt & Fleming (1923), mainly in connection with sugar cookies. The best evidence was derived from the microscopical examination of doughs after mixing, during proof and during baking. Burhans & Clapp (1942) described an initial fine dispersion of fat during doughmixing and its subsequent 'beading' along the surfaces of the starch granules during fermentation, while alignment of starch granules and gluten strands in the gas cell walls was taking place. During baking, the shortening previously dispersed along the starch granules coalesced into droplets. These phenomena were held to support the lubrication hypothesis. However, this idea failed to explain why small amounts of solid or semi-solid fats were superior improvers to larger amounts of liquid oils. The effectiveness of castor oil as an improver for LFP doughs was attributed to its lubricant properties (Fisher & Jones, 1932). Alternatively, high viscosity was regarded as an important property of a bakery fat by Baker & Mize (1942), who attributed the effect of castor oil to its retention of a high viscosity at elevated temperatures. However, it was pointed out (Lord, 1950) that dough expansion is rapid at temperatures at which many effective fats are liquefied, and Cookson & Coppock (1954) found no correlation between the viscosity of melted fats and their effects in baking; they concluded that the similar effects of castor oil and semi-solid fats were not linked to viscosity. Recent experiments in this laboratory have shown that castor oil is an 'exception to the rule' that solid fats are essential for the production of bread of good volume by the CBP. The hydroxyl group in ricinoleic acid is presumably responsible for this anomalous behaviour.

The 'pore-sealing' hypothesis

A successor to the 'lubrication hypothesis', first advanced by Baker & Mize (1939 and 1942) and later extended by Baldwin *et al.* (1963), is the 'pore-sealing' hypothesis, according to which fats exert their effects by melting at a critical stage in the expansion of the dough during baking and, by flowing into developing 'pores' in the structure, seal these off so that the escape of gas is retarded. This highly attractive hypothesis does not appear to be consistent with the very low level of 'solid' fat present at the end of proof (*e.g.* $<1\%$ solids content at 43°C in a highly effective shortening). Triglycerides are effective in the CBP when their melting points exceed the proof temperature of the dough. In general this rule applies to hydrocarbons, but reduction of proof temperature to a point below the melting point of an ineffective hydrocarbon did not result in an increase in loaf volume (Elton & Fisher, 1968). Melting point and chain length are interrelated, and it is conceivable that while melting point as such is not the critical property, orientation or mobility of the chains in the region of the melting or slip point may be important.

It has been shown that glyceryl tripalmitate, melting point 65°C, is a good improver, while palmitic acid, with nearly the same melting point (64°C) has no effect (Bell & Fisher, unpublished work). Interactions involving the carboxyl group may hinder the adoption of a favourable configuration or spacing in dough structures. The expansion of a dough in the early stages of baking will be shown, in a later section, to be directly related to the ability of the dough to retain carbon dioxide. Carbon dioxide escapes either through pores formed in the dough during its expansion or by passage through barriers formed by its structural elements, particularly the gluten network. The ordering of these structural elements during doughmixing may be influenced by the presence of fat, which may later also affect the ability of the dough to expand without rupturing or becoming excessively permeable.

'Structural support' hypotheses

A hypothesis which has so far gained only limited acceptance is related to the mechanical support supposed to be given by solid fats or waxes to the structure of the rising dough, on the analogy of the use of hair-like material to hold plaster together (Bayfield & Young, 1964; Pomeranz *et al.*, 1966). Similarly, Ballschmieter (1965) has suggested that fats might support the dough structure as the gluten denatures.

FURTHER ASPECTS OF THE ACTION OF FAT

Delayed release of carbon dioxide

The idea that the effect of fat is to delay the release of carbon dioxide from the fully-proved dough, when it is first placed in the oven, has recently been tested (Bell *et al.*, 1974; Daniels & Fisher, 1976).

Graphs showing the course of the evolution of carbon dioxide from the doughs, plotted as a function of time, were sigmoid in form (Fig. 3). There was a short induction period at the start of baking, during which no appreciable loss of carbon dioxide occurred. With all samples of flour of normal baking behaviour (fresh, or kept at −20°C for 18 months) the induction period was longer with doughs containing fat than with those without fat. This was not the case with flour which had deteriorated in baking quality through long storage. The induction period probably corresponded to the period of rising internal pressure observed by Baker & Mize (1939) and also to the period of rapid dough expansion observed by Elton & Fisher (1966). Results obtained with a number of flours have shown that the effects of fat on both loaf volume and induction period are statistically significant, and it was also shown that there is a high positive correlation between loaf volumes and induction periods.

These results provide an experimental demonstration that the final volume of a loaf is dependent on the permeability of the dough to carbon dioxide in the earliest

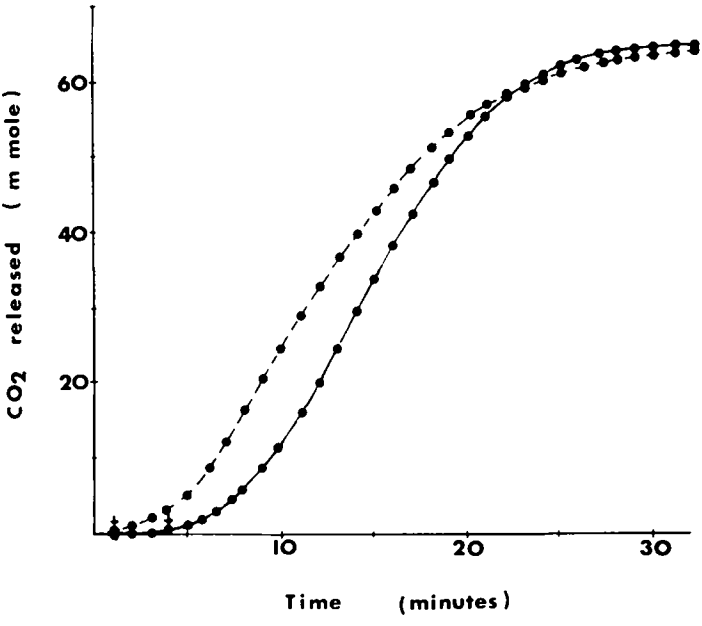

Fig. 3. The release of carbon dioxide during baking, from doughs mixed with and without fat. Without fat: -----. With fat: ———. The end of the induction period is indicated by an arrow.

stages of baking. They also confirm that shortening fat exerts its main effect at this stage. The amount of carbon dioxide evolved from the dough during proof is very small, and measurements of gas production and gas retention have shown no difference attributable to the presence or absence of fat at this stage (Fisher, 1969). The effect of fat, in delaying the release of carbon dioxide, only becomes apparent when the dough expands rapidly.

The form of the carbon dioxide release curves led to the hypothesis (Daniels & Fisher, 1976) that the end of the observed induction period corresponds to a change in dough properties at a definite temperature. Accordingly, the length of the induction period and the transition temperature would be interrelated: in particular, in a dough made from fresh flour, the presence of a suitable shortening would raise it. However, we have since found that differences in loaf volume are manifested during isothermal expansion of doughs prepared with and without fat, and the effects of temperature are therefore less direct than were suggested in this hypothesis.

Interactions between fat and other dough components

It is usually held that the gluten network is responsible for the gas-retaining properties of a dough, the level of protein largely determining the baking potential of a flour. Strong binding of lipids to gluten proteins occurs during doughmixing,

particularly affecting the more polar phospholipids and glycolipids of flour, but little of the shortening lipid becomes bound in the dough. We have already mentioned evidence that free, rather than bound, shortening lipids are important in baking. The effects of shortening therefore do not appear to be attributable to strong binding with gluten proteins, although weaker interactions with either proteins or other components may be important.

Interaction between the shortening and starch, even if not strong enough to be classed as 'lipid binding' in terms of solvent extraction, may contribute to the effect of fat in baking. As mentioned earlier, there is evidence that the fat globules tend to concentrate at the surface of the starch granules (Burhans & Clapp, 1942). Jongh (1961) and Jackson & Landfried (1965) have shown that monoglycerides and triglycerides have beneficial effects in the baking of gluten-free starch loaves, the former mainly with respect to crumb structure and the latter with respect to loaf volume. Addition of the corresponding unsaturated lipids did not benefit starch loaves. There is thus a close similarity between the responses to fat observed in baking ordinary flour doughs and gluten-free starch batters. Saturated monoglycerides were preferentially bound to starch, and unsaturated monoglycerides to protein, in continuous-mix bread (Baldwin *et al.*, 1965). However, although commercial shortenings usually contain monoglycerides, recent experience with model fats consisting of pure triglycerides shows that incorporation of monoglycerides is not obligatory for the production of volume-improving effects in the CBP (Bell *et al.*, 1974).

The fat response of aged flour

A major aspect of the loss of baking quality seen in flours stored for long periods is the diminution in the response of the doughs to the addition of fat. Thus a sample of flour, 6 years old, gave a lower loaf volume, and the dough showed a shorter induction period for carbon dioxide release, when the usual level of fat was used, than when no fat was added (Daniels & Fisher, 1976). A sixfold increase in the level of added fat gave normal loaf volumes. The extensive lipolysis that had occurred in this flour may have affected its baking behaviour in two ways: the directly adverse effects of the unesterified fatty acids and the diminution in the amount of free lipid in the dough due to the loss of flour triglycerides. Additions of unsaturated fatty acids to fresh flour doughs were deleterious in baking by LFP (Sullivan *et al.*, 1936; Barton-Wright, 1938); however, Cookson & Coppock (1954) stated that free fatty acids in reasonable quantity had no adverse effect with flour of 85% extraction rate. Recent experiments have indicated that addition of unsaturated acids, together with fat, to fresh flour doughs leads to poor loaf volume in the CBP. It may be relevant to the suggested involvement of starch in the effects of fat that unesterified fatty acids are almost exclusively bound to starch in continuous-mix bread (Baldwin *et al.*, 1965); such binding is believed to occur at the doughmaking stage. One may speculate that with stored flour more unesterified fatty acid may become bound to

the starch than with fresh flour. The starch would thus become more lipophilic, influencing its interaction with added shortening. The baking performance of the flour might be indirectly affected in this way.

Foam-like properties of dough

Retardation of the release of carbon dioxide was earlier postulated as perhaps involving the production of numerous small gas cells in the dough through the emulsifying properties of commercial shortenings (Elton & Fisher, 1968). MacRitchie (1975) has found that polar lipids promote the formation of stable foams from the aqueous phase separated from doughs by centrifugation. This property doubtless contributes to the beneficial effects of such compounds in bread-making. It was suggested that non-polar lipids dissolve, and thus inactivate, anti-foaming agents in the dough, rather than themselves having a direct foam-promoting action.

It is notable that the presence of a solid (or perhaps liquid-crystalline) phase significantly affects the behaviour of other lipid-containing systems (Holland & Herrington, 1953). The stability of ice-cream emulsions during manufacture is promoted by the use of a suitable partially solidified oil (Berger & White, 1971). Evidence was given that in such a *cooled* system concentric shells of crystalline fat occur in the fat globules, surrounded by an emulsifier film, this arrangement apparently helping to stabilise a thin protein layer at the oil–water interface.

In another field of research, it was shown that increasing the degree of unsaturation of a lecithin membrane increased its permeability to small molecules (Finkelstein & Cass, 1968). Similar results have been obtained with biological membranes and liposome model systems (de Gier, 1973).

CONCLUSIONS

None of the numerous hypotheses discussed above appears to provide a wholly satisfactory explanation of the action of fats in breadmaking. While oxidative changes in the flour lipids undoubtedly occur in the course of doughmixing, we believe that these chemical reactions are not directly involved in the effects of shortenings.

The generalisation that the effectiveness of a lipid as a baking improver depends on the presence of a solid phase at the dough temperature appears to apply only to non-polar lipids, including triglycerides and wax esters, and possibly hydrocarbons. Slightly more polar lipids, such as free fatty acids or the hydroxyesters present in castor oil, behave differently, perhaps because they interact with different sites in the dough matrix.

Although the solid components of a shortening are of primary importance in influencing loaf volume, the liquid components have secondary effects that may be

significant in certain situations. They appear to be necessary for the dispersion of the solid components. Furthermore, when excessive lipid binding occurs, as in doughs made from long-stored flour, augmenting the free lipid fraction by the use of additional oil may be beneficial. It may well be that the physical properties of the liquid components also contribute to the overall effectiveness of a shortening fat.

The fact that semi-solid fats are necessary for the successful use of the rapid breadmaking processes suggests that, in the absence of fat, these processes give rise to structural defects in the 'cellular' structure of the dough which are not produced when solid fat is used. These adverse effects, which occur when high levels of work input are employed in mixing, have been attributed to excessive lipid binding. It is also possible that the solid (or liquid-crystalline) fat may interact with the hydrated dough components in its vicinity and induce the formation of an ordered structure. Such a structure might be resistant to disruption even after the temperature had risen sufficiently to melt the fat that had originally promoted its formation.

ACKNOWLEDGEMENTS

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REFERENCES

AXFORD, D. W. E., CHAMBERLAIN, N., COLLINS, T. H. & ELTON, G. A. H. (1963). Special report: Continuous breadmaking—The Chorleywood Process, *Cereal Sci. Today*, **8**, 265–70.

BAKER, J. C. & MIZE, M. D. (1939). Effect of temperature on dough properties. II, *Cereal Chem.*, **16**, 682–95.

BAKER, J. C. & MIZE, M. D. (1942). The relationship of fats to texture, crumb and volume of bread, *Cereal Chem.*, **19**, 84–94.

BALDWIN, R. R., JOHANSEN, R. G., KEOGH, W. J., TITCOMB, S. T. & COTTON, R. H. (1963). Special report: Continuous breadmaking—The role that fat plays, *Cereal Sci. Today*, **8**, 273–6, 284, 296.

BALDWIN, R. R., TITCOMB, S. T., JOHANSEN, R. G., KEOGH, W. J. & KOEDDING, D. (1965). Fat systems for continuous mix bread, *Cereal Sci. Today*, **10**, 452–7.

BALLSCHMIETER, H. M. B. (1965). Über die backverbessernde Wirkung von Fetten bei Hefebroten, *Brot Gebäck.*, **19**, 125–31.

BARTON-WRIGHT, E. C. (1938). Studies on the storage of wheaten flour. III. Changes in the flour and the fats and the influence of these changes on gluten character, *Cereal Chem.*, **15**, 521–41.

BAYFIELD, E. G. & YOUNG, W. E. (1964). Fluid shortenings for white bread, *Cereal Sci. Today*, **9**, 363–71, 381.

BELL, B. M., DANIELS, D. G. H. & FISHER, N. (1974). Studies on mechanically developed doughs: Shortening distribution and carbon dioxide release during baking, *J. Am. Oil Chem. Soc.*, **51**, 530A.

BERGER, K. G. & WHITE, G. W. (1971). An electron microscopical investigation of fat destabilization in ice cream, *J. Fd Technol.*, **6**, 285–94.

BURHANS, M. E. & CLAPP, J. (1942). A microscopic study of bread and dough, *Cereal Chem.*, **19**, 196–216.

CHAMBERLAIN, N., COLLINS, T. H. & ELTON, G. A. H. (1962). The Chorleywood Bread Process, *Bakers' Dig.*, **36** (5), 52.
CHAMBERLAIN, N., COLLINS, T. H. & ELTON, G. A. H. (1965). The Chorleywood Bread Process: Improving effects of fat, *Cereal Sci. Today*, **10**, 415–19, 490.
CHAMBERLAIN, N. (1970). Baking: The significance of modern processing methods. In *Proteins in human food*, ed. R. A. Lawrie, London, Butterworths.
CHUNG, O. K. & TSEN, C. C. (1975). Changes in lipid binding and protein extractability during dough mixing in presence of surfactants. *Cereal Chem.*, **52**, 549–60.
COOKSON, M. A. & COPPOCK, J. B. M. (1954). The role of glycerides in baking. I. The effects of added glycerinated fats in bread and flour confectionery, *J. Sci. Fd Agric.*, **5**, 8–19.
COOKSON, M. A. & COPPOCK, J. B. M. (1956). The role of lipids in baking. III. Some breadmaking and other properties of defatted flours and of flour lipids, *J. Sci. Fd Agric.*, **7**, 72–87.
DAFTARY, R. D., POMERANZ, Y., SHOGREN, M. D. & FINNEY, K. F. (1968). Functional (breadmaking) properties of lipids. II. The role of flour lipid fractions in breadmaking, *Fd Technol. Champaign*, **22**, 327–30.
DANIELS, D. G. H. & FISHER, N. (1976). The release of carbon dioxide from dough during baking, *J. Sci. Fd Agric.*, **27**, 351–7.
DANIELS, N. W. R., RICHMOND, J. W., EGGITT, P. W. R. & COPPOCK, J. B. M. (1969). Studies on the lipids of flour. IV. Factors affecting lipid binding in breadmaking, *J. Sci. Fd Agric.*, **20**, 129–36.
DANIELS, N. W. R., FRAZIER, P. J. & WOOD, P. S. (1971). Flour lipids and dough development, *Bakers' Dig.*, **45** (4), 20–26.
DE GIER, J. (1973). Comparative permeability studies on liposomes and biological membranes with various lipid compositions, *Biochem. Soc. Trans.*, **1**, 331–3.
ELTON, G. A. H. & FISHER, N. (1966). A technique for the study of the baking process, and its application to the effect of fat on baking dough, *J. Sci. Fd Agric.*, **17**, 250–4.
ELTON, G. A. H. & FISHER, N. (1968). Effect of solid hydrocarbons as additives in breadmaking, *J. Sci. Fd Agric.*, **19**, 178–81.
FINKELSTEIN, A. & CASS, A. (1968). Permeability and electrical properties of thin lipid membranes, *J. Gen. Physiol.*, **52**, 145S–173S.
FISHER, E. A. & JONES, C. R. (1932). The use of oils, fats and emulsions of oils and fats in breadmaking, *Bakers' nat. Ass. Rev.*, 427–9, 459–62.
FISHER, N. (1964). Final Technical Report on Project No. UR-E29-(10)-14 to the US Dept. of Agriculture, p. 38.
FISHER, N. (1969). Final Technical Report on Project No. UR-E29-(10)-76 to the US Dept. of Agriculture, p. 129.
GROSSKREUTZ, J. C. (1960). The physical structure of wheat protein, *Biochim. Biophys. Acta*, **38**, 400–9.
GROSSKREUTZ, J. C. (1961). A lipoprotein model of wheat gluten structure, *Cereal Chem.*, **38**, 336–49.
HESS, K., KIESSIG, H. & HANSSEN, E. (1952). Die Samen proteine der Gramineen und ihre Wandlungen bein Teigen und Backen, *Naturwiss.*, **39**, 135–6.
HESS, K. (1954). Protein, Kleber, und Lipoid in Weizenkorn und Mehl, *Kolloid Z.*, **136**, 84–99.
HOLLAND, R. F. & HERRINGTON, B. L. (1953). Churning time of milk fat at different temperatures, *J. Dairy Sci.*, **36**, 850–3.
HOSENEY, R. C., FINNEY, K. F. & POMERANZ, Y. (1970). Functional (breadmaking) and biochemical properties of wheat flour components. VI. Gliadin–lipid–glutenin interaction in wheat gluten, *Cereal Chem.*, **47**, 135–40.
JACKSON, G. R. & LANDFRIED, B. W. (1965). The effect of various glycerides on the baking properties of starch doughs, *Cereal Chem.*, **42**, 323–30.
JELACA, S. & DODDS, N. J. H. (1969). Studies of some improver effects at high dough temperatures, *J. Sci. Fd Agric.*, **20**, 540–5.
JOHNSON, A. H. & WHITCOMB, W. O. (1931). Wheat and flour studies. XIX. Studies of the effect on their breadmaking properties of extracting flours with ether, with special reference to the gas-retaining powers of doughs prepared from ether-extracted flours, *Cereal Chem.*, **8**, 392–403.
JONGH, G. (1961). The formation of dough and bread structure. I. The ability of starch to form structures, and the improving effect of glyceryl monostearate, *Cereal Chem.*, **38**, 140–52.
LORD, D. D. (1950). The action of polyoxyethylene monostearate upon starch with reference to its softening action in bread, *J. Colloid Sci.*, **5**, 360–75.
MACRITCHIE, F. (1975). A mechanism for the action of lipids in baking. Paper presented to the ICC Symposium 'Lipids in Wheat Technology', 27–30 May, Wageningen, The Netherlands.
MANN, D. L. & MORRISON, W. R. (1974). Changes in wheat lipids during mixing and resting of flour–water doughs, *J. Sci. Fd Agric.*, **25**, 1109–119.

OLCOTT, H. S. & MECHAM, D. K. (1947). Characterization of wheat gluten. I. Protein–lipid complex formation during doughing of flours. Lipoprotein nature of the glutenin fraction, *Cereal Chem.*, **24**, 407–14.

PLATT, W. & FLEMING, R. S. (1923). The action of shortening in the light of the newer theories of surface phenomena, *Ind. Eng. Chem.*, **15** (4), 390–4.

POMERANZ, Y., FINNEY, K. F. & HOSENEY, R. C. (1970). Molecular approach to breadmaking, *Science*, **167**, 944–9.

POMERANZ, Y., RUBENTHALER, G. L. & FINNEY, K. L. (1966). Studies on the mechanism of the bread-improving effect of lipids, *Fd Technol. Champaign*, **20**, 1485–8.

STANDING, M. A. (1973). The microstructure of dough and bread using histophysical techniques, *J. Sci. Fd Agric.*, **24**, 984.

SULLIVAN, B., NEAR, C. & FOLEY, G. H. (1936). The role of the lipids in relation to flour quality, *Cereal Chem.*, **13**, 318–31.

TRAUB, W., HUTCHINSON, J. B. & DANIELS, D. G. H. (1957). X-ray studies on the wheat protein complex, *Nature, Lond.*, **179**, 769–70.

WOOD, P. S., DANIELS, N. W. R. & GREENSHIELDS, R. N. (1974). The use of radiotracers to study lipid binding in wheat-flour doughs, *J. Sci. Fd Agric.*, **25**, 1045–6.

MEALINESS AND SOGGINESS IN SWEET POTATO

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ABSTRACT

Sweet potato (var. Jersey) and yam (var. Velvet) are 'mealy' and 'soggy' varieties, respectively, of Ipomoea batatas *Poir. In the raw state the storage roots of both have virtually the same histological structure: more or less abundant starch grains in the parenchyma cells and few intercellular spaces. After baking, the sweet potato shows rounded cells which are full of gelatinised starch and are separated by numerous small, capillary-like intercellular spaces. The baked yam has very little starch, shrivelled cells, ruptured and shrunken cell walls and many large intercellular cavities. The relationships of histological structure to textural properties in these roots and in the mealy and soggy varieties of the tuber of the Irish potato are discussed.*

INTRODUCTION

The texture of the cooked tuber of the Irish potato (*Solanum tuberosum* L.) has been considered by this laboratory in earlier publications (Sterling, 1955; Sterling & Bettelheim, 1955). The explanation that was advanced in those publications was that, upon heating, the starch grains within the cells gelatinised and swelled greatly, exerting a strong swelling pressure on the cell wall: the greater the starch content, the greater the pressure. The strength of the middle lamella between adjoining cells was also considered to be important, so that a weak middle lamella could permit the turgid cells to round off and separate from one another. The resultant creation of a network of capillary spaces would lead to a mealy quality, *i.e.* the baked or boiled potato would feel dry on the tongue as the superficial layer of moisture on the latter migrated into the capillaries of the vegetable. Tissues with unseparated cells would be soggy, *i.e.* they would feel moist in the mouth.

In recent years, the explanation of the mechanism leading to mealiness has been

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challenged (Bartolome & Hoff, 1972; Hoff, 1972), and a refutation of the challenge has in turn been published (Reeve, 1972).

To investigate this problem further, we decided to make a histological examination of the baked sweet potato (*Ipomoea batatas* Poir.). It is well known that there are two main varietal types of sweet potato, called respectively 'sweet potato' and 'yam'. The varietal type, sweet potato, is mealy and mildly sweet upon baking, whereas the varietal type, yam, becomes soggy and very sweet when baked.

MATERIALS AND METHODS

Storage roots of sweet potato, var. Jersey, and yam, var. Velvet, were obtained upon the local market in December, 1975. These were washed and cut in half. Depending on the diameter of the segment, two or more cores were cut with a cork borer, $\phi = 1$ cm, perpendicular to the cut surface. The cores were removed and sliced transversely with three cuts, almost all the way through, to demarcate two more or less centrally located discs, each 3 mm high. The cores were then replaced in the large original segment.

The segments were put on a teflon sheet, with their cut surfaces against the sheet. Some were held raw for an hour and the rest were baked for an hour in a pre-heated electric oven set at 350°C. At the end of this period, the cores were isolated by cutting away the surrounding tissue. The discs were removed by a slight finish-cut through the cores and were quartered by two perpendicular diametral cuts. These disc quarters served as histological specimens. They were first placed in Navaschin's solution (Johansen, 1940) under house vacuum (about 0·22 atm). After 24 h the specimens were gradually dehydrated in ethanol and then in turn transferred gradually to pure xylene and finally to paraffin, in which they were embedded. Sections were cut at 13 μm and stained with methylene blue.

RESULTS

The raw sweet potato and the raw yam are shown in Figs 1A and 1B. There is no obvious microscopic difference between the two varieties. Both display parenchymatous tissue as well as parenchyma-cambial intergrades, which typify the highly meristematic properties of this tissue (Artschwager, 1924). There are noticeably more intercellular air spaces in the sweet potato than in the yam, but by and large the raw tissue is fairly compact. Starch grains are more numerous in the sweet potato than in the yam; all are marked by an air space at the hilum, which is presumably caused by desiccation when the tissues are prepared for sectioning.

After baking, the sweet potato shows full-sized, apparently turgid cells separated by capillary spaces (Fig. 2A). Gelatinised starch fills the cells, and the cell walls are

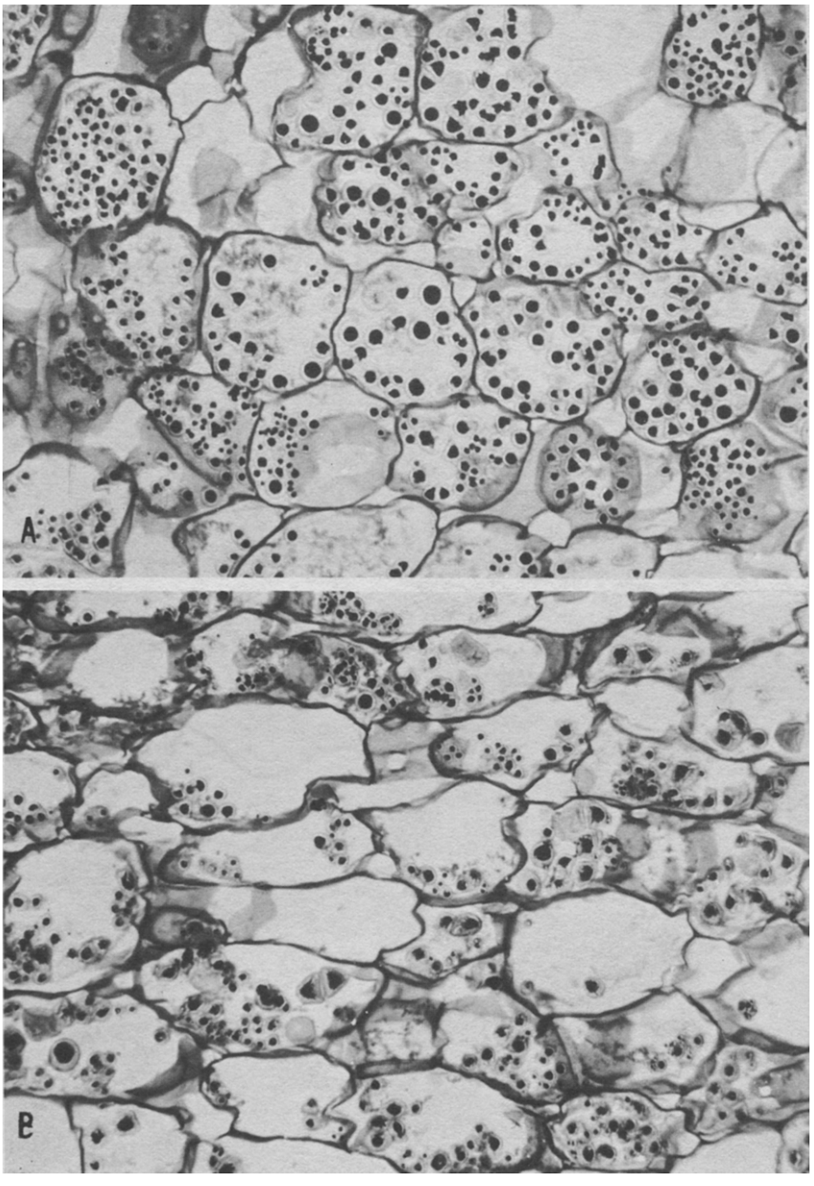

Fig. 1A. Longitudinal section of raw sweet potato (var. Jersey), showing many starch grains, some intercellular spaces. × 216. B. Longitudinal section of raw yam (var. Velvet), with less numerous starch grains and very few intercellular spaces. × 216.

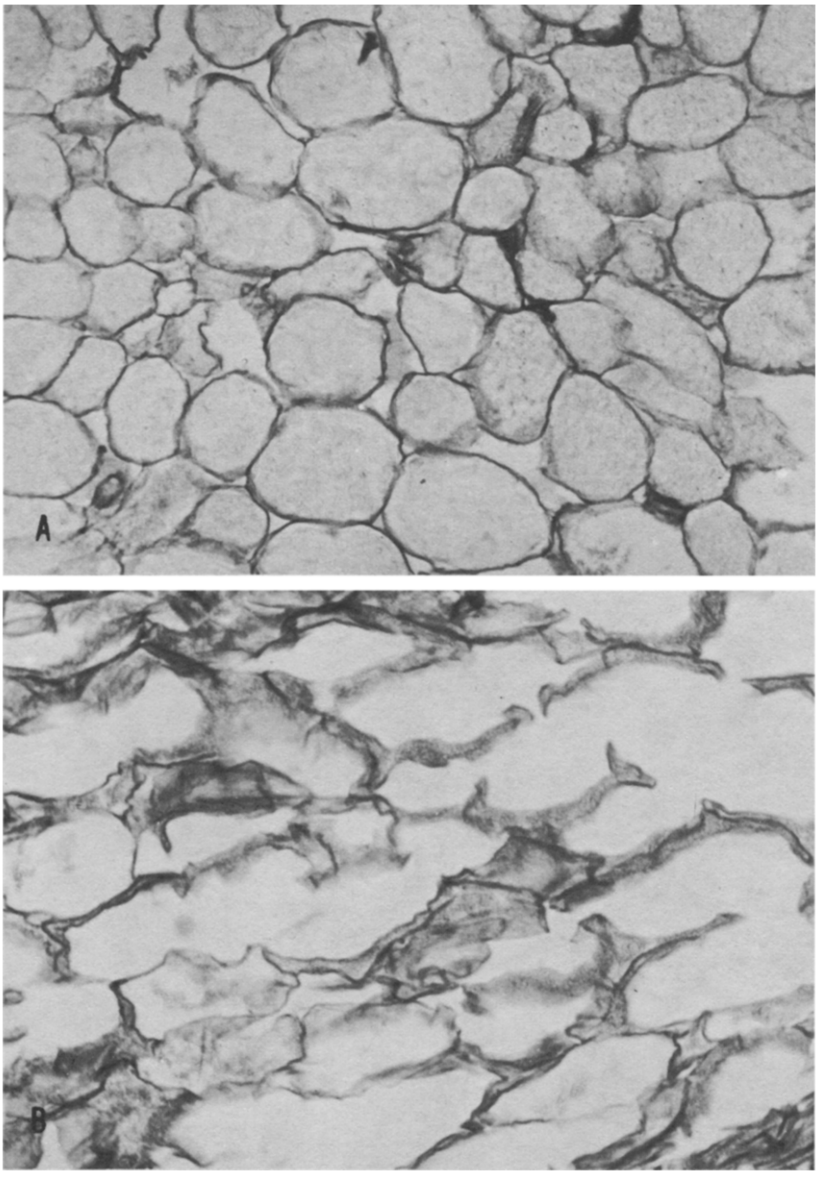

Fig. 2A. Longitudinal section of baked sweet potato (var. Jersey). Starch is gelatinised; many intercellular spaces are present and cells are rounded. ×216. B. Longitudinal section of baked yam (var. Velvet). Little starch is apparent; cell walls are shrunken and disintegrating; and many large, irregular cavities have been created. × 216.

Fig. 3. Portions of large cavities in longitudinal section of baked yam (var. Velvet). × 216.

intact. The baked yam experiences many changes—very little starch is present in its cells; the cells are shrunken irregularly; their walls are disintegrating; and many large cavities traverse the parenchymatous tissue (Figs 2B and 3).

DISCUSSION

Even though the sweet potato is a storage-modified root and the Irish potato is a storage-modified shoot, the cellular aspects of the mealy varieties of both are similar. In each there is a separation of whole cells which appear to be turgid and rounded off. The cells are filled with gelatinised starch. Although the baking process does not introduce water into the tissues, probably a high vapour pressure is created within each cell from water already present in that cell. The effect of the gelatinised starch may be to impede the loss of water by restricting its diffusion, thus permitting the creation of a more or less high internal vapour pressure, depending on the amount of starch.

The microscopic observations do not allow a resolution of the question of the existence of a hydrostatic pressure when starch swells in an unlimited amount of

water during gelatinisation. However, since gelatinisation of starch is an energy-absorbing process (Collison & Dickson, 1971; Mullen & Pacsu, 1942), and since swelling indicates an osmotic pressure of the gel system (Hermans, 1949), it seems evident that the swelling starch grain is indeed capable of exerting a hydrostatic pressure. If enough water is immediately available, that pressure, produced by the many swelling starch grains in a cell, may be capable of causing the cell to burst (Reeve, 1954).

The soggy quality of the baked yam has a different cause from that of the baked Irish potato. While the Irish potato is soggy because its cells do not separate from one another, in the yam, on the other hand, both starch and cell wall tend to break down. Cavities are formed, but they do not make the tissue feel dry; therefore, those cavities are probably filled with sweetened juice derived from the cell sap and from presumably saccharified starch. (The resemblance of this structure to that of the ripe fruit of pear (Sterling, 1954) and of ripe stone fruits (Addoms *et al.*, 1930; Sterling, 1953), which also form juice-filled cavities, is remarkable, because in all these cases there is a partial disintegration of the cell walls.) The mechanism of the disintegration is worthy of exploration.

REFERENCES

ADDOMS, R. M., NIGHTINGALE, G. T. & BLAKE, M. A. (1930). Development and ripening of peaches as correlated with physical characteristics, chemical composition, and histological structure of the fruit flesh. II. Histology and microchemistry, *Bull. N.J. Agr. Exp. Sta.*, **507**.

ARTSCHWAGER, E. (1924). On the anatomy of the sweet potato root, with notes on the internal breakdown, *J. Agr. Res.*, **27**, 157–66.

BARTOLOME, L. G. & HOFF, J. E. (1972). Firming of potatoes: Biochemical effects of preheating, *J. Agr. Food Chem.*, **20**, 266–70.

COLLISON, R. & DICKSON, A. (1971). Heats of dehydration of starch gels, *Stärke*, **23**, 203–5.

HERMANS, J. J. (1949). Thermodynamics of long-chain molecules. In *Colloid science*, ed. H. R. Kruyt, **2**, 49–92, New York, Elsevier.

HOFF, J. E. (1972). Starch 'swelling pressure' of cooked potatoes, *J. Agr. Food Chem.*, **20**, 1283–4.

JOHANSEN, D. A. (1940). *Plant microtechnique*, New York, McGraw-Hill.

MULLEN, J. W., II & PACSU, E. (1942). Starch studies. Gelatinization of starches in water and in aqueous pyridine, *Ind. Eng. Chem.*, **34**, 807–12.

REEVE, R. M. (1954). Histological survey of conditions influencing texture in potatoes. I. Effects of heat treatments on structure, *Food Res.*, **19**, 323–32.

REEVE, R. M. (1972). Pectin and starch in preheating firming and final texture of potato products, *J. Agr. Food Chem.*, **20**, 1282.

STERLING, C. (1953). Developmental anatomy of the fruit of *Prunus domestica* L., *Bull. Torrey Bot. Club*, **80**, 457–77.

STERLING, C. (1954). Sclereid development and the texture of Bartlett pears, *Food Res.*, **19**, 433–43.

STERLING, C. (1955). Effect of moisture and high temperature on cell walls in plant tissues, *Food Res.*, **20**, 474–9.

STERLING, C. & BETTELHEIM, F. A. (1955). Factors associated with potato texture. III. Physical attributes and general conclusions, *Food Res.*, **20**, 130–7.

BOOK REVIEWS

Concise Guide to Food Legislation. By D. Pearson. Weybridge, Surrey. University of Reading, National College of Food Technology. 38 pp. Price: £1.30.

In spite of its all-embracing title, this booklet only covers the food law of England and Wales in any detail. Unfortunately, this is not made clear in the text, and there is no indication that the Food and Drugs Act, 1955, and the subordinate regulations, are not applicable to Scotland or Northern Ireland; nor is any reference made to those countries' similar legislation. The section on EEC legislation is minimal, and for Overseas Regulations only two references to other works in which the information may be found are given. However, the statutory requirements for food sold in England and Wales are covered accurately, and the main compositional requirements, maximum levels for permitted additives and maximum contaminant levels are clearly set out. In addition, the relevant Food Orders and Regulations are listed, as are the Food Standards Committee and Food Additives and Contaminants Committee reports, and LAJAC codes of practice.

It must be stressed that, although accurate, the information is not, by the nature of the booklet, comprehensive. Anyone needing to comply with the regulations for a particular product would find the guide useful in indicating which Statutory Instruments, etc, should be studied, but the specific information given in the guide would, in most cases, be insufficient by itself. Nevertheless, the guide should prove a useful and convenient source of reference to those dealing with food legislation.

J. B. HIRONS

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There has been a need for a lucid description of the effects of sugar structures on their reactive properties in foods. Ever since Emil Fisher proposed the projection

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formula, numerous models have been advanced for the structure of sugars. The authenticity of conformational models has been debated vehemently among many eminent scientists. Needless to say, the conformational structures of sugars not only influence their individual reactive properties but affect the properties of polysaccharides of which they are the basic units. This book fills a significant gap in the food chemistry literature by providing a clear description of the complex relationship between structure and properties of sugars in foods.

The first two chapters deal with the fundamental chemistry and structure of sugars. A chronological description of how the various structural formulas of glucose evolved logically leads into a detailed discussion of the configurational and conformational isomers of aldoses and ketohexoses. Simple rules are presented for manipulating the various structural formulas of the sugars. The authors have done a commendable job of explaining this complex—and often confusing—subject. Their examples illustrate the manner in which structure predetermines and directs chemical and physical properties. These two chapters are well organised and clearly presented; they alone justify the book.

The distribution and properties of sugars are presented in the next chapter. Tables showing the free sugars composition of fruits, vegetables and legumes emphasise the great variation in sugar content among organisms. General physical properties, including optical rotation, specific and molecular rotation, solubility, crystallisation, water activity and viscosity, are explained. Actions of alkalis and acids, oxidation and condensation reactions, and acetal and ketal formation are among the chemical reactions described.

Probably the most worthwhile chapter for those interested in the more applied aspects of sugar chemistry is that describing the inherent chemical reactions that sugars can undergo in foods. The effects of reaction conditions (*i.e.* solvent, polarity, pH, temperature, sugar concentration and time elapsed after dissolution) on mutarotation, enolisation and isomerisation, dehydration and fragmentation, anhydride formation and polymerisation reactions are well explained and documented.

The nutritional, metabolic and medicinal properties are covered in the next two chapters. Interestingly, the authors consider the sweetness of the sugars to be a basic nutritional attribute. They emphasise the hypothesis that sugar sweetness is related to the stereostructure and to the intrinsic chemical properties of sugars. A brief but clear section on digestion of carbohydrates and absorption of sugars follows. These chapters should have been combined and reorganised to eliminate the overlap between them.

The chapter on non-enzymatic browning emphasises the importance of potential chemical reactions on the colour, flavour, texture and nutritive qualities of foods. The chapter is well written and comprehensive, but would have been more logical in the development of the subject matter if it had followed the chapter on the intrinsic chemical reaction of sugars. The last chapter deals with the enzymatic hydrolysis of oligosaccharides and fermentation of sugars.

The book is well written. The illustrations, particularly stereochemical structures, are very well done. The treatment of the subject matter, along with the extensive bibliography and index, should provide researchers and students with a valuable reference and text on the chemistry of sugars in foods.

L. F. HOOD

Gas and Liquid Chromatography Abstracts—Cumulative Indexes 1969–1973. Edited by C. E. H. Knapman. London, Applied Science Publishers, 1976. 381 pp. Price: £16.00.

The rapid growth of scientific literature over the last decade has made it a major task for scientists to keep abreast of developments in their field. Sophisticated information retrieval systems are available but generally these are not readily accessible to the bench scientist. The appearance in the late 1950s of *Gas Chromatography Abstracts* and their expansion in 1970 to include liquid chromatography provided a valuable source of information for chromatographers. As a logical development to shorten search times, the publishers introduced five-year cumulative indexes. This present volume is the third in the series and covers the period 1969–1973. It is produced in the same style and format as the earlier volumes but, with 381 pages, is considerably larger than these. The volume comprises cumulative author and subject indexes with gas and liquid chromatography treated separately, although in a similar manner. The major part of the book is devoted to gas chromatography and liquid chromatography only occupies 22 pages.

My main criticism of this volume lies in the principles upon which the subject index is based. These have been adopted from the early volumes of *Gas Chromatography Abstracts* and were originally evolved to facilitate the transfer of the subject index to a card system. The subject index is divided into seven sections, each of which is further subdivided. The resulting multiplicity of indexes is confusing in a larger volume, despite the provision of a subject index classification. The high degree of cross referencing involved has the disadvantage of considerably lengthening the book. Inconsistencies in indexing also occur. Thus, 'phenolic amines in fruit juices' is indexed in the Gas Chromatography Section once under 'Apparatus and Technique' and twice under 'Applications and Specialised Separations', but not at all under 'Sample Type'.

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D. MCHALE

OBJECTIVE SPECIFICATION OF FOOD FLAVOUR. ANALYSIS OF GAS CHROMATOGRAPHIC PROFILES OF SOY SAUCE FLAVOUR BY STEPWISE REGRESSION ANALYSIS

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ABSTRACT

The relationship between gas chromatographic profiles and sensory tests, which were carried out by an ordering method, was investigated by stepwise regression analysis. The results indicated that sensory qualities were linearly related to gas chromatographic profiles. The multiple correlation coefficient (R) *increased with the increase of step number, and exceeded* 0·9 *at step* 10. R *reached* 0·968 *at the last step number,* 43. *On the other hand, the standard error of estimate reached a minimum value at step* 28 *and then began to increase gradually. The most predictive regression model for sensory test panel acceptability was calculated for each step and the resulting calculated models were tested by substituting the gas chromatographic data. The results showed good accuracy for the estimation of sensory quality.*

INTRODUCTION

The flavour of processed foods is very complicated, being derived from the integrated effects of many aromatic compounds produced by chemical and enzymatic reaction, or bacterial effects. As a result, adequate relationships have not yet been established between the organoleptic qualities of flavour and the quantity of each peak on the gas chromatograms of most processed foods. Recently, however, several attempts have been made to evaluate the flavour quality of foods on the basis of whole gas chromatograms by applying multivariate analysis methods.

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Powers & Keith (1968) applied stepwise discriminant analysis for potato chips and coffee flavour. Using a similar technique, Young *et al.* (1970) reported on the discrimination of two kinds of cola beverage; Powers *et al.* (1971) on the changing of peanut flavour and Bendnarczyk & Kramer (1971) on the changing of ginger oil flavour.

Concerning soy sauce flavour, Saito & Tanaka (1967) studied the relationship between sensory evaluation, which was carried out by an olfactory method, and 17 water-soluble components by multiple regression analysis. According to their report, the contributing proportion for the analysis was only 46%, indicating that there was no linear combination between the quality of soy sauce and the quantities of the water-soluble components. However, Aishima & Nobuhara (1976) observed a linear relationship between the gas chromatographic (GLC) profiles of soy sauce flavour and sensory scores by multiple regression analysis and reported the presence of a peak which possessed a correlation coefficient (r) of more than 0·7. This indicates that one peak is more closely related to the whole flavour of soy sauce than the 17 water-soluble components reported by Saito & Tanaka (1967). Consequently, it was shown that the quality of soy sauce flavour could be assessed objectively by GLC analysis. Further, Aishima & Nobuhara calculated the contributing proportions as quantitative criteria which express the relative significance of each peak of the gas chromatogram for the flavour quality. This is the first report showing that the contributing proportion could become a new standard for the trapping of GLC peak components or for the investigation of flavour components by GC–MS.

According to the data given in this report, there are several peaks which show only a very low contributing proportion and very low correlation coefficients between the quantities of the flavour components and sensory scores. Therefore, there exists the possibility of accurate estimation without using all the peaks on the chromatograms. In other words, the objective evaluation would be obtained more practically and more easily by using some particular peaks as independent variables in the regression model. Therefore, stepwise regression analysis was examined for the analysis of GLC profiles of soy sauce flavour in the present paper.

MATERIALS AND METHODS

Materials and sensory tests

Ten different brands (A, B, C, D, E, F, G, H, I and J) of fermented soy sauce filled in 2-litre glass bottles on the market were divided into two groups in which brands A and B were contained commonly. One group consisted of A, B, C, D, E and F and the other of A, B, G, H, I and J. Each group was evaluated by an ordering method of preference by about 150 well-trained members of a sensory test panel at the Kikkoman Shoyu Co. Ltd. The sum of orders produced for each sample

was divided by the number of sensory test panel members and the quotient was used as the sensory score in this study. The same sensory test was repeated five times on the samples collected separately on the market.

Flavour isolation and GLC analysis

The isolation and concentration of soy sauce flavour for GLC analysis was performed by the method described in detail in our previous paper (Aishima & Nobuhara (1976)). The flavour concentrate was prepared by distillation of soy sauce under a reduced pressure and the distillate was trapped in the cold traps cooled with ice-water and dry ice-acetone. Then the flavour was extracted with dichloromethane from the distillate and the extract concentrated under reduced pressure. The resulting flavour concentrate maintained the characteristics of the original flavour of each sample.

One portion of the flavour concentrate was used for GLC analysis. The analysis was carried out under the conditions shown in Fig. 1. Each gas chromatogram was recorded by a JEOL-252A two-pen recorder and the ratio of two pens settled as one to ten. The peaks selected as the independent variables were numbered. Next, the heights of the numbered peaks were measured and then represented as a percentage of their total.

Stepwise regression analysis

N sensory scores corresponding to each soy sauce sample could be described as $Y = (y_1, y_2, \ldots, y_i, \ldots, y_n)$, $1 \geqq i \geqq n$. If the gas chromatogram of each flavour concentrate shows m peaks, each gas chromatogram could be described as $(x_{i1}, x_{i2}, \ldots, x_{ij}, \ldots, x_{im})$, $1 \geqq j \geqq m$, and consequently the gas chromatograms for whole samples could be shown as the following matrix:

$$\begin{vmatrix} x_{11} & x_{12} \cdots x_{1j} \cdots x_{1m} \\ \vdots & \vdots \quad \vdots \quad \vdots \\ x_{i1} & x_{i2} \cdots x_{ij} \cdots x_{im} \\ \vdots & \vdots \quad \vdots \quad \vdots \\ x_{n1} & x_{n2} \cdots x_{nj} \cdots x_{nm} \end{vmatrix}$$

where $i = 1$ to 60, $j = 1$ to 39 in this study.

The multiple regression model is generally shown as below:

$$Y = \alpha_1 X_1 + \alpha_2 X_2 + \cdots + \alpha_l X_l + \cdots + \alpha_m X_m + \beta$$

where α_l = regression coefficient; β = intercept; Y = dependent variable; and X_l = independent variable.

The α_l and β are computed by the linear least squares method, and the equation is soluble for $n > m$. The correlation coefficient between the real Y and the estimated Y from the computed multiple regression model is designated as a multiple

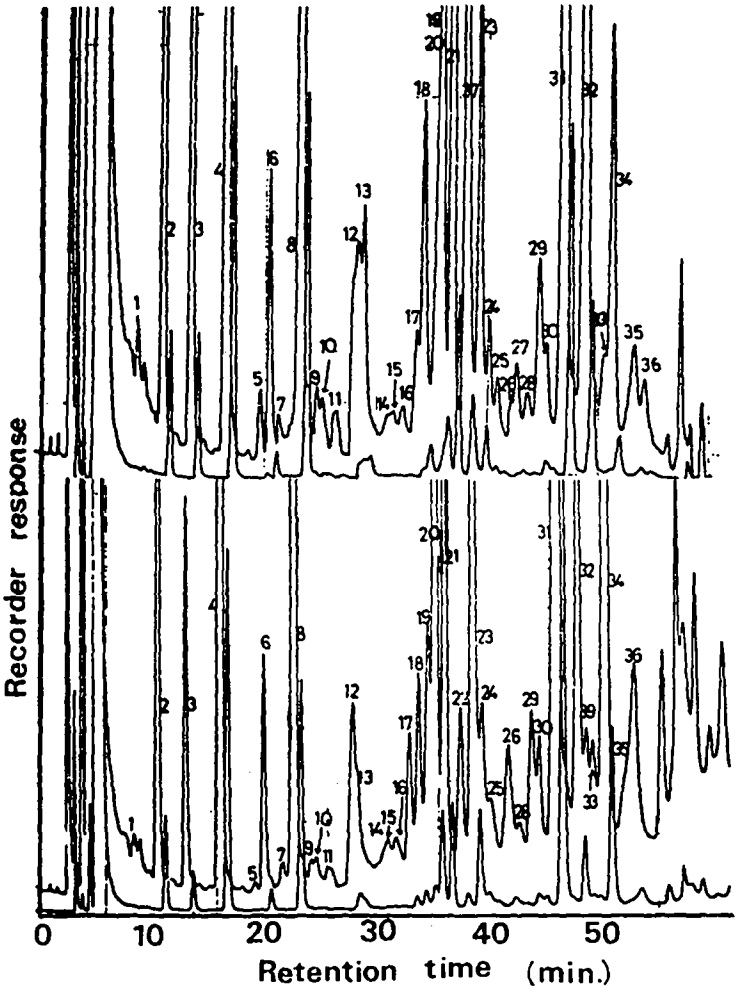

Fig. 1. Gas chromatograms of soy sauce flavour. Conditions—column: glass, 3 mm × 2 m, 20% PEG 20M on Chromosorb W-AW-DMCS; oven temperature: 50–200°C (3°C/min); carrier gas: N_2, 40 ml/min; instrument number: JEOL JGC 1100. Upper pattern is recorded 1·1 mV as full scale and that of the lower is 11 mV.

correlation (R). $R^2 \times 100$, which means the ratio of variance of Y explained by the regression model, is called the contributing proportion.

Smaller numbers of independent variables are desirable for the easier estimation of Y using computed multiple regression models for quality tests or controls of manufacturing processes. To date many kinds of multivariate analysis methods have been developed for this purpose, but stepwise regression analysis (SRA) is

considered as the most appropriate method for selecting a subset of significant variables from a set of variables (Draper & Smith, 1966). For selecting the variables in SRA, several different algorithms have been developed, but an increasing and decreasing method is regarded as being the most adequate method when considering the efficiency of computers and the accuracy of the analysis. Therefore, in the present paper, the relationships between GLC profiles and sensory scores were analysed by an increasing and decreasing SRA method. The SRA is performed by trial-and-error peak selection to obtain the most suitable subset of variables on the basis of *F*-value from the analysis of variance on each independent variable at each step because the degree of significance of entered variables is changed by the entry of another variable. Therefore, the entry of the variable which is not yet included in the regression and the removal of the variables which are already included in the regression are determined from the settled conditions of *F*-value on each variable.

The settled conditions are as described below. The variable having the highest *F*-value among the variables which are not yet included in the regression is selected for entry. If *F*-values of variables which were already included in the regression are under 0·005, the variables are removed from the regression. If *F*-values of all the variables which are not yet included in the regression are under 0·01, the SRA is stopped at that step. The maximum number of the step is settled as 80. The SRA was carried out by UNIVAC-1108, using the BMD02R program (Anon., 1972).

The contributing proportion for each independent variable is calculated by the method described in our previous paper (Aishima & Nobuhara, 1976).

RESULTS AND DISCUSSION

Sensory test and GLC analysis

Sensory scores of the most liked and the most disliked samples are 1·80 and 5·52, respectively. The GLC profiles among the different brands were clearly discriminated from each other, although there was also a slight difference in samples of the same brand. The GLC pattern on one of the most preferred samples is shown at the top, and that on one of the most disliked samples at the bottom in Fig. 1. The values of their sensory scores were 1·91 and 4·48, respectively.

The correlation coefficients between the sensory score of each sample and the quantity of each peak of its GLC pattern are shown in Table 1. Since the sensory tests are performed by an ordering method, a positive correlation in a peak means that the peak gives a preferable contribution with increase of the quantity and a negative correlation means that the peak gives the reverse contribution. The correlation coefficient of peak 3 shows over 0·7, indicating that the correlation coefficient of only one component exceeds the multiple correlation coefficient from 17 water-soluble components reported by Saito & Tanaka (1967). There are several

TABLE 1

MEAN PEAK QUANTITIES (%) AND STANDARD DEVIATIONS FOR 39 PEAKS, AND CORRELATION COEFFICIENTS BETWEEN PEAK QUANTITIES AND SENSORY SCORES FOR EACH PEAK

Peak number	*Mean* (%)	*Standard deviation*	*Correlation coefficient* (r)
1	0·321	0·216	0·068
2	2·640	1·568	−0·466**
3	1·767	1·368	−0·710**
4	9·205	3·353	−0·473**
5	0·208	0·420	0·259*
6	0·947	0·360	0·016
7	0·208	0·420	0·071
8	10·722	2·845	−0·538**
9	0·212	0·181	−0·290*
10	0·306	0·172	−0·574**
11	0·459	0·399	0·031
12	0·928	0·724	0·085
13	0·868	0·594	0·099
14	0·260	0·175	0·052
15	0·281	0·240	0·116
16	0·240	0·159	0·055
17	0·698	0·376	0·220
18	1·919	0·663	−0·359**
19	4·351	6·342	0·362**
20	3·803	1·068	0·488**
21	9·187	2·694	0·172
22	0·872	0·451	0·214
23	3·455	1·094	0·228
24	1·121	0·555	0·199
25	0·514	0·438	0·188
26	0·460	0·301	0·037
27	0·157	0·192	0·105
28	0·330	0·266	0·184
29	1·325	0·427	0·052
30	0·692	0·362	0·269*
31	21·824	5·399	0·044
32	10·366	4·417	0·166
33	0·216	0·361	−0·018
34	5·779	2·649	0·146
35	0·454	0·542	0·142
36	0·604	0·826	0·027
37	1·501	1·522	−0·568**
38	0·107	0·330	0·233
39	0·339	0·429	0·272*

* $P < 0·05$.
** $P < 0·01$.

peaks which show significant relations with the sensory scores such as peaks 8, 10 and 37, whereas peaks 1, 6 and 7 do not show any significant relations with the sensory scores. The quantities of peaks 3 and 8 were plotted against the sensory scores in Fig. 2. As is apparent from that figure, it is impossible to estimate the flavour quality of soy sauce samples on the basis of the quantity of only one peak, in spite of the highly significant relation between the quantity of the peak and the sensory score.

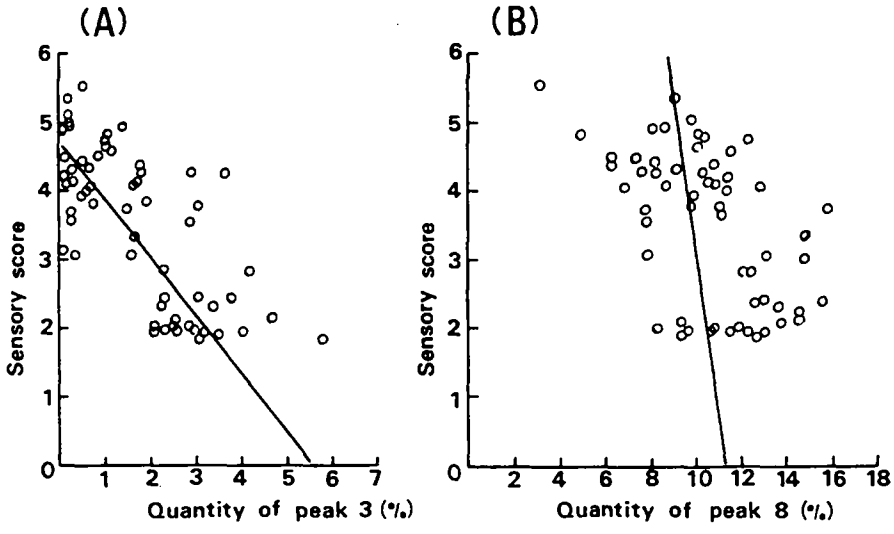

Fig. 2. Sensory scores versus quantities of peaks. ** $P < 0.01$.
(A) $r = -0.710^{**}$; $Y = -0.872X_3 + 4.821$. (B) $r = -0.538^{**}$; $Y = -1.376X_8 + 15.532$.

Multiple regression model and multiple correlation coefficient (R)

In general, many factors may be selected and tested as independent variables. Some factors may then be eliminated because they have no significance for the intended analysis. In the case of soy sauce flavour, about 50 peaks were observed on the gas chromatogram of each sample and 39 of these were selected as independent variables. Among these 39 peaks there exist several groups which correlate with each other. Therefore, each of these groups should be represented by one typical peak. Further, there exist several peaks which show approximately zero values of the contributing proportion (Aishima & Nobuhara, 1976). Therefore, these peaks should be removed from the regression analysis. Thus, the calculation of the multiple regression model was carried out by using 39 independent variables with the expectation that this would not lower the accuracy of the estimation.

The correlation matrix for the variables in the regression model at step 10 is shown in Table 2. Three multiple regression models computed at steps 5, 10 and 43 are shown in Fig. 3. In addition, in this figure the significance of the regression coefficients is examined on the basis of *F*-value. According to Saito & Tanaka (1967), 17 water-soluble components show no significance for the regression coefficients. In Fig. 3, however, most of the regression coefficients show significance at steps 5 and 10. Further, even at step 43, many of these coefficients show significance.

The multiple correlation (R), the increase in R and the contributing proportion on the entered variables at each step are shown in Fig. 4. Peak 3, which showed the

TABLE 2

MATRIX OF CORRELATION COEFFICIENTS AMONG VARIABLES ENTERED INTO THE REGRESSION MODEL COMPUTED AT STEP 10

		Peak number									
		2	3	4	6	8	18	20	26	29	37
Peak number	2	1·000	0·792**	0·647**	0·172	0·299*	−0·093	−0·172	−0·091	−0·166	0·181
	3		1·000	0·486**	0·105	0·440**	0·115	−0·333**	−0·152	−0·236*	0·440**
	4			1·000	0·321*	0·276*	−0·134	−0·165	−0·004	−0·246*	0·258*
	6				1·000	0·194	−0·260*	−0·078	0·210	0·134	−0·095
	8					1·000	0·142	−0·196	−0·161	−0·354**	0·165
	18						1·000	−0·220	−0·164	0·194	0·321*
	20							1·000	0·214	−0·140	−0·370**
	26								1·000	−0·151	−0·120
	29									1·000	0·024
	37										1·000

* $P < 0·05$.
** $P < 0·01$.

Step number 5

$$Y = -0{\cdot}34242X_3^{**} - 0{\cdot}10063X_8^{**} - 0{\cdot}27508X_{18}^{**} + 0{\cdot}18613X_{20}^{**} - 0{\cdot}16178X_{37}^{**} + 5{\cdot}24460$$

Step number 10

$$Y = 0{\cdot}21691X_2^{**} - 0{\cdot}49577X_3^{**} - 0{\cdot}12568X_4^{**} + 0{\cdot}79301X_6^{**} - 0{\cdot}13469X_8^{**} - 0{\cdot}19317X_{18}^{*} + 0{\cdot}18297X_{20}^{**} - 0{\cdot}64005X_{26}^{**} - 0{\cdot}56075X_{29}^{**} - 0{\cdot}06544X_{37} + 6{\cdot}46128$$

Step number 43

$$Y = -0{\cdot}67905X_1 + 0{\cdot}11246X_2 - 0{\cdot}34891X_3^{**} - 0{\cdot}03407X_4 + 3{\cdot}32445X_5^{**} + 1{\cdot}04569X_6^{**} - 0{\cdot}15934X_8^{**} + 0{\cdot}38308X_9 - 0{\cdot}82184X_{10} - 1{\cdot}78821X_{11}^{**} - 0{\cdot}35205X_{12}^{*} - 0{\cdot}02815X_{13} - 0{\cdot}58607X_{14} + 0{\cdot}29482X_{15} + 0{\cdot}73454X_{16} + 0{\cdot}67578X_{17} - 0{\cdot}25561X_{18} + 0{\cdot}01219X_{19} - 0{\cdot}07238X_{20} - 0{\cdot}05584X_{21} + 0{\cdot}71894X_{22}^{**} + 0{\cdot}30140X_{23}^{**} + 0{\cdot}36594X_{24} + 0{\cdot}18797X_{25} - 0{\cdot}64726X_{26}^{*} + 1{\cdot}10172X_{27}^{*} + 0{\cdot}79105X_{28} - 1{\cdot}00033X_{29}^{**} - 0{\cdot}54843X_{30} - 0{\cdot}03543X_{31} + 0{\cdot}03727X_{32} + 0{\cdot}19355X_{33} + 0{\cdot}05264X_{34} + 0{\cdot}34151X_{35} - 0{\cdot}08671X_{37}^{*} - 0{\cdot}74229X_{38} + 0{\cdot}06977X_{39} + 5{\cdot}83343$$

Fig. 3. Multiple regression models computed at step numbers 5, 10 and 43.
$^{*}P < 0{\cdot}05$. $^{**}P < 0{\cdot}01$.

highest value for the contributing proportion, is entered at the first step in the regression model. The R-value increases with the increase of the step number, and exceeds 0·9 by the entry of peak 2 at step 10. The contributing proportion attains 90% at step 19. After this, peaks 20, 7 and 13 are removed from the regression model at steps 26, 37 and 39, respectively. The R-value attains 0·968 at the last step, 43,

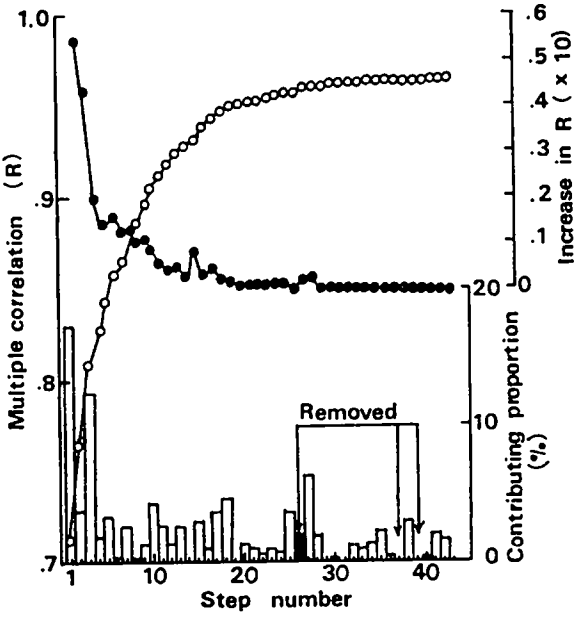

Fig. 4. Multiple correlation (R), increase in R and contributing proportion for entered or removed variable at each step. —○—○— Multiple correlation. —●—●— Increase in R.

indicating that over 93% ($0{\cdot}968^2 \times 100$) of the variance of sensory scores is explained by the regression model shown in Fig. 3.

F-ratio and F-value

The *F*-ratio and *F*-value calculated from the analysis of variance at each step are shown in Fig. 5. The *F*-value is 59 for peak 3 at the first step and then decreases to around 11 for peaks 37 and 8 (steps 2 and 3), respectively. Peak 11 is entered into

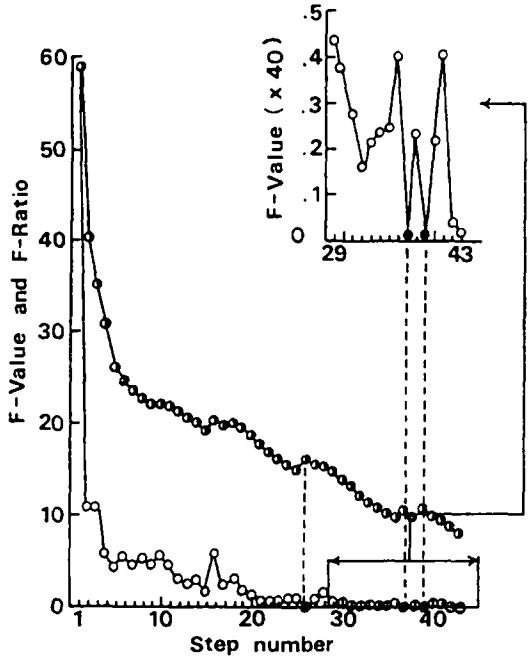

Fig. 5. *F*-value and *F*-ratio with entry or removal of variable for each step. —◑—◑— *F*-ratio. —○—○— *F*-value to enter. —●—●— *F*-value to remove.

the regression model at step 16, and the great efficiency caused by the entry of peak 11 is apparent from the increase in *R*. The changes of *F*-values from steps 29 to 43 are magnified 40 times and shown at the upper right of Fig. 5. The changes of the *F*-values caused by the entrance of the variables included in or removed from the regression are shown in this figure.

The *F*-ratio shows a tendency to decline with increase in the degree of freedom, but is increased by the entry or removal of the efficient peaks such as the peaks at steps 16 and 26. The *F*-value of peak 13 is entered at step 43, the last step. However,

the F-values of peaks 7 and 36 which are not entered in the regression model were under 0·01. Therefore, the SRA was stopped at this step.

Standard error of estimate

The change of the standard error of estimate on the steps is shown in Fig. 6. The standard error of estimate is defined as the standard deviation of residuals (e_a) expressed in the equation

$$e_a = Y \text{ estimated} - Y \text{ observed}$$

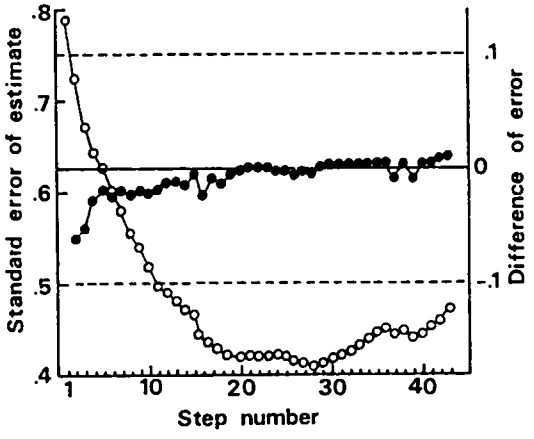

Fig. 6. Decrease and increase in standard error of estimate. —○—○— Standard error of estimate. —●—●— Difference of standard error of estimate between neighbouring two steps.

The accuracy of the estimation becomes higher with the increase in the numbers of the variables entered in the regression model. After the standard error of the estimate attained the maximum value at step 28, it increased again gradually. Consequently, it is apparent that the increase of the efficient peaks in the regression model results in the decrease of the standard error of the estimate, accompanying a considerable increase in R. On the other hand, the standard error of the estimate increases in spite of the increase in R in the case of the entry of the inefficient peaks. Except in the case of analysing the relation between the whole of the chromatographic peaks and the sensory score, the selection of the adequate peaks as independent variables is very important for the accuracy of the estimation.

Accuracy of estimation

The sensory scores, estimated by substituting the quantities in the peaks for the

six which consist of different steps, are shown in Table 3. The correlation coefficients between the real sensory scores and the estimated scores are shown in the last column. These values increase with the increase of the steps, reach a maximum at step 30 and then decrease. Such change of the correlation coefficients is coincident with that of the standard error of the estimate.

TABLE 3

EVALUATED AND ESTIMATED SCORES FOR SIX DIFFERENT STEPS

Sensory score	*Estimated score*					
	Step number					
	1	5	10	20	30	40
1·80	1·22	2·14	1·88	1·80	1·68	1·58
1·99	1·20	2·75	2·17	1·73	1·91	1·87
2·15	3·05	2·59	2·02	2·90	2·52	2·57
2·43	2·78	2·34	2·15	2·31	2·69	2·51
3·32	3·57	3·51	3·52	2·83	3·60	3·59
3·78	4·07	3·95	4·32	4·16	4·02	3·95
4·12	4·41	3·78	3·59	4·23	4·35	4·22
4·25	3·47	4·04	4·45	4·48	4·42	4·56
4·50	4·00	4·67	4·86	4·73	4·79	4·64
4·80	4·46	3·83	4·40	4·92	4·84	4·90
Correlation coefficient	0·878**	0·849**	0·956**	0·961**	0·991**	0·989**

Numbers underlined mean estimation as wrong orders. ** $P < 0{\cdot}01$.

Since the selection of the peaks as the independent variables is the most important matter for the analysis of soy sauce flavour, all the information on the statistical and chemical analysis must be utilised to find the most adequate subset of the peaks. The accuracy of the SRA or multiple regression analysis seems to depend on influential factors such as the methods for the isolation and concentration of flavour, the accuracy of GLC analysis, the quantification problem in the sensory tests, etc. The inaccuracy of the sensory tests by olfactory judgement seems to be an especially restrictive factor for the accuracy of the regression analysis, because the olfactory sensation is very delicate and complicated, and, moreover, the mechanisms of olfactory receptors and their neurones are not yet understood.

When the samples possess similar flavour qualities and similar thresholds, it is very difficult to differentiate them by GLC profiles, because of the limitations of reproducibility and accuracy in GLC analysis. This problem now remains an important one in the pattern analysis of gas chromatograms.

ACKNOWLEDGEMENTS

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REFERENCES

AISHIMA, T. & NOBUHARA, A. (1976). *Food Chemistry* (in press).
ANON. (1972). *Univac Manual*, Class R, 25–35.
BENDNARCZYK, A. A. & KRAMER, A. (1971). Practical approach to flavor development. *Food Technol.*, **25**, 1098–107.
DRAPER, N. R. & SMITH, H. (1966). *Applied regression analysis*, 163–80. New York, J. Wiley & Sons, Inc.
POWERS, J. J. & KEITH, E. S. (1968). Stepwise discriminant analysis of chromatographic data as an aid in classifying the flavor quality of foods. *J. Fd Sci.*, 33, 207–12.
POWERS, J. J., COX, R. J., ELLAND, M. C. & ACKROYED-KELLY, P. (1971). Exposure of peanuts and peanut volatiles to light: Influence on flavor and gas chromatographic profile. *Flavour Industry*, 2, 87–92.
SAITO, N. & TANAKA, T. (1967). Studies on sensory evaluation of soy sauce (I). *J. Ferment. Technol.*, **45**, 246–53.
YOUNG, L. L., BARGMANN, R. E., & POWERS, J. J. (1970). Correlation between gas chromatographic patterns and flavor evaluation of chemical mixtures and cola beverages. *J. Fd Sci.*, 35, 219–28.

STRUCTURAL FUNCTIONS OF TASTE IN THE SUGAR SERIES: TASTE PROPERTIES OF SUGAR ALCOHOLS AND RELATED COMPOUNDS

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ABSTRACT

The sweetness of simple polyhydric alcohols is examined in relation to conformation and configuration. Comparative studies of the relative sweetness of these and polyhydric glycosides are also carried out and further evidence is obtained regarding the importance of the glycosidic linkage in taste stimulation. Although the AH,B systems in these sugars are located at the polyhydric aglycones, the sugar moieties seem to exert a strong influence on their tastes.

INTRODUCTION

The basic structural entity of all compounds which elicit the sensation of sweetness has been proposed (Shallenberger & Acree, 1967) to be an AH,B system conventionally used to define the hydrogen bond. Provided the AH,B arrangement has an appropriate geometrical conformation with an A and B separation of between 2·5 and 4·0 Å, initiation of the sweet taste response is due to an interaction between this sweet unit and the receptor site involving the formation of two simultaneous hydrogen bonds. With sugars and sugar derivatives, the axial-equatorial and di-equatorial α-glycol groupings fulfil this geometrical requirement for eliciting the sweet taste response. Chemical modification of sugar molecules at selected sites could not only enable conclusions to be drawn about the stereospecific moieties within a sugar ring which elicit this response, but could also lead to the location of

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Shallenberger's AH,B system. Studies on deoxy sugars (Birch & Lee, 1974) and methyl sugars (Lindley *et al.*, 1976) suggested that the C-3 hydroxyl group is of unique importance (possibly in association with the C-4 hydroxyl group). A similar conclusion has also been reported by Evans (1963) and by Horowitz & Gentili (1971). Furthermore, recent studies (Birch & Lindley, 1973; Birch & Lee, 1974) suggested that the preferable structural feature for sweetness may be a five- or six-membered ring with a polar substituent including an AH,B system outside the ring, such as those found in saccharin, cyclamates and other artificial sweeteners. Fructose, the sweetest simple sugar known, and other ketoses have such a structure and studies on these (Lee & Birch, 1976) showed that the C-1 and C-2 hydroxyl groups exerted a major controlling influence on the interaction with the receptor site to elicit the sweet taste response. Glycosides which possess polyhydric aglycones may also possess such a property. The polyhydric aglycones are simple sugar alcohols, many of which are widespread naturally-occurring materials and many are utilised industrially in foods and drinks and in pharmaceuticals. It is, therefore, of particular fundamental interest that their sensory properties should be studied. Accordingly, this paper describes the structural functions of taste in a number of these compounds.

MATERIALS AND METHODS

All the parent sugars used in this study were crystalline materials purchased from British Drug Houses, Biochemicals, Poole, Dorset, Great Britain, and Pfanstiehl Laboratories, Illinois, USA. All these compounds were purified by repeated recrystallisations before tasting. Sugar alcohols were synthesised by treating the parent sugars with sodium borohydride for 12–18 h at room temperature, deionising with Amberlite IR 120 (H^+), filtering and evaporating to a syrup, which was distilled successively with small volumes of methanol to remove the boric acid as methyl borate. Maltitol was purified by acetylation; the nonaacetate was recrystallised twice from ethanol and deacetylated using sodium methoxide, followed by deionising with Permutit Biodeminrolit mixed bed ion-exchange resin (CO_3^{2-} form), filtering and evaporating to give pure syrupy maltitol. Erythritol and glycerol glycosides were prepared by lead tetraacetate oxidation of the respective disaccharides, followed by sodium borohydride reduction (Charlson & Perlin, 1956; Charlson *et al.*, 1956, 1962). 4-*O*-β-D-mannopyranosyl-D-erythritol was obtained as a gift from Dr P. A. J. Gorin, National Research Laboratories, Saskatoon, Saskatchewan, Canada. Known compounds prepared agreed in melting points and optical rotations with literature reports.

Taste testing

Panellists (ten in all) were selected and trained from college personnel according to a previous publication (Birch *et al.*, 1972).

Tasting of 'solid' sugars

All sugars to be tasted were dissolved in a small volume of water and evaporated to an amorphous solid or glass at 50° *in vacuo*. This is to ensure uniformity of samples, thus minimising errors due to rate of solution. A few milligrammes of each substance were placed on the tongue of each panellist and the tastes were classified according to one of the following descriptions: trace sweet (tr. S), sweet (S) or very sweet (SS) or trace bitter (tr. B), bitter (B) or very bitter (BB). Zero (0) is no response either way. Results reported are those obtained in at least 75% of total judgements, each panellist carrying out duplicate tasting sessions.

Difference testing

When comparing the intensity of sweetness, the two-sample difference testing was used (Byer & Abrams, 1953).

The number of correct selections of the sweeter sample within a pair in excess of chance expectation is defined in terms of standard deviations, σ, where

$$\sigma = \frac{n - Np}{(Npq)^{\frac{1}{2}}}$$

where N = total number of judgements, n = number of correct selections, p = probability of 'correct selection' by chance and q = probability of 'incorrect selection' by chance. The level of significance was then obtained by referring to the table compiled by Yule & Kendall (1950).

In both types of testing, rinsing the mouth with tap water between substances and pausing one minute before passing on to the following substances were required. Swallowing was permitted.

Relative sweetness intensity

Relative sweetness was calculated from threshold concentration (relative to the threshold of sucrose). Threshold determination was carried out using the triangular test (Byer & Abrams, 1953). Three solutions, two alike and one odd, were presented simultaneously in a circular arrangement. The judges were asked to determine which was the odd sample.

RESULTS AND DISCUSSION

That a variety of non-ionised aliphatic alcohols such as ethylene glycol, glycerol, erythritol and various sugar alcohols are sweet is well established (Slosson, 1890; Carr *et al.*, 1936) (Table 1). However, comparative studies of these—in particular, the sugar alcohols and their anhydrides—have not been widely reported. Carr *et al.* (1936) tried to correlate the number of carbon atoms or hydroxyl groups in the molecule with taste but concluded that there was no relationship between spatial

structure and sweet and bitter taste in this class of compound. However, recent studies (Lindley *et al.*, 1976) showed that the sweetness of xylitol, arabitol and ribitol could be satisfactorily explained by applying the concepts of Shallenberger's hydrogen bonding theory (Shallenberger, 1963, 1964; Shallenberger & Acree, 1967).

In ethylene glycol, spectroscopic measurements indicated that considerable intramolecular hydrogen bonding exists (Kuhn, 1952), thus suggesting that steric and dipolar repulsion of the hydroxyl groups are more than outweighed by the

TABLE 1

RELATIVE SWEETNESS OF SUGAR ALCOHOLS

Compounds	*Sweetness*
Sucrose	100
Ethylene glycol	130*†
Glycerol	108*†
Erythritol	238*†
Pentaerythritol	110*†
L-Arabitol	56
Ribitol	52
Xylitol	95
Sorbitol	54
Galactitol	46
Mannitol	62

* Carr *et al.* (1936).
† These values are anomalously high and have not been substantiated by other workers.

energy gained in the formation of the hydrogen bond. Since intramolecular hydrogen bonding can only occur if the molecule is in a *cis* or *gauche* conformation, it is clear that the latter is more likely to be the favoured structure; considerable steric and dipolar repulsion of the hydroxyl groups would be encountered in the *cis* conformation (Eliel, 1962). The *gauche* conformation (with its interorbital A–B spacing of 2·8–2·9 Å) is probably therefore the structural function of sweetness in these molecules. The intense sweetness of erythritol (which is nearly twice as sweet as ethylene glycol) (Table 1) could only be rationalised on the basis that the two terminal α-glycol groups have the *gauche* conformation, due to the 1,3-diaxial non-bonded interactions discussed below. The sweetness of pentaerythritol, on the other hand, poses an interesting problem. This alcohol forms tetragonal crystals (Cox *et al.*, 1937; Nitta & Watanabe, 1937; Shiono *et al.*, 1958) with intermolecular hydrogen bonds, O–H · · · O distance 2·69 Å, binding the molecules into layers. In solution, however, steric and dipolar repulsion causes the hydroxyl groups to orientate themselves to give a least energetically demanding structure, probably stabilised by intramolecular hydrogen bonding; thus several AH,B systems satisfying Shallenberger's requirement for sweetness are made available for interaction with the taste receptor. However, the sweetness of pentaerythritol has not been

reported by other workers and our test showed it to be even less sweet than glucose. It is questionable whether a molecule containing only primary alcohol groupings would possess such an intense sweetness as reported.

With increase in chain length, the stereochemistry becomes more complex. Like the polymethylene hydrocarbons, acyclic carbohydrates tend to adopt planar zigzag conformations wherein all the carbon atoms lie in the same plane (Aroney *et al.*, 1966; Rudrum & Shaw, 1965). However, the non-bonded interactions between 'parallel' hydroxyl groups in the 1,3 positions, *i.e.* the *syn*-axial interaction, are the most highly destabilising and in xylitol and ribitol this interaction introduces a considerable strain into the planar zigzag conformers, which is relieved by torsion around their C_3–C_4 bonds (Kabayama & Patterson, 1958), thus leading to the structure shown in Fig. 1. *Arabino* or *lyxo* configurations, on the other hand, will

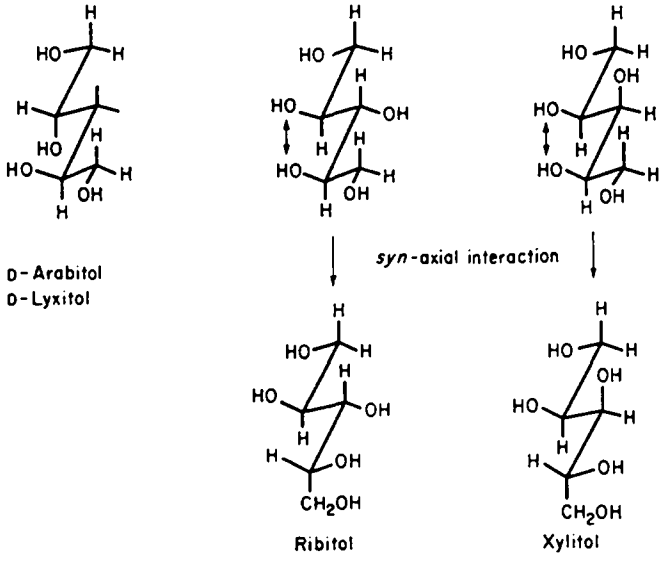

Fig. 1

exist preferentially as planar zigzag conformers. Amongst the hexitols, nmr and X-ray crystallographic studies showed that *galacto* and *manno* configurations exist as planar zigzag conformers; the others should contain appreciable amounts of sickle and other bent carbon–carbon conformers (Horton & Wander, 1969; Azarinia *et al.*, 1970). Thus, with steric factors and increased hydrogen bonding due to increased chain length, a decrease in sweetness would be expected. It is interesting to note that none of the alditols, including the heptitol, persitol (Lee, 1976), are

ever bitter. This, together with the taste of the homologous series of glycols shown in Table 2, which showed that increase in chain length by addition of methylene groups causes a gradual change from sweetness to bitterness, clearly is in accordance with an earlier prediction (Birch & Lindley, 1973; Lee & Birch, 1976) that increased

TABLE 2

TASTE OF GLYCOLS IN RELATION TO THEIR $\Delta\nu$ (cm^{-1})* VALUES

Compounds	*Taste***	$\Delta\nu$ (cm^{-1})†
Ethylene glycol	Very sweet	32
Trimethylene glycol	Sweet	78
Propylene glycol (1,2)	Sweetish	—
Tetramethylene glycol	Trace sweet	156
Hexamethylene glycol	Bitter	—

* Difference in the frequence of absorption (cm^{-1}) between free and intramolecularly bonded hydroxyl groups in the infra-red spectra.
** Cohn (1914).
† Kuhn (1952).

lipophilicity causes the molecule to either align itself on the taste receptor surface or to bind fresh sites (bitter sites). Kubo & Kubota (1969) suggested that intramolecular hydrogen bonding is a prerequisite for bitterness, the AH proton to B orbital distance being 1·5 Å. It is significant that Kuhn (1952) reported that the increase in $\Delta\nu$ (the infra-red absorption for the free and intramolecularly bonded hydroxyl

TABLE 3(a)

RELATIVE SWEETNESS OF POLYHYDRIC GLYCOSIDES

Compounds	*Sweetness*
Maltitol	63
Lactitol	34
Cellobiitol	11
Melibiitol	9

TABLE 3(b)

TASTE PROPERTIES OF POLYHYDRIC GLYCOSIDES

Compounds	*Sweetness*	*Bitterness*
2-*O*-α-D-glucopyranosyl D-glycerol	Tr. S	0
2-*O*-β-D-glucopyranosyl D-glycerol	Tr. S	0
2-*O*-α-D-galactopyranosyl D-glycerol	Tr. S	0
2-*O*-α-D-glucopyranosyl D-erythritol	Tr. S	0
2-*O*-β-D-glucopyranosyl D-erythritol	Tr. S	0
2-*O*-α-D-galactopyranosyl D-erythritol	Tr. S	0
4-*O*-β-D-mannopyranosyl D-erythritol	0*	B
3-*O*-β-D-galactopyranosyl D-arabitol	Tr. S	Tr. B

* Some panellists reported a trace of sweetness.

groups) upon the replacement of hydrogens by methylene groups is believed to be due not entirely to the change in polarity of the hydroxyl groups but to the decrease in O–H · · · O distance, that is an increase in the strength of the intramolecular hydrogen bond.

The sensory evaluations of glycosides containing polyhydric aglycones are shown in Tables 3(a) and 3(b). Just as with alkyl, phenyl and benzyl glycosides (Birch & Lindley, 1973), these results clearly show that increasing the chain length of the polyhydric aglycones does not sterically exclude the molecule from binding to the receptor site. In fact, sweetness appears to increase with increasing chain length. The glycerol and erythritol glycosides (Figs 2 and 3) appear to have only trace

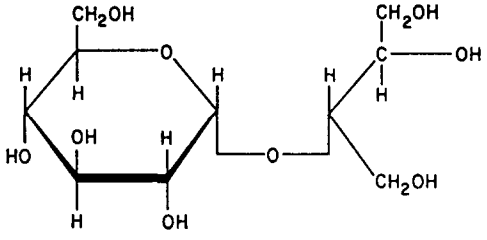

Fig. 2. 2-*O*-α-D-glucopyranosyl D-erythritol.

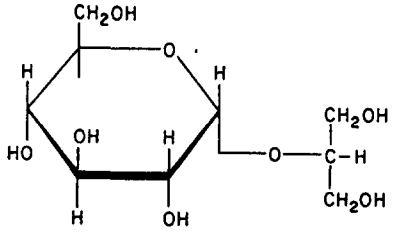

Fig. 3. 2-*O*-α-D-glucopyranosyl D-glycerol.

sweetness while maltitol and lactitol (Figs 4 and 5) are definitely sweet. Results in Tables 3(a) and 3(b) also give further support to an earlier hypothesis (Lee & Birch, 1976) that the glycosidic linkage in glycosides and disaccharides does indeed govern the relative sweetness. This is very clearly indicated by the greater sweetness intensity of maltitol and maltose compared to cellobiitol and cellobiose. Similar observations are also obtained from α- and β-glycerol and erythritol glycosides (Table 4). Studies on taste receptors of blowfly (*Phormia regina*) (Dethier, 1963) have similarly indicated the lesser effectiveness of β-linked sugars in stimulating the sugar receptors and it was proposed (Evans, 1963; Pflumm, 1972) that this was due

to steric hindrance by the β-substituents. It is also significant that 4-*O*-β-D-mannopyranosyl D-erythritol, like β-D-mannose, possessed a fairly strong bitter taste. If the mechanism of stimulation by β-D-mannose proposed by Birch & Lindley (1973) is correct, then it is not critical that a hydroxyl group be available at C-1; an oxygen atom is sufficient. This is also the case in sugar–cation complexes (Angyal, 1976) and sugar–inorganic oxyacid complexes (Bourne *et al.*, 1960). Angyal showed

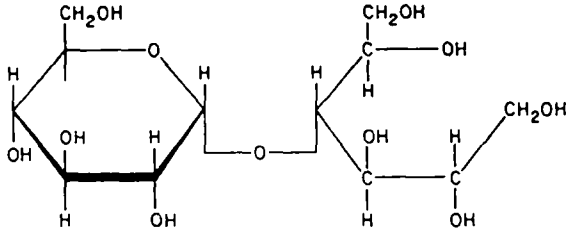

Fig. 4. Maltitol.

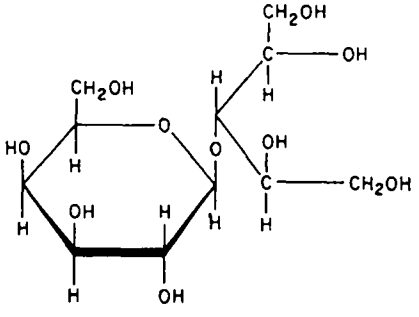

Fig. 5. 3-*O*-β-D-galactopyranosyl D-arabitol.

TABLE 4

COMPARISON OF SWEETNESS BETWEEN α- AND β-POLYHYDRIC GLYCOSIDES USING THE PAIRED COMPARISON TEST

Sugars	*Judgements*	*Selection of α-glycoside as sweeter*	*Selection of β-glycoside as sweeter*	*σ units*	*Significance*
2-*O*-α-D-glucopyranosyl D-glycerol 2-*O*-β-D-glucopyranosyl D-glycerol	40	27	13	2·21	Significant
2-*O*-α-D-glucopyranosyl D-erythritol 2-*O*-β-D-glucopyranosyl D-erythritol	50	33	17	2·20	Significant

that α,α-*allo*,*allo*-trehalose and its 4,6:4′,6′-dibenzylidene derivative complexes with calcium chloride to form pentadentate complexes involving C-2,2′, C-3,3′ and the glycosidic oxygen while Bourne *et al.* (1960) obtained a tridentate complex involving C-2, C-3 and the anhydro-ring oxygen atom when 1,6-anhydro-β-D-mannopyranose was reacted with molybdate; the cyclic complex across the 2- and 3-hydroxyl groups is stabilised by hydrogen bonding between a molybdenum-hydroxyl group and the oxygen atom of the anhydro-ring. Further studies with similar types of sugars are needed to determine the importance and role played by the β-D-mannopyranosyl moiety and its glycosidic oxygen in sweetness and bitterness.

Results in Tables 3(a), 4 and 5 further suggest that the AH,B systems in polyhydric glycosides are located at the aglycone end. Although lactitol, cellobiitol and melibiitol are not significantly different in taste from their respective parent sugars

TABLE 5

COMPARISON OF SWEETNESS BETWEEN SUGAR ALCOHOLS AND THEIR PARENT SUGARS USING PAIRED COMPARISON TEST

Sugars	*Judgements*	*Selection of sugar as sweeter*	*Selection of alcohol as sweeter*	*σ units*	*Significance*
Maltose	32	9	23	2·48	Highly
Maltitol	32	8	24	2·83	significant
Lactose	30	13	17	0·73	—
Lactitol	30	14	16	0·37	—
Cellobiose	24	11	13	0·41	—
Cellobiitol	24	10	14	0·82	—
Melibiose	24	14	10	0·82	—
Melibiitol	24	15	9	1·22	—

Second row figures in each set are those obtained with 'solid' sugars.

(both when equimolar solutions and 'solid' sugars were tasted) maltitol was very much sweeter than maltose (Table 5). Furthermore, successive removal of hydroxymethyl groups from the aglycone to produce arabitol, erythritol and glycerol glycosides resulted in a definite reduction in sweetness (although no absolute sweetness scores were obtained for these sugars due (mainly) to lack of materials). Although the AH,B system(s) may be located on the aglycone, the sugar moiety of these glycosides appears to have a strong influence in determining the taste. As seen above, 4-*O*-β-D-mannopyranosyl D-erythritol, like β-D-mannose, is bitter. The taste of flavanone glycosides gives further proof of this (Horowitz & Gentili, 1971). The flavanone 7-β-neohesperidosides are all bitter and their dihydrochalcones are all intensely sweet while the flavanone rutinosides such as hesperidin and naringenin 7-β-rutinoside and their dihydrochalcones are all tasteless. Furthermore, the sugar moiety of lactitol, melibiitol and 3-*O*-β-D-galactopyranosyl D-arabitol (all these are

TABLE 6

COMPARISON OF SWEETNESS BETWEEN SUGAR ALCOHOLS AND THEIR PARENT SUGARS USING PAIRED COMPARISON TEST

Sugars	*Judgements*	*Selection of sugar as sweeter*	*Selection of alcohol as sweeter*	*σ units*	*Significance*
D-Glucose	32	16	16	0	—
Glucitol	32	15	17	0·35	—
D-Galactose	32	13	19	1·06	—
Galactitol	32	15	17	0·35	—
D-Mannose	32	10	22	2·12	Significant
Mannitol	32	11	21	1·76	
D-Xylose	32	0	32	5·66	Very highly
Xylitol	32	0	32	5·66	significant
D-Ribose	24	11	13	0·41	—
Ribitol	24	10	14	0·82	—
L-Arabinose	24	10	14	0·82	—
Arabitol	24	11	13	0·41	—

Second row figures in each set are those obtained with 'solid' sugars.

much less sweet than maltitol, Table 3(a)) is a galactopyranoside. It seems that the axial hydroxyl group of this sugar exerts a strong effect on the binding of the sugar to the receptor site. How or why this is so is still not very clear. The low sweetness of 'galacto' sucrose and raffinose (Lee & Birch, 1976) is probably a structurally related phenomenon.

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REFERENCES

ANGYAL, S. J. (1976). The separation and synthesis of sugar derivatives by use of metal complexes. Paper presented at the Chemical Society Carbohydrate Group Spring Meeting at Swansea, Great Britain, 23–25 March.

ARONEY, M. J., LEFARVE, R. J. W. & SAXBY, J. D. (1966). Molecular polarisability. The configuration of chloromethyl methyl as a solute. *J. Chem. Soc.* (B), 414–16.

AZARINIA, N., JEFFREY, G. A., KIM, H. S. & PARK, Y. J. (1970). Conformational studies of alditols; crystal structure of D-glucitol, D-iditol and allitol. *Joint ACS/CIC Conference, Abstract of Papers, CARB.* 26.

BIRCH, G. G., COWELL, N. D. & YOUNG, R. H. (1972). Structural basis of interaction between sweetness and bitterness in sugars. *J. Sci. Fd Agric.*, 23, 1207–12.

BIRCH, G. G. & LINDLEY, M. G. (1973). Structural functions of taste in the sugar series. Effect of aglycones on the sensory properties of simple glycosidic structures. *J. Fd Sci.*, 38, 665–7.

BIRCH, G. G. & LEE, C. K. (1974). Structural functions of taste in the sugar series. Sensory properties of deoxy sugars. *J. Fd Sci.*, **39**, 947–9.
BOURNE, E. J., HUTSON, D. H. & WEIGEL, H. (1960). Paper ionophoresis of sugars and other cyclic polyhydroxy compounds in molybdate solution. *J. Chem. Soc.*, 4252–6.
BYER, A. J. & ABRAMS, D. (1953). A comparison of the triangular and two-sample taste test methods. *Food Tech.*, **7**, 185–7.
CARR, C. J., BECK, F. F. & KARANTZ, JR., J. C. (1936). Sugar alcohols. V. Chemical constitution and sweet taste. *J. Amer. Chem. Soc.*, **58**, 1394–5.
CHARLSON, A. J. & PERLIN, A. S. (1956). Lead tetraacetate oxidation of reducing disaccharides. *Can. J. Chem.*, **34**, 1200–8. The configuration of glycosidic linkages in oligosaccharides. Application of Jackson and Hudson's oxidation method to reducing disaccharides. *Can. J. Chem.*, **34**, 1804–10.
CHARLSON, A. J., GORIN, P. A. J. & PERLIN, A. S. (1956). The configuration of glycosidic linkages in oligosaccharides by degradation of reducing disaccharides to 2-*O*-glycosyl glycerols. *Can. J. Chem.*, **34**, 1811–18.
CHARLSON, A. J., GORIN, P. A. J. & PERLIN, A. S. (1962). Determination of the configuration of glycosidic linkages in oligosaccharides. In *Methods in carbohydrate chemistry*, ed. R. L. Whistler & M. L. Wolfrom, **1**, 419–26. New York, London, Academic Press.
COHN, G. (1914). *Die organischen geschmackstoffe*. Siemenroth, Berlin.
COX, E. G., LLEWELLYN, F. J. & GOODWIN, T. H. (1937). The crystalline structure of the sugars. Pentaerythritol and the hydroxyl bond. *J. Chem. Soc.*, 882–94.
DETHIER, V. G. (1963). *The physiology of insect senses*. New York, John Wiley & Sons.
ELIEL, E. L. (1962). *Stereochemistry of carbon compounds*. New York, McGraw-Hill.
EVANS, D. R. (1963). In *Olfaction and taste*, ed. Y. Zotterman, **1**, 165–76. Oxford, Pergamon Press.
HOROWITZ, R. M. & GENTILI, B. (1971). Dihydrochalcone sweeteners. In *Sweetness and sweeteners*, ed. G. G. Birch, L. F. Green & C. B. Coulson. London, Applied Science Publishers.
HORTON, D. & WANDER, J. D. (1969). Conformation of acylic derivatives of sugars. Conformation of peracetylated aldose dithioacetals in solution. *Carbohyd. Res.*, **10**, 279–88.
KABAYAMA, M. A. & PATTERSON, D. (1958). The thermodynamics of mutarotations of some sugars. Theoretical considerations. *Can. J. Chem.*, **36**, 563–73.
KUBO, I. & KUBOTA, T. (1969). Bitterness and chemical structure. *Nature*, **223**, 97.
KUHN, L. P. (1952). The hydrogen bond. Intra- and intermolecular hydrogen bonds in alcohols. *J. Amer. Chem. Soc.*, **74**, 2492–9.
LEE, C. K. & BIRCH, G. G. (1976). Structural functions of taste in the sugar series. Taste properties of ketoses. *J. Pharmaceut. Sci.*, **65**, 1222–5.
LEE, C. K. (1976). Unpublished results.
LINDLEY, M. G., BIRCH, G. G. & KHAN, R. (1976). The sweetness of sucrose and xylitol. Structural considerations. *J. Sci. Fd Agric.*, **27**, 140–4.
NITTA, I. & WATANABE, T. (1937). Hydrogen bridges in solid pentaerythritol. *Nature*, **140**, 365.
PFLUMM, W. W. (1972). In *Olfaction and taste*, IV, 364–70, ed. D. Schneider. Wissenschaffiche Verlagsgesellschaft mblt, Stuttgart.
RUDRUM, M. & SHAW, D. F. (1965). The structure and conformation of some monosaccharides in solution. *J. Chem. Soc.*, 52–7.
SHALLENBERGER, R. S. (1963). Hydrogen bonding and the varying sweetness of the sugars. *J. Fd Sci.*, **28**, 584–9.
SHALLENBERGER, R. S. (1964). Sweetening agents in food. *Agric. Food Review*, **2**, 11–20.
SHALLENBERGER, R. S. & ACREE, T. E. (1967). Molecular theory of sweet taste. *Nature*, **216**, 480–2.
SHIONO, R., CRUIKSHANK, D. W. J. & COX, E. G. (1958). A refinement of the crystal structure of pentaerythritol. *Acta Cryst.*, **11**, 389–91.
SLOSSON, E. E. (1890). The relative sweetness of different alcohols. *Trans. Kansas Acad. Sci.*, **12**, 104–5.
YULE, G. U. & KENDALL, M. G. (1950). *An introduction to the theory of statistics*. New York, Hafner Publishing Co.

MEASUREMENT OF THE FAT CONTENT OF MEAT USING ULTRASONIC WAVES*

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ABSTRACT

The velocity of 2·5 MHz ultrasound in muscle, fatty tissue and muscle/fatty tissue mixtures has been measured over the temperature range −20°C to +40°C. At a set temperature there is a relation between the velocity of ultrasound and the composition of the tissue, and this relation was studied at −9°C, 0°C and +37°C. Measurements at 37°C appeared to have the best predictive value, and at that temperature, a linear relation existed between the reciprocal of the velocity and the volume fraction of fat. This relation is such that a measurement of the time taken for a pulse to travel through meat consisting of multiple, parallel layers of muscle and fatty tissue of arbitrary fatness could be used to estimate the overall mean volume fraction of fat. This property could be useful in assessing at selected sites the composition of the soft tissue of living animals. Muscles are acoustically anisotropic and the slight difference between velocities along, and parallel to, the fibres has been measured. The effect of dehydration has also been investigated.

1. INTRODUCTION

Measurement of the velocity of transmitted ultrasound has been used successfully to determine the composition of a number of foods (Zacharias *et al.*, 1972), *e.g.*

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wines (Winder *et al.*, 1970) and milk (Fitzgerald, 1961; Winder *et al.*, 1961), and has been suggested as a means of measuring the composition of meat and living tissue (Stouffer, 1966; Henry *et al.*, 1967). The feasibility of the latter was supported by recent measurements on muscle and subcutaneous fatty tissue at 37°C (Miles *et al.*, 1974). These data showed little scatter between samples, no more than might be caused by differences in composition, and a reasonable difference (10%) between the velocities in the two tissue types.

The present investigation has examined the potential and limitations of using the velocity of ultrasound to determine the amount of fat in mixtures of fatty tissue and muscle.

2. MATERIALS AND METHODS

2.1. *Freshly dissected tissues*

2.1.1. *Muscles:* Twenty whole beef *M. semitendinosus* were trimmed of all visible external fat and cut into three or four pieces to allow velocity measurements along and across the fibres. Samples were sealed under vacuum in plastic pouches and the velocity of ultrasound measured at −9°C, 0°C and 37°C. Opposite faces of the frozen samples were made parallel by freezing them sandwiched between flat wooden boards.

2.1.2. *Comminuted tissue:* Sections of *M. longissimus thoracis* from the 11th to 13th ribs were taken from 26 sides of beef and 16 *M. semitendinosus* were dissected whole and trimmed of all visible external fat. Samples of subcutaneous and intermuscular fatty tissue were collected separately by dissection of the round joints of 13 beef sides. All tissues were bowl-chopped separately for one minute and packaged under vacuum to remove trapped air. Measurements of the velocity of ultrasound were made as in Section 2.1.1.

2.1.3. *Mixtures:* Quantities of minced muscle and fatty tissue were prepared separately. The minced muscle consisted mostly of *M. pectoralis profundus*, *M. latissimus dorsi* and *Mm. obliquus abdominis externus et internus* and the fatty tissue was both subcutaneous and intermuscular. Weighed quantities were mixed in a bowl-chopper for one minute to obtain homogeneous mixtures varying in fat content from 0 to 60% in 5% steps. Samples were taken for chemical and ultrasonic analysis. The velocity of ultrasound was measured at −10°C and +37°C.

2.2. *Dehydrated muscle*

A quantity of comminuted beef *M. longissimus dorsi*, prepared as above, was divided into three and freeze-dried for different lengths of time to produce dehydrated tissue ranging from 30 to 57% water. The velocity of ultrasound was measured in each sample over the temperature range −20°C to +40°C.

2.3. *Fat*

Beef fat was prepared from subcutaneous adipose tissue by rendering, filtering and drying in a vacuum oven. The velocity of ultrasound was measured over the temperature range −20°C to +40°C.

2.4. *Velocity measurements*

The group velocity of pulses of ultrasound was measured by timing them as they travelled through a known thickness of sample. Lead zirconate titanate transducers 20 mm in diameter and nominally resonating at 2·5 MHz were used. Transit times were measured with an accuracy of ±0·1 μs with a 10 MHz clock (Miles and Cutting, 1974). Samples were typically 4 to 6 cm thick and always greater than 2·5 cm. Constant temperatures of −9°C and 0°C were achieved by storing and measuring the packaged samples in a refrigerated room, the air of which was controlled to within ±1°C. Measurements at 37°C were made by immersing the packaged samples in an oil bath, thermostatically controlled to within ±0·1°C. To study the effect of temperature, samples were held in a specially constructed holder that could be controlled to within ±0·05°C of any temperature in the range −20°C to +40°C.

2.5. *Chemical analysis*

Samples were freeze-dried and the extractable fat determined by Soxhlet extraction with 40° to 60° petroleum spirit (Hanson, 1973).

3. RESULTS AND DISCUSSION

3.1. *Effect of temperature*

As the temperature was raised from −15°C to −1°C (Fig. 1), the velocity of ultrasound in fatty tissue decreased, slowly at first, but then more rapidly as −1°C, the initial freezing point of the tissue water, was approached. Further heating, from −1°C to +40°C, continued to cause a decrease. This behaviour contrasts with that of lean meat (Miles & Cutting, 1974), which shows an increase with temperature in this temperature range.

As a result, curves representing the velocities in fatty tissue and in muscle intersect twice in the temperature range −15°C to +40°C, once just below the initial freezing point of the tissue water and again between body and chill temperatures (Fig. 1). At these two temperatures measurement of the velocity of ultrasound cannot be used to discriminate between fatty tissue and muscle.

3.2. *Comminuted mixtures of fatty tissue and muscle*

The velocity of ultrasound in homogeneous mixtures of fatty tissue and muscle

at −10°C and +37°C fell as the proportion of fat increased. Within the experimental uncertainties at both temperatures the reciprocal of the velocity squared was linearly related to fatness (Fig. 2). At 37°C, but not at −10°C, there was also a linear relation between the reciprocal of the velocity itself and the fat content expressed either as a mass or a volume fraction (*cf.* Fig. 3).

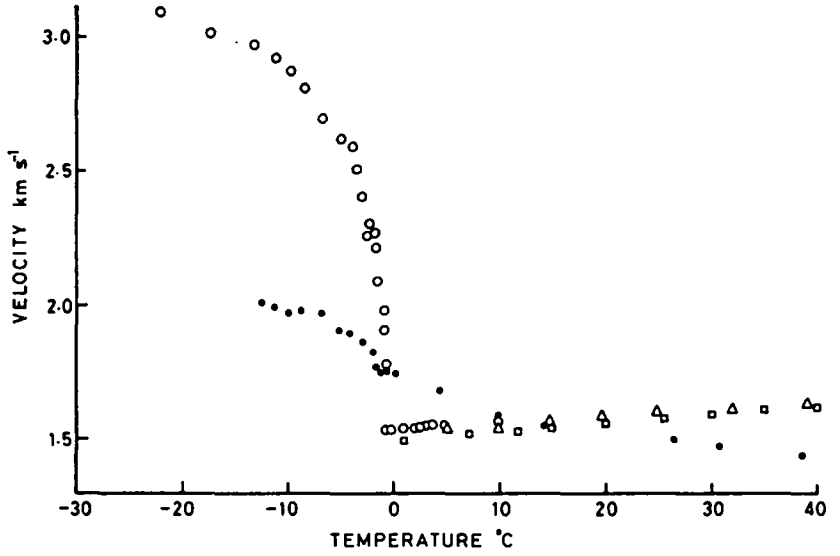

Fig. 1. Velocity of ultrasound in beef muscle and fatty tissue. ○ Comminuted *M. longissimus thoracis* 6·8% fat, 70·2% water (data of Miles & Cutting, 1974). Δ Intact *M. semimembranosus* (2 MHz). □ Intact *M. extensor carpi radialis* (2 MHz). ● Comminuted subcutaneous adipose tissue 74·3% fat.

3.3. *Comminuted muscle and fatty tissue*

The above data showed the form of the relationship between velocity and composition while replicated measurements of the same tissue taken from different carcasses showed the magnitude of the variation at constant composition (Table 1). The data of Table 1 show that at a given temperature the velocity of ultrasound was very much the same in different samples of the same tissue type. Even so, small differences did exist and these were, to a large extent, caused by small differences in composition. The residual standard deviations in Table 1 describe numerically the scatter at constant fatness, and it is this scatter that will ultimately limit the accuracy of fatness prediction from velocity.

In Fig. 3 all measurements at 37°C of individual tissues and mixtures of fatty tissue and muscle are superimposed to show that the same linear relation between the reciprocal of the velocity and the fatness holds for all the tissues tested.

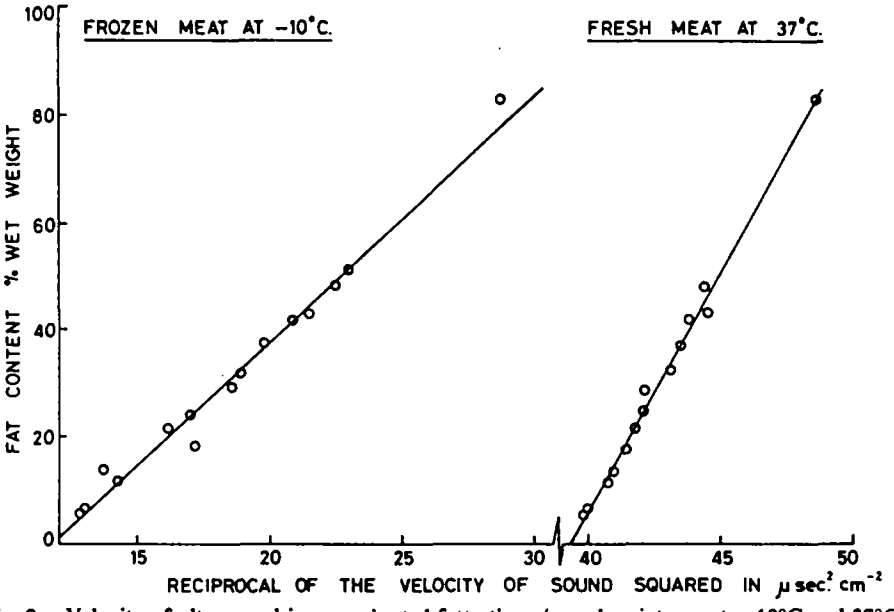

Fig. 2. Velocity of ultrasound in comminuted fatty tissue/muscle mixtures at −10°C and 37°C.

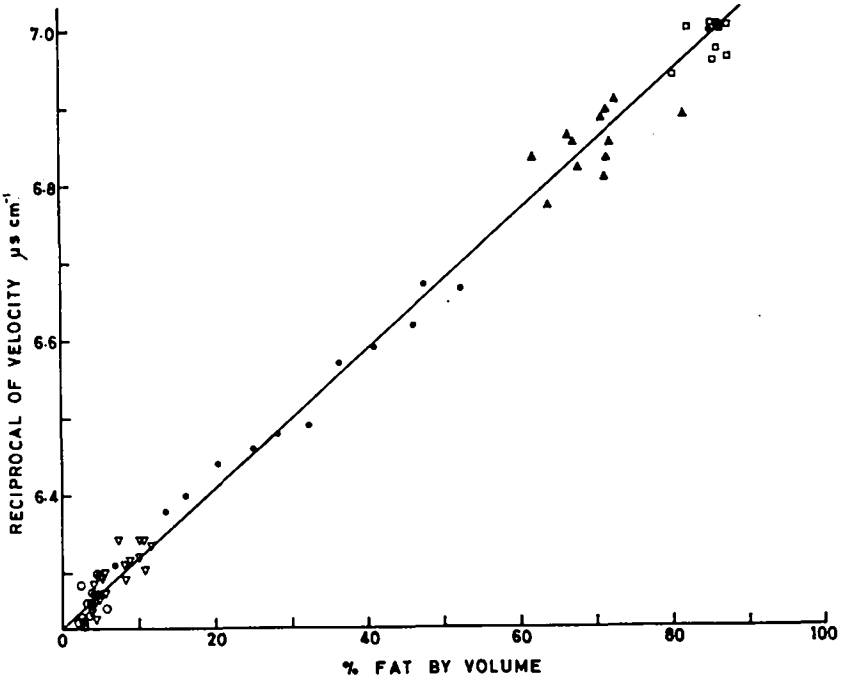

Fig. 3. Relationship between the reciprocal of the velocity of ultrasound and the volume fraction of fat in beef muscle, fatty tissue and muscle/fatty tissue mixtures at 37°C. ∇ *M. longissimus thoracis*. ○ *M. semitendinosus*. ▲ Intermuscular fatty tissue. □ Subcutaneous fatty tissue. ● Muscle/fatty tissue mixtures.

TABLE 1

EFFECT OF COMPOSITION ON THE VELOCITY OF SOUND IN COMMINUTED BEEF MUSCLE AND FATTY TISSUE

Tissue	*Temp. of measurement* (°C)	B ± SE ($\mu s^2\ cm^{-2}$/% *fat*)	A ± SE ($\mu s^2\ cm^{-2}$)	r (*significance*)	n	rsd ($\mu s^2\ cm^{-2}$)	*Mean % extractable fat* ± sd	*Mean % water* ± sd	*Mean* $\frac{1}{v^2}$ ± sd ($\mu s^2\ cm^{-2}$)
LD	−9			(NS)	18		6·0 ± 2·2	70·9 ± 2·0	12·6 ± 0·6
	0	−0·10 ± 0·02	42·1 ± 0·3	−0·702 (***)	26	0·24	5·6 ± 2·4	71·2 ± 1·8	41·5 ± 0·3
	37	0·14 ± 0·02	38·8 ± 0·3	0·848 (***)	19	0·22	5·9 ± 2·4	71·0 ± 1·9	39·6 ± 0·4
ST	−9			(NS)	12		3·0 ± 1·0	73·4 ± 0·9	12·4 ± 0·9
	0	−0·23 ± 0·11	42·8 ± 0·5	−0·508 (*)	16	0·38	2·9 ± 0·9	73·4 ± 0·8	42·1 ± 0·4
	37	0·18 ± 0·06	38·7 ± 0·3	0·651 (*)	13	0·21	2·9 ± 1·0	73·5 ± 0·9	39·2 ± 0·3
IM	−9			(NS)	13		66·8 ± 5·3	25·5 ± 4·0	22·7 ± 0·4
	0	−0·07 ± 0·02	39·5 ± 1·5	−0·714 (**)	12	0·35	67·5 ± 4·8	24·9 ± 3·6	34·8 ± 0·5
	37	0·06 ± 0·03	42·9 ± 1·7	0·600 (*)	12	0·45	66·5 ± 5·4	25·7 ± 4·1	46·9 ± 0·5
SC	−9			(NS)	12		83·9 ± 3·5	11·1 ± 2·5	26·8 ± 1·2
	0			(NS)	12		83·9 ± 3·5	11·1 ± 2·5	33·5 ± 0·8
	37			(NS)	11		83·2 ± 2·6	11·7 ± 1·8	49·0 ± 0·5
LD + ST	−9			(NS)	30		4·8 ± 2·4	71·9 ± 2·0	12·5 ± 0·7
	0	−0·15 ± 0·02	42·4 ± 0·3	−0·732 (***)	42	0·33	4·6 ± 2·4	72·1 ± 1·9	41·8 ± 0·5
	37	0·15 ± 0·02	38·8 ± 0·2	0·867 (***)	32	0·21	4·7 ± 2·4	72·0 ± 2·0	39·5 ± 0·4
IM + SC	−9	0·19 ± 0·03	10·2 ± 2·3	0·841 (***)	25	1·24	75·0 ± 9·8	18·6 ± 8·0	24·7 ± 2·2
	0	−0·07 ± 0·01	39·7 ± 1·3	−0·732 (***)	24	0·64	75·7 ± 9·3	18·0 ± 7·7	34·2 ± 0·9
	37	0·12 ± 0·01	39·3 ± 1·0	0·908 (***)	23	0·52	74·5 ± 9·5	19·0 ± 7·9	47·9 ± 1·2

LD *M. longissimus thoracis* excised from the 11th, 12th and 13th rib positions.
ST *M. semitendinosus*, intact.
IM Intermuscular fatty tissue excised from the round.
SC Subcutaneous fatty tissue excised from the round.

Data are fitted to the equation $\frac{1}{v^2} = Bx + A$.

x mass fraction of fat.
v velocity.
SE standard error.
sd standard deviation.

3.4. *Heterogeneous mixtures*

Consider a slab of meat at 37°C bounded by two parallel planes, $X = 0$ and $X = L$. Suppose the meat consists of multiple parallel layers of muscle and fatty tissue, there being an arbitrary number of layers and these arranged in any combination. All the tissues may be of different arbitrary fatness. The results of Fig. 3 show that in all these tissues the volume fraction of fat, Y, could be estimated from the same relation:

$$Y = \frac{b}{v} + a$$

where a and b are constants and v is the velocity of ultrasound.

The mean volume fraction of fat is given by

$$\bar{Y} = \frac{\int_0^L \left(\frac{b}{v} + a\right) dx}{\int_0^L dx} = b\left(\frac{\bar{1}}{v}\right) + a$$

where $(\bar{1}/v)$ is the transmission time divided by the distance travelled, a quantity that can be measured directly.

3.5. *Anisotropy*

The velocity of ultrasound measured across the fibres of beef *M. semitendinosus* was lower than that along the fibres at each of the three temperatures tested, −9°C, 0°C and +37°C, although at −9°C the difference was not statistically significant at the $p < 0{\cdot}05$ level. In fresh muscle the effect was slight, differences between directions being only 0·6% and 0·4% at 37°C and 0°C, respectively. These results (Table 2) conform with earlier measurements on dog and rabbit thigh muscles at

TABLE 2

VELOCITY† OF ULTRASOUND MEASURED ACROSS AND ALONG THE FIBRES OF BEEF *M. semitendinosus*

Temperature in °C	37	0	−9
Number of muscles	16	8	4
Mean fat content %	2·1	2·2	3·7
Mean water content %	74·9	75·0	73·0
Transverse, km/s	1·595	1·525	2·87
Axial, km/s	1·605	1·531	2·93
Significance of difference	XXX	XXX	NS

† Harmonic mean.

26°C (Goldman & Richards, 1954). In frozen muscle the velocity of ultrasound varied with time after freezing. For example, 8 days after freezing the velocity in one sample was still increasing at a rate of about 0·3% per day.

3.6. *Dehydration*

As expected, the velocity in frozen muscle was decreased by dehydration (Fig. 4) and the initial freezing point was depressed. Above freezing, dehydration caused a slight increase in velocity over the entire temperature range 0°C to 40°C. The dependence of velocity on the proportion of fat in dehydrated meat was completely different from that in the native material. For example, at 37°C a velocity of 1·59 km/s for dehydrated meat composed of 30·2% fat and 35·5% water would be typical of fresh muscle with a composition of 6% fat and 71% water.

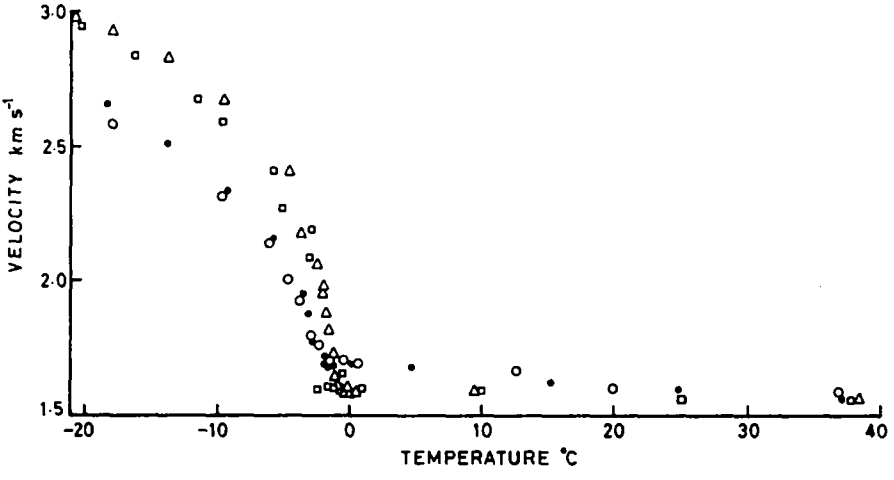

Fig. 4. Velocity of ultrasound in comminuted beef muscle at various levels of dehydration. Δ Native muscle at 62·3% water, 17·7% fat. □ 56·7% water, 20·3% fat. ● 48·3% water, 24·2% fat. ○ 30·3% water, 35·5% fat.

To explain these results it is useful to use a triangular graph on which the three mass fractions, fat, fat-free dry solids and water, may be represented by a single point (Fig. 5). When plotted in this way the composition of native muscle and adipose tissue taken from healthy and mature animals shows approximately linear relationships between the three components (Callow, 1962). It is a property of this type of graph that the composition which is produced by mixing two tissues represented by two points in the triangle lies on the line joining these points, and it therefore follows that any mixture of any of the samples shown in Fig. 5 would lie within the narrow band ABCD.

When the fresh sample *P* was dehydrated, its composition followed a completely different locus, progressing along the line PQ formed by producing the line defined by the point P and that apex of the triangle where the water content is 100% (Fig. 6).

It is now possible to mark points of equal velocity at 0°C on PQ and the band ABCD and join them up to give the direction of contours of equal velocity. For example, the line FQ represents a line of compositions for which the velocity of ultrasound at 0°C is 1·69 km/s. Similarly, a set of contours can be drawn at 37°C such as the line QM. This shows that dehydration loci, such as PQ, cut the 37°C

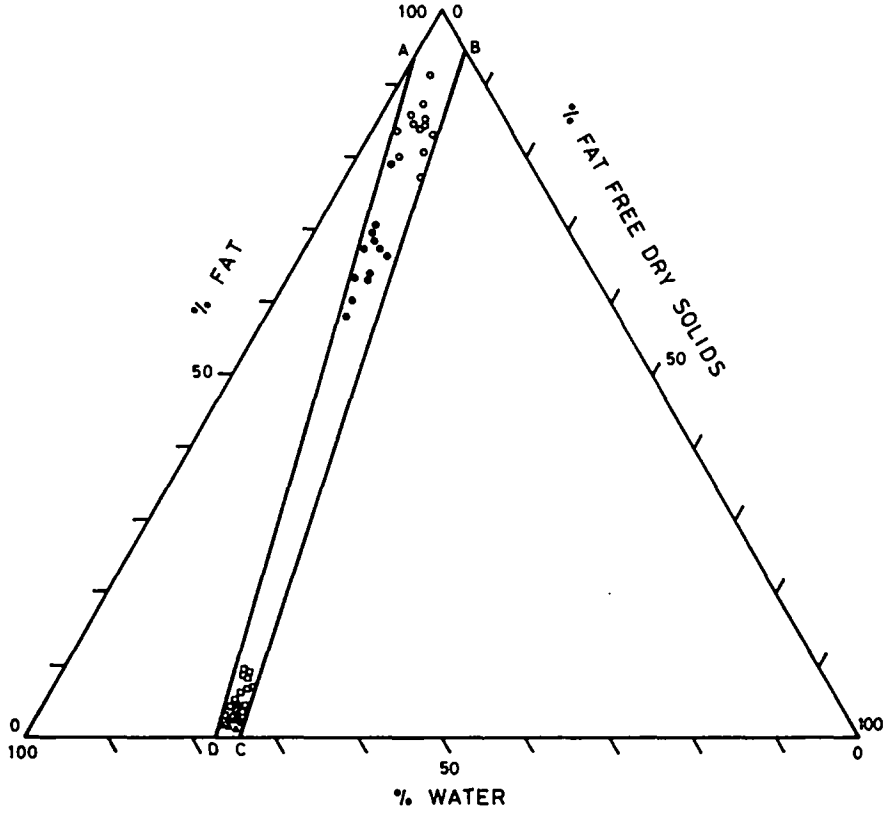

Fig. 5. The compositions of fatty tissue and muscle fall in the narrow band ABCD. □ *M. longissimus thoracis.* ▲ *M. semitendinosus.* ● Intermuscular fatty tissue. ○ Subcutaneous fatty tissue.

velocity contours at glancing angles so that dehydration caused little change in velocity at this temperature. At 0°C, where the corresponding angles were large, dehydration caused relatively large changes. This graph also shows that the velocity contours of 0°C cut those at 37°C at fairly large angles, indicating that velocity

measurements at these two temperatures might be used to determine the composition of samples lying in areas other than ABCD.

A change in temperature from 0°C to 37°C caused the velocity contours to shift and rotate clockwise. Therefore, at some intermediate temperature, the rotation would be such that a velocity contour is roughly concurrent with the band ABCD.

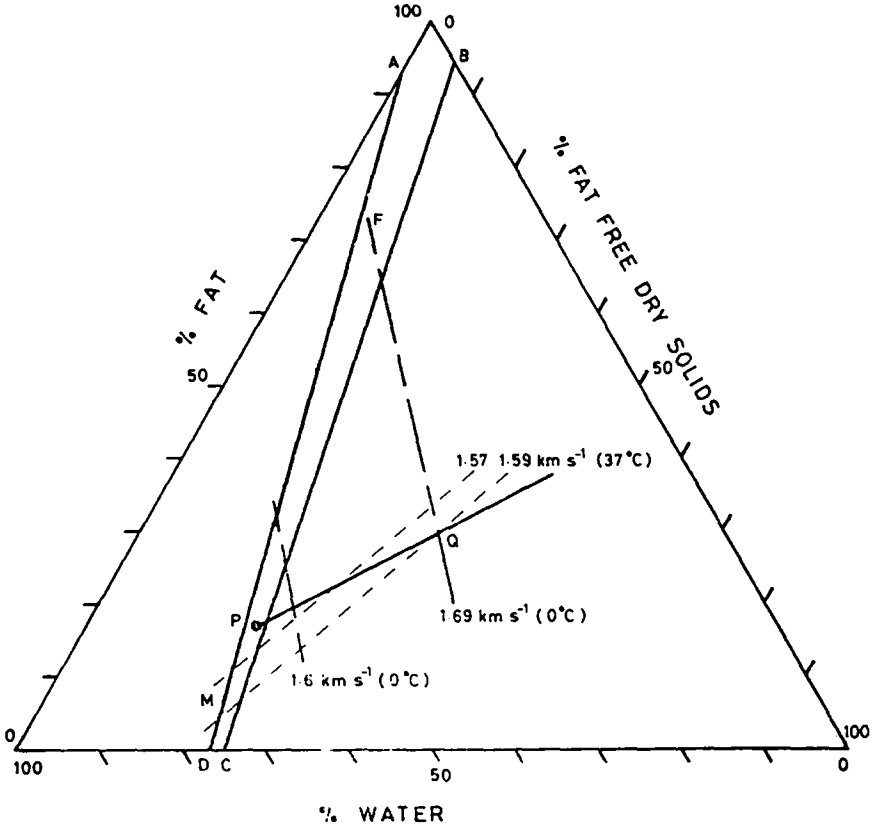

Fig. 6. Diagram used to explain the effect of dehydration. Dashed lines FQ and MQ represent contours of equal velocity. PQ locus followed when tissue of composition *P* is progressively dehydrated. The area ABCD is defined in Fig. 5.

At this temperature the velocity in fatty tissue would be the same as that in muscle (*cf.* Fig. 1). At another temperature when the velocity in fat is the same as that in water (*cf.* Fig. 7) the velocity contours will be roughly aligned with the loci of equal fat-free dry solids, and the velocity will depend on the proportion of fat-free dry solids only.

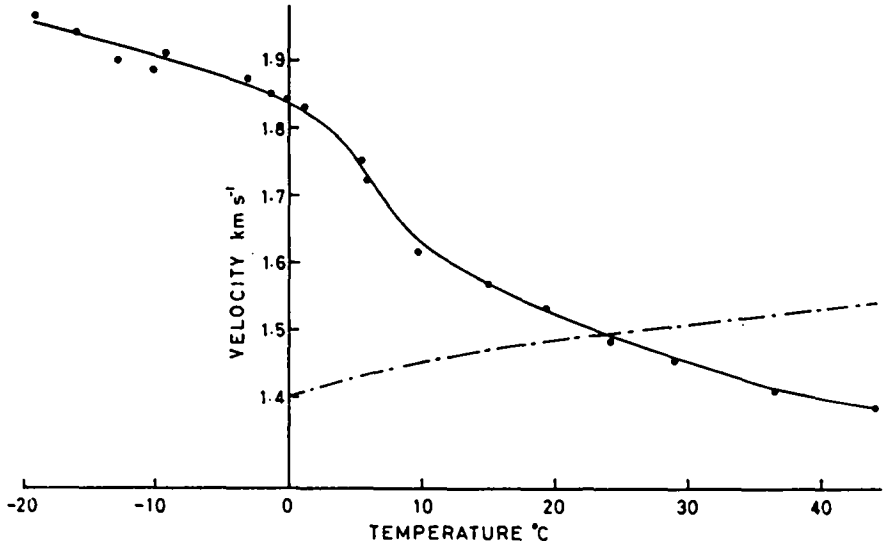

Fig. 7. Velocity of ultrasound in beef fat (●) and water (– · – · –). Fat rendered from subcutaneous adipose tissue, filtered and dried. Water data (Greenspan & Tschiegg, 1957).

3.7. *Fatness prediction*

These results show that the velocity of ultrasound may be used to give a rough but rapid estimate of the composition of mixtures of fatty tissue and muscle. If measurements are made *in vivo*, or the material has been freshly dissected, a single velocity measurement will suffice to locate the fat level within a few percentage points, but this procedure assumes that a linear relation exists between the proportions of water, fat and fat-free dry solids. This cannot be justified for tissues from immature animals and could be influenced by other factors, such as a pathological condition. In this case, or if the meat has been dehydrated, it would be necessary to make velocity measurements at two temperatures.

Of the three temperatures investigated, −9°C, 0°C and 37°C, single measurements at 37°C appeared to have the best predictive value and, at body temperature, a linear relation existed between the reciprocal of the velocity and the volume fraction of fat. This relation should be useful in assessing the composition of the soft tissues of living animals at selected sites. In making such estimates, it would be necessary to make a slight adjustment to the prediction equation to allow for the acoustic anisotropy of the muscle.

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REFERENCES

CALLOW, E. H. (1962). *Food Processing and Packaging*, April, 123–7; May, 166–81.
FITZGERALD, J. W. (1961). *J. Dairy Sci.*, **44**, 1165.
GOLDMAN, D. E. & RICHARDS, J. R. (1954). *J. acoust. Soc. Am.*, **26**, 981–3.
GREENSPAN, M. & TSCHIEGG, C. E. (1957). *J. Res. Nat. Bur. Standards*, **59**, 249–54.
HANSON, N. W. (1973). *Official standardised and recommended methods of analysis*, 2nd edn. London, Society for Analytical Chemistry.
HENRY, E. A. & BIAGINI, H. G. (1967). U.S. Patent 3345 863.
MILES, C. A. & CUTTING, C. L. (1974). *J. Fd Technol.*, **9**, 119–22.
MILES, C. A. & FURSEY, G. A. J. (1974). *Anim. Prod.*, **18**, 93–6.
STOUFFER, J. R. (1966). In *Diagnostic ultrasound*, ed. G. C. Grossman, 310–6. New York, Plenum Press.
WINDER, W. C., COSIGNY, N. P. & RODRIGUEZ-LOPEZ, B. (1961). *J. Dairy Sci.*, **44**, 1165.
WINDER, W. C., AULIK, D. J. & RICE, A. C. (1970). *Am. J. Enol. Vitic.*, **21**, 1–11.
ZACHARIAS, E. M. & PARNELL, R. A. (1972). *Food Technology*, April, 160–6.

USE AND ABUSE OF ASCORBIC ACID—A REVIEW

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ABSTRACT

The antiscorbutic and 'extra-antiscorbutic' functions of ascorbic acid are reviewed. Although megatherapy remains unproven there is substantial evidence that ascorbic acid may affect metabolism in other ways, e.g. by enhancing heavy metal absorption from the alimentary canal. There is little generally accepted evidence that megadoses of ascorbic acid are of themselves toxic.*

INTRODUCTION

The relationship between vitamin C (L-xyloascorbic acid, ascorbic acid, AA) and scurvy is well characterised. The recommended daily intake of AA in the United Kingdom is 30 mg—some three times the amount required to provide protection against scurvy in the Medical Research Council's 'Sheffield Experiment' (Bartley *et al.*, 1953). Recently, the role of AA in biological hydroxylations has been fairly clearly delineated in biochemical terms and the impaired formation of collagen in scorbutic animals is, in all probability, a consequence of defective hydroxylation of collagen proline and lysine (Barnes & Kodicek, 1972). Defective hydroxylation of collagen is presumably the primary biochemical lesion in scurvy and could account for the majority of the features usually associated with classical scurvy—defective wound healing, loosening of teeth, increased fragility of blood vessels, etc. Whether or not this is the sole biochemical function of AA is, however, another matter.

* The optimum recommended daily intake of this vitamin is still not internationally agreed. (Ed.)

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Early writers on scurvy frequently referred to features of the disease—such as mental fatigue and lassitude—that are not immediately attributable to defective hydroxylation of the 'collagen type'. The last decade has witnessed a sudden increase in interest in such 'extra-antiscorbutic' involvements of AA.

There is certainly some presumptive evidence for the existence of such relationships. Tetrahydropteridine ($DMPH_4$) and tetrahydrofolate, which have 56% and 43% respectively of the activity of AA in promoting protocollagen proline hydroxylase activity, are ineffective in prolonging the life of AA-deficient guinea-pigs (Davies *et al.*, 1976). A substantial number of clinical and nutritional studies would also appear to indicate that AA may function in, or influence, systems other than the simple prevention of scurvy.

A less widely acceptable feature of these claims is the belief that 'extra-antiscorbutic' activities require intakes of AA considerably in excess of those necessary for the prevention of scurvy. The ingestion of large amounts of a vitamin for a specific purpose is often described as 'vitamin megatherapy'; in the case of AA it has resulted in a substantial increase in consumption of the vitamin in recent years. Much AA is also used by food manufacturers as a processing aid in the brewing, baking and meat-curing industries; this is unlikely to increase the daily consumption of AA significantly as most of the AA will have been destroyed during the food processing; it does imply, however, that the average consumer ingests substantial amounts of AA breakdown products—a point which should be of interest to both food chemists and nutritionists. This review will seek to comment on (i) the claims of the AA megatherapists; (ii) the possible disadvantages of AA megatherapy; and (iii) the intake of AA breakdown products resulting from both megatherapy and the use of AA as a manufacturing aid.

ASCORBIC ACID MEGATHERAPY: THE STONE–PAULING HYPOTHESIS

The arguments for AA megatherapy were first adumbrated by Irwin Stone and later re-presented and elaborated by Linus Pauling. Stone's original thesis appeared in a number of speculative papers published between 1965 and 1967 (Stone, 1965, 1966*a*, 1966*b*, 1967). Stone regards the current recommended daily intake for AA (30 mg in the UK, 45 mg in the USA) as grossly inadequate. He bases his argument primarily on a consideration of those species able to synthesise their own AA; man, and some three or four other species, has lost the ability to produce AA endogenously and is perforce dependent upon dietary supplies of the vitamin. Stone points out that animals producing their own AA do so at a comparatively high rate; per 70 kg body weight (the weight of an average man) the daily production would be 1·8 g (rat), 15·8 g (rabbit) and 19·3 g (mouse) (Stone, 1974). Man's daily intake, according to Stone, should therefore be of this order to restore the nutritional *status quo* existing before man lost his capacity to produce AA. An integral

feature of Stone's argument is the assumption that animals always synthesise metabolites at an optimal rate—a superficially plausible argument but one that would be challenged by many biochemists and particularly, perhaps, by clinicians striving to depress tissue levels of endogenously-produced uric acid and cholesterol.

Stone also quotes, with approval, Bourne's estimation that the natural diet of the gorilla provides it with a daily intake of some 4·5 g AA (Bourne, 1949)—and this, he argues, should be taken as an index to man's 'natural requirement'. Pauling has extended this argument by calculating that if man ate a 'natural' diet of nuts, grains, fruit and vegetables his AA intake would be some 2·3 g/day—about eighty times the recommended daily allowance in the UK (Pauling, 1970, 1974). Jukes has criticised Pauling's calculations on two counts: (i) that Pauling's 'natural diet' contains horticultural innovations that would not have been present in primitive man's dietary pattern and (ii) that the comparatively high intake of AA by primitive man was fortuitous and nutritionally non-significant. As Jukes points out: 'If an animal is consuming a mixture of plant foods to satisfy the need for calories, the non-caloric ingredients will be ingested at levels that do not necessarily correspond to a quantitative nutritional requirement' (Jukes, 1974). Jukes' point is a valid one; 'natural diets' contained comparatively high amounts of phytic acid, fibre, organic acids, carotenes, etc., but it would be difficult to substantiate a claim that the 'natural' intake of these substances represented a nutritionally-optimal level. Again, the Stone–Pauling hypothesis contains a puzzling internal inconsistency. If animals such as the rabbit are already obtaining such significant amounts of AA from their natural diet, why do they require to synthesise AA at such high production rates? It is difficult to avoid the conclusion that the level of intake of dietary AA, and probably also its rate of endogenous biosynthesis, are fortuitous happenings, contingent upon other more fundamental processes, and in themselves of little quantitative nutritional significance.

EXTRA-ANTISCORBUTIC INVOLVEMENTS OF AA

It would be tedious, and scientifically unrewarding, to record the vast range of physiological patterns and pathological states that are modifiable by AA. Wilson has produced a documented list of some thirty disease states in which the blood ascorbic acid levels are reduced (Wilson, 1974); some clinicians have claimed that in such conditions supplementary doses of AA to restore the tissue levels have a therapeutic value—a somewhat remarkable *non sequitur*. Stone, in a well-documented but somewhat uncritical survey of the field, has listed some 500 publications dealing with the cure or amelioration of diseased states by AA (Stone, 1972). Few of these clinical claims have been subjected to extensive, controlled clinical tests. Difficulty in assessing the response to AA therapy is in any case likely to be exacerbated by two factors, namely: (i) the possibility that AA functions in a

'derived' or secondary capacity (*e.g.* by the formation of active breakdown products or metabolites) and (ii) by a very considerable individual variation in absorption, tissue retention and metabolism of AA.

There are, however, certain clearly defined areas where the physiological consequences of AA megatherapy in both man and experimental animals have been examined scientifically. The influence of AA on iron metabolism is often quoted as a case in point. There is considerable evidence that increasing the ingestion of AA enhances both the intestinal absorption of iron and its subsequent mobilisation in the tissues. There is some evidence that in both cases the AA acts by converting the iron into the ferrous form (or maintaining it in that form in the case of absorption), thus facilitating its release from bound and transport forms (Hughes, 1974). There is no considerable evidence that in iron metabolism massive doses of AA are of any greater value than more realistic intakes of the order normally associated with the attainment of AA tissue saturation. In contrast with this are some five fairly clearly delineated situations where it is claimed that measurable effects are obtained only after the administration of megadoses of the vitamin.

(*a*) *Lipid metabolism and atherogenesis*

Early observations that cholesterol accumulated in the tissues during avitaminosis C stimulated interest in the possible use of AA to modify the level of cholesterol in the body—an obviously important relationship as high blood cholesterol levels appear to be associated with atherogenesis and its clinical sequelae (DHSS Report, 1974). Initial studies suggested that AA megatherapy depressed blood cholesterol in young subjects but raised it in atherosclerotic patients (Spittle, 1971); more recent studies have not confirmed these claims (Kotze *et al.*, 1974; Crawford *et al.*, 1975). Nor is there evidence in non-supplemented subjects of any correlation between plasma AA and plasma cholesterol (Elwood *et al.*, 1970). In human studies there are interpretative difficulties too. A change in plasma cholesterol is meaningless unless it can be related to the overall distribution of cholesterol in the body. A lowering of plasma cholesterol could be a reflection of a general lowering of tissue cholesterol. It could equally well be a consequence of an enhanced uptake of cholesterol by the tissues—a presumably undesirable happening in terms of cardiovascular dysfunction.

Ginter has recently published a detailed monograph describing his careful studies in this field (Ginter, 1975). He ascribes to AA a significant role in the conversion of cholesterol to bile acids in the liver and shows that in guinea-pigs one consequence of chronic hypovitaminosis C (low intakes of AA over a prolonged period) is hypercholesterolaemia. He has some evidence that in humans AA megatherapy brings about a depression of blood cholesterol. His main thesis, however, is that in man the emphasis should be on the avoidance of hypovitaminosis C rather than on AA megatherapy. AA megatherapy, however, is not without its fervent adherents in this context (*e.g.* Rinse, 1973; Spittle, 1973).

(*b*) *Cerebral function*

There are indications that AA has involvements in brain metabolism. Lassitude and fatigue were features of classical scurvy and, more recently, there are indications of a possible relationship between mental depression and AA levels (Brook, 1972). Pauling quotes earlier studies where mental activity and I.Q. could be correlated with AA intake; he himself has elaborated a system of orthomolecular psychiatry—the treatment of mental diseases such as schizophrenia by the provision of the optimum molecular environment in the brain—and he attaches considerable importance to AA megatherapy as a means of achieving this state (Pauling, 1968; Hoffer, 1975). To date, orthomolecular theories have made few inroads into orthodox psychiatry (*see e.g.* Jenner, 1973) and evidence for Pauling's theory that learning and increased mental acuity could benefit from AA megatherapy is meagre. Massive doses of AA failed to influence the learning capacity of guinea-pigs in a maze (Adlard *et al.*, 1974)—perhaps a not entirely unexpected finding as brain AA is less dependent upon dietary intake than that of other organs and even doses of the order prescribed by the megatherapists would be unlikely to produce any substantial shift in the cerebral AA concentration (Hurley *et al.*, 1972).

(*c*) *Protection against infection*

In this context the main involvement has been in the prevention and/or treatment of the common cold. Here the number of studies is legion—with many of them, in terms of structure and interpretation, falling far short of the normal canons of scientific experimentation. Studies designed to assess the influence of AA megatherapy on the incidence and course of the common cold suffer from a built-in disadvantage from the start. To make a careful clinical assessment of each subject at each stage of the trial often means that the trial has to be structured in terms of a statistically unsatisfactory number of participants—perhaps as few as 20–30 per group. The alternative is to use as large a number of subjects as possible—say, 100–200 per group—and to rely on subjective evaluations, usually achieved by each subject recording details on a standard-type 'symptoms card'. It would be pointless to enumerate all the trials that have been reported in the scientific literature. Suffice it to say that Berry and Darke in 1968 in a survey of trials carried out to that date concluded that 'to date there is no satisfactory evidence that any increase in the present recommended allowance of 30 mg of ascorbic acid is necessary' (Berry & Darke, 1968). Pauling's assessment of more or less the same data was quite different and led him to state that megadoses of AA could reduce both the frequency and severity of colds (Pauling, 1970). Recently a careful and comprehensive study of the whole situation appeared in the American Press; the authors concluded that their review of those 'controlled studies . . . that meet some reasonable criteria of design reveals little convincing evidence to support claims of clinically important efficacy' (Dykes & Meier, 1975). The reaction of the scientific and medical press to Pauling's claims has, on the whole, been one of benevolent hostility: many reviewers have

echoed Tyrrell's remark that, in this context, 'belief . . . is not evidence' (Tyrrell, 1974).

(*d*) *Ascorbic acid and detoxication*

Low tissue concentrations of AA in guinea-pigs result in an impairment of microsomal hydroxylation in the liver and a reduction in detoxication capacity (Degkwitz & Staudinger, 1974; Fielding & Hughes, 1975) and there is some evidence that elevated dietary intakes of AA may protect the body against toxic substances. A megadose of AA given to mice (a species capable, incidentally, of producing its own AA) prevented the formation of bladder tumours following the implantation of 3-hydroxy anthranilic acid (Schlegel *et al.*, 1970). AA has a protective influence in the hepatotoxic effect caused by sodium nitrite plus aminopyrene in rats (Kamm *et al.*, 1973). An extension of this finding prompted Edgar to suggest that 'AA, if present in sufficient concentrations, may also afford protection from the adverse effects of other environmental alkylating agents . . . and the maintenance of high tissue levels of ascorbic acid may be of value for this reason' (Edgar, 1974). However, unbridled theorising in this context is not without its difficulties. Lewin, in a closely reasoned attempt to anticipate nature, concluded that 'toxic cations such as Hg^{2+}, Pb^{2+} and Cd^{2+} may complex with AA and be removed from the body—thus justifying the intake of large quantities of AA' (Lewin, 1974*a*). This is patently not so: in the case of at least mercury, AA megadoses increase the uptake of mercury by guinea-pigs, and hence its toxicity, considerably—a point to be discussed below.

(*e*) *Longevity and ageing*

Claims that AA megadoses may prolong the life span appear to be derived from three distinct, but not mutually exclusive, arguments, namely:

(i) That AA megadoses, in an undefined way, retard the tissue changes that are characteristic of ageing.

(ii) That the well-documented negative correlation between tissue AA and age (Andrews & Brook, 1966; Hughes & Jones, 1971) suggests that 'old' tissues are metabolically disadvantaged with respect to AA and that raising the AA concentration to levels characteristic of 'young' tissues would increase the 'metabolic well-being' of the subject with a consequent increase in life span.

(iii) That flooding the tissues with AA and maintaining blood AA at maximum levels would lessen the need for energy-assisted 'AA-conservation' systems—such as tubular reabsorption of AA in the kidney; the resultant overall reduction in the 'energy burden' could, it is suggested, be conducive to a prolongation of life.

This latter argument has been used by Pauling, who has estimated that AA megatherapy will extend his life-span by 4–6 years (Pauling, 1970, 1974). It is

difficult entirely to discount arguments of this type, particularly as our knowledge of the biochemical basis of ageing is far from complete. As Lewin has shown, the AA molecule possesses a high degree of functional versatility (Lewin, 1974*a*), and it could well influence the degenerative changes or 'accumulation of errors' that are a central feature of some modern theories of ageing (Hayflick, 1975). An interaction between AA and –S–S groups (or between dehydro AA and –SH groups) could well be of significance in any mechanism of ageing involving the integrity of –SH groups or the degree of cross-linking between proteins and nucleic acids. However, AA megadoses have no measurable effect on tissue –SH groups in guinea-pigs (Davies *et al.*, 1976) nor do they influence the life-span of male guinea-pigs (Davies & Hughes, 1976*a*).

Again, the negative correlation between tissue AA and age does not necessarily imply a relative deficiency of AA in old animals. There is evidence that 'old' tissues have a lower natural retention capacity for AA than 'young' tissues (Hughes, 1973) and recent work has indicated that 'saturation levels' of 'old' tissues are significantly lower than those of 'young' ones (Davies *et al.*, 1976). Attempting to raise the AA levels of 'old' tissues to those of young ones could therefore be a physiologically non-productive exercise.

TISSUE SATURATION

In sum, there is evidence that AA may influence cholesterol metabolism, iron absorption and mobilisation, certain detoxication systems and possibly also cerebral function and the response of the body to specific infections. In these extra-antiscorbutic activities chronic hypovitaminosis C could disadvantage a person and there is therefore a strong *prima facie* case for ensuring that AA tissue levels are adequate. That AA can apparently influence such a wide range of superficially unrelated happenings is perhaps a reflection of its functional versatility (Lewin, 1974*a*). The uneven response pattern elicited in many AA clinical trials (such as common cold trials) is probably attributable in part to biochemical individuality (Williams & Deason, 1967) and in part to a non-direct mediation on the part of AA. A non-direct influence implies that the AA acts by modifying a 'mediation molecule' in a secondary system; Lewin's suggestion that AA influences the activity of cyclic AMP and cyclic GMP is an example of a possible non-direct influence (Lewin, 1974*a*, 1974*b*).

Mechanisms of this type could well require tissue AA concentrations in excess of those estimated to offer protection against scurvy; in such circumstances tissue saturation with AA could well be a desirable state. What is not so easily acceptable is the assertion that megadoses are required to achieve saturation. Earlier studies using labelled AA indicated that only a small proportion of an administered megadose was incorporated in the body pool of AA (Hodges *et al.*, 1971) and more

recently it has been shown that in adult females 80 mg of AA (given as orange juice) produced essentially the same level of leucocyte AA as a megadose of 1 g AA daily (Baird *et al.*, 1976): this confirms earlier suppositions that 100–200 mg AA daily is sufficient to ensure tissue saturation (Chatterjee, 1967; Vilte, 1967).

Man, in the course of evolution, has presumably adapted himself to survive on comparatively low intakes of AA by the development in his tissues of a greater abstraction/retention capacity towards AA than would be necessary if his body were continually exposed to daily intakes of AA of the order of that ingested by herbivorous animals.

POSSIBLE DISADVANTAGES OF AA MEGATHERAPY

From time to time fears have been expressed about the possible disadvantages of sustained AA megatherapy, although the actual number of cases where a direct adverse effect has been clearly demonstrated are few. High dose AA therapy exacerbated the condition of a patient with sickle cell thalassemia (Goldstein, 1971), Horrabin has warned against the danger of megadose-induced deep vein thrombosis (Horrobin, 1973) and Jackisch reported that a daily dose of 1 g AA dried up the mucous secretions of the nose and mouth and reduced his production of semen (Jackisch, 1971)—a finding later confirmed by Schrauzer and Rhead (Schrauzer & Rhead, 1973). The possibility that AA megatherapy induces infertility in women has also been discussed in the medical press (Briggs, 1973*a*, 1973*b*; Hoffer, 1973). On the other hand, a number of workers have been unable to detect any untoward effects in patients given megadoses of AA over comparatively long periods (*e.g.* Hoffer, 1971).

Other supposed potential dangers have a more substantial substratum of scientific theory and practice. Briggs has from time to time cogently presented them in letters and notes in the medical press (*e.g.* Briggs, 1974). Three of the more plausible arguments merit some discussion.

(i) *An increased susceptibility to scurvy on cessation of AA megatherapy*

It is reasoned that the overall AA metabolism of the body becomes geared to high intakes of AA and that an abrupt cessation of megatherapy may precipitate a condition of AA deficiency. This is the thesis presented by Rhead and Schrauzer, who referred to individual cases where scorbutic symptoms emerged on cessation of AA megadosing (Rhead & Schrauzer, 1971). These workers later reported that the blood AA levels of subjects who had received AA megadoses (1–3 g daily for prolonged periods of up to 54 months) fell to below-control values on cessation of dosing—thus confirming the results of an earlier study by Masek and Hruba (Masek & Hruba, 1969, 1974; Schrauzer & Rhead, 1973). Complementary studies with guinea-pigs have, however, given conflicting results (Gordonoff, 1960; Lang,

1965; Hornig *et al.*, 1973; Nandi *et al.*, 1973; Sorensen *et al.*, 1974). Cochrane, in discussing systemic conditioning in the context of prenatal and neonatal nutrition, has suggested that infantile scurvy could be a consequence of *in utero* conditioning to material megatherapy (Cochrane, 1965).

(ii) *An increased formation and excretion of oxalic acid*

Oxalic acid is an end-product of AA metabolism in man and the influence of increased AA intakes on oxalic acid excretion has received some attention. It is assumed that any substantial increase in oxalic acid formation could conduce renal calcification and the formation of calculi. Our knowledge of the catabolism of AA in man is still incomplete (ARC/MRC Committee Report, 1974). Oxalic acid is only one of a number of potential end-products and little is known of its comparative toxicity *vis-à-vis* that of other breakdown products and metabolites such as diketogulonic acid, lyxonic acid, xylonic acid—and possibly other, hitherto unidentified, compounds. On balance, it would appear that fairly substantial intakes of AA (4–9 g daily) are necessary to produce any significant change in the urinary excretion of oxalic acid (Lamden & Chrystowski, 1954; Takenouchi *et al.*, 1966; Wyngaarden & Elder, 1966), although a recent study has indicated that a daily intake of 1 g AA by adult females produced a significantly greater output of urinary oxalic acid than a daily supplement of orange juice containing 100 mg AA (Baird *et al.*, 1976). The significance of these findings is, however, somewhat obscure as there is no general agreement on how much urinary oxalate is physiologically acceptable. It has been suggested that even a comparatively small increase in oxalic acid excretion could disadvantage a person 'with a high normal or elevated level of urinary oxalate' (Smith, 1972). Increases in oxalic acid formation should, of course, always be considered in relation to the body calcium status. In adults, a high urinary oxalate in the presence of elevated calcium levels could enhance the formation of calcium oxalate which, because of its limited solubility in urine, could increase any tendency to stone formation: in young, growing subjects with a marginal or non-optimal calcium intake excess oxalic acid formation could precipitate nutritional hypocalcosis.

The normal oxalic acid output is *ca.* 30 mg/24 h (Hodgkinson & Williams, 1972; Baird *et al.*, 1976); megadoses of 1–5 g AA daily may increase this to 35–50 mg. Recently, Briggs reported that out of 67 volunteers screened by him for AA-induced hyperoxaluria three (including a father and his son) excreted greatly increased amounts of oxalic acid (600–700 mg daily) after ingesting 4 g AA daily for 7 days (Briggs, 1976). AA-induced hyperoxaluria of this order could well be regarded as a contraindication for AA megatherapy—always assuming, of course, that the oxalic acid is not being produced at the expense of other, more toxic, metabolites.

(iii) *An increased toxicity of metals*

There is substantial evidence that increased intakes of AA enhance the uptake of

certain metals from the gastrointestinal tract (Hughes, 1974). The physiological significance of this type of relationship depends upon the nature of the metal involved. In the case of iron it is usually regarded as a beneficial one; but any AA-enhanced uptake of a toxic metal can only disadvantage the body. Recent work has shown that AA megadoses given to guinea-pigs exposed to dietary mercury (inorganic or organic) doubled the deposition of mercury in the tissues and halved the survival time of the guinea-pigs (Blackstone *et al.*, 1974; Murray & Hughes, 1976).

These findings are in direct contradiction to Lewin's claim (derived from a theoretical consideration of AA–metal relationships) that 'high intake of ascorbic acid can thus be utilised for redressing adverse effects from environmental toxic metal pollution' (Lewin, 1974*a*). Practising nutritionists may draw some satisfaction from this demonstration of the apparent supremacy of *praxis* over *theoria*; extrapolation of this finding to the human level would imply that exposure to a high environmental mercury concentration should be a definite contraindication for AA megatherapy. The mode of action of AA in this context is not known: it possibly acts by maintaining the integrity of thiol groups essential to the membrane transport system. It would be of interest to determine (a) whether AA enhances the passage of mercury across membranes other than that of the gastrointestinal tract and (b) whether the membrane transport of other toxic metals, such as cadmium and lead, is influenced in a similar way. The influence of *in utero* exposure to maternal megatherapy on the uptake of toxic metals by the foetal brain could also be of profound importance in this context.

BREAKDOWN PRODUCTS OF ASCORBIC ACID

Commentators have, in the past, confined their attention to the biological activity of the AA molecule *per se*; there has been virtually no consideration of the possible physiological significance of its breakdown products and metabolites. This is surprising when one considers that comparatively large amounts of AA are used industrially as a permitted additive under conditions which produce a considerable range of breakdown products. It has been estimated that about half of the AA produced synthetically is used today in the food industry, the bulk of it as a technological aid (Klaui, 1974). The main areas of application are in the soft drinks industry, in meat curing and pickling, in the fermentation industries and to improve the baking quality of flour (Klaui, 1974). In meat curing about 0·2 g AA/kg is used in practice, although the EEC list permits substantially higher concentrations (Counsell, 1971; Klaui, 1974; Rankin, 1974); in the baking industry some 70% of British breads are prepared from AA-treated flour (0·075 g AA/kg flour) (Thewlis, 1974). In many cases the AA is almost completely broken down during the processing and its nutrient contribution is correspondingly reduced. Thewlis has shown

that there was virtually complete destruction of added AA during the baking of bread and that 87% of the original AA could be accounted for as CO_2 (24%), L-threonic acid (52%) and 2,3-diketogulonic acid (11%). Other breakdown products, such as L-xylonic acid, have been characterised in different systems; one group of workers isolated as many as fifteen different products from the *in vitro* breakdown of AA (Tatum *et al.*, 1969). From additive AA the average consumer will ingest daily some tens of milligrammes of a number of AA breakdown products whose metabolic and physiological significance is far from clearly defined. Of relevance in this context is the recent report that breakdown products of AA are mutagenic in bacterial and animal cells, a finding which, if verified, could be of some significance both in the causation of disease and in the ageing process (Stick *et al.*, 1976; Burnet, 1974).

Breakdown products and metabolites should also be considered in relation to AA megadoses; an increased AA intake will presumably result in the formation of greater amounts of breakdown products. The formation of such compounds will in turn be influenced by factors such as the gastrointestinal pH and the rate of movement of the intestinal contents: the extent of production of AA breakdown products and their rate of elimination from the body are therefore evidently singularly individual matters. It is not inconceivable that some of these breakdown products are physiologically more potent than AA itself and they could well be responsible for some of the 'extra-antiscorbutic' functions attributed to AA. If this should be so, then their uneven pattern of formation from AA could in part account for the inconsistencies and variations in response to AA megatherapy reported by different investigators.

CONCLUSIONS

A substantial proportion of persons have AA intakes below the recommended daily allowance of 30 mg (Allen *et al.*, 1968). Dietary supplementation is obviously desirable in such cases. There is also evidence that AA has metabolic involvements other than the prevention of classical scurvy. It is not unreasonable to assume that tissue saturation with AA is the best physiological insurance against incomplete fulfilment of these 'extra-antiscorbutic' functions. Such evidence as is available, however, would appear to suggest that tissue saturation can be achieved with a daily intake of 100–200 mg AA. There is no compelling reason for believing that megadoses are necessary—although the uneven pattern of physiological response often elicited by moderate AA supplementation has sometimes been interpreted as indicating an insufficient dose. This is a spurious argument for the results of AA megatherapy are no more clearly defined than those of moderate supplementation.

AA megatherapy is unproven. Whether or not it will suffer the same fate as previous nutritional panaceas—such as tar-water in the eighteenth century (Prior,

1746) or white mustard seed in the nineteenth (Cooke, 1826)—will depend primarily upon a more complete characterisation of the supposed extra-antiscorbutic involvements of AA. Further clinical trials and experimental studies designed to reveal a simple causal relationship are unlikely to advance matters beyond the observational stage. The uneven response patterns characteristic of such studies to date would suggest that in future work the emphasis should be shifted to an examination of derived and peripheral relationships. In particular, it would be of value to consider:

(1) the possibility that AA breakdown products and/or metabolites possess a hitherto undefined pharmacological potency;
(2) the possibility that AA influences metabolism indirectly and that measurable effects are always derived or secondary ones;
(3) the extent to which population and individual differences—both acquired and genetic—may influence the requirement for, and the metabolism and physiology of, ingested AA.

Information of this type would contribute to the resolution of current inconsistencies in response patterns reported by different workers and should enable us to define more clearly the true extra-antiscorbutic role of AA—and in particular with respect to the efficacy of megadoses.

Similarly, it is unlikely that AA megadoses are of themselves toxic. But here again the situation cannot be studied in isolation: individual differences in metabolism, dietary patterns and near-toxic levels of other substances are examples of factors which could induce an unfavourable response in the presence of AA megadoses. The possible toxicity of breakdown products and/or metabolites derived both from additive AA and AA megadoses would merit further study as, too, would the influence of AA megadoses on the passage of metals across biological membranes and their subsequent uptake and retention by the tissues. This could well prove to be a most significant aspect of AA megatherapy. An AA-enhanced uptake of metals (particularly toxic ones) could be of especial significance with regard to (a) foetal tissues exposed to *in utero* megadoses and (b) the possibility that an increased uptake of certain metals may be a contributory factor in the aetiology of conditions such as heart disease.

REFERENCES

ADLARD, B. P. F., MOON, S. & SMART, J. L. (1974). Discrimination learning in ascorbic acid deficient guinea-pigs. *Nature*, **247**, 398.

AGRICULTURAL RESEARCH COUNCIL/MEDICAL RESEARCH COUNCIL COMMITTEE REPORT (1974). *Food and nutrition research*, 72. London, HMSO.

ALLEN, R. J. L., BROOK, M. & BROADBENT, S. R. (1968). The variability of vitamin C in our diet. *Br. J. Nutr.*, **22**, 555–63.

ANDREWS, J. & BROOK, M. (1966). Leucocyte–vitamin C content and clinical signs in the elderly. *Lancet*, **1**, 1350–1.

BAIRD, I. M., HOWARD, A. N., HUGHES, R. E. & WILSON, H. K. (1976). Unpublished data.
BARNES, M. J. & KODICEK, E. (1972). Biological hydroxylations and ascorbic acid with special regard to collagen metabolism. *Vitams Horm.*, **30**, 1–43.
BARTLEY, W., KREBS, H. A. & O'BRIEN, J. R. P. (1953). Spec. Rep. Ser. Med. Res. Coun. (Lond.), No. 280.
BERRY, W. T. C. & DARKE, S. J. (1968). Vitamins in health and disease. *Practitioner*, **201**, 305.
BLACKSTONE, SUE, HURLEY, R. J. & HUGHES, R. E. (1974). Some inter-relationships between vitamin C (L-ascorbic acid) and mercury in the guinea-pig. *Fd Cosmet. Toxicol.*, **12**, 511–16.
BOURNE, G. (1949). Vitamin C and immunity. *Br. J. Nutr.*, **2**, 346.
BRIGGS, M. H. (1973*a*). Vitamin C and infertility. *Lancet*, **ii**, 677.
BRIGGS, M. H. (1973*b*). Fertility and high-dose vitamin C. *Lancet*, **ii**, 1083.
BRIGGS, M. H. (1974). More vitamin C. *Med. J. Aust.*, **1**, 722–3.
BRIGGS, M. (1976). Vitamin C-induced hyperoxaluria. *Lancet*, **i**, 154.
BROOK, M. (1972). Vitamin C deficiency in Britain. In *Nutritional deficiencies in modern society*, ed. A. N. Howard & I. McLean Baird, 45. London, Newman Books Ltd.
BURNET, M. (1974). *Intrinsic mutagenesis: a genetic approach to ageing*. Lancaster, M.T.P.
CHATTERJEE, G. C. (1967). In *The vitamins*, ed. W. H. Sebrell & R. S. Harris, Vol. 1, 399. New York, London, Academic Press.
COCHRANE, W. A. (1965). Overnutrition in prenatal and neonatal life: a problem? *Can. med. Ass. J.*, **93**, 893–9.
COOKE, C. T. (1826). *Observations on the efficacy of white mustard seed . . . on the general management of health and life*, 3rd edn. Gloucester.
COUNSELL, J. N. (1971). Meat processing with ascorbic acid. *Proc. Biochem.*, **6**, 25, 28.
CRAWFORD, G. P. M., WARLOW, C. P., BENNETT, B., DAWSON, A. A., DOUGLAS, A. S., KERRIDGE, D. F. & OGSTON, D. (1975). The effect of vitamin C supplements on serum cholesterol coagulation, fibrinolysis and platelet adhesiveness. *Atherosclerosis*, **21**, 451–4.
DAVIES, J. E. W., PULSINELLI, J. & HUGHES, R. E. (1976). Ascorbic acid saturation levels in young and old guinea-pigs. *Proc. Nutr. Soc.* (in press).
DAVIES, J. E. W., WILSON, H. K. & HUGHES, R. E. (1976). The effect of food intake and of dietary supplements on the survival time of scorbutic guinea-pigs. *Nutr. Metab.*, **20**, 62–6.
DEGKWITZ, E. & STAUDINGER, HJ. (1974). Role of vitamin C on microsomal cytochromes. In *Vitamin C*, ed. G. G. Birch & K. Parker, 161–78. London, Applied Science Publishers.
DEPARTMENT OF HEALTH AND SOCIAL SECURITY REPORT (1974). *Diet and coronary heart disease*. London, HMSO.
DYKES, M. H. M. & MEIER, P. (1975). Ascorbic acid and the common cold. Evaluation for its efficacy and toxicity. *J. Am. med. Ass.*, **231**, 1073–9.
EDGAR, J. A. (1974). Ascorbic acid and biological alkylating agents. *Nature (Lond.)*, **248**, 136.
ELWOOD, P. C., HUGHES, R. E. & HURLEY, R. J. (1970). Ascorbic acid and serum cholesterol. *Lancet* **i**, **2**, 1197.
FIELDING, A. M. & HUGHES, R. E. (1975). The absence of an inhibitory effect of metyrapone (2-methyl-1,2-di-(3-pyridyl)propan-2-one) on hepatic microsomal hydroxylation in scurvy. *Experientia*, **31**, 1394–5.
GINTER, E. (1975). *The role of vitamin C in cholesterol catabolism and atherogenesis*. Bratislava, Czechoslovakia, Veda, Vydavatelstvo Slovenskej Akademie Vied.
GOLDSTEIN, M. L. (1971). High-dose ascorbic acid therapy. *J. Am. med. Ass.*, **216**, 332–3.
GORDONOFF, T. (1960). Darf man wasserlosliche vitamine uberdosieren. *Schweiz. Med. Wschr.*, **90**, 726–9.
HAYFLICK, L. (1975). Current theories of biological ageing. *Federation Proc.* (*Fedn Proc. Fedn Am. Socs Exp. Biol.*), **34**, 9–13.
HODGES, R. E., HOOD, J., CANHAM, J. E., SAUBERLICH, H. E. & BAKER, E. M. (1971). Clinical manifestation of ascorbic acid deficiency in man. *Amer. J. clin. Nutr.*, **24**, 432–43.
HODGKINSON, A. & WILLIAMS, A. (1972). Determination of oxalic acid in urine. *Clinica chim. Acta*, **36**, 127.
HOFFER, A. (1971). Ascorbic acid and toxicity. *New Engl. J. Med.*, **285**, 635–6.
HOFFER, A. (1973). Vitamin C and infertility. *Lancet*, **ii**, 1146.
HOFFER, A. (1975). Orthomolecular medicine: What it is, how does it work? Impact of science on society. *Imp. Science Soc.*, **25**, 3.
HORNIG, D., WEISER, H., WEBER, F. & WISS, O. (1973). Effect of massive doses of ascorbic acid on its catabolism in guinea-pigs. *Int. Z. VitamForsch.*, **43**, 28–33.
HORROBIN, D. F. (1973). D.V.T. after vitamin C? *Lancet*, **ii**, 317.
HUGHES, R. E. (1973). Assessment of vitamin C status. *Proc. Nutr. Soc.*, **32**, 243–8.
HUGHES, R. E. (1974). In *Vitamin C*, ed. G. G. Birch & K. Parker, 68–77. London, Applied Science Publishers.

HUGHES, R. E. & JONES, P. R. (1971). The influence of sex and age on the deposition of L-xylo-ascorbic acid in tissues of guinea-pigs. *Br. J. Nutr.*, **25**, 77–83.
HURLEY, R. J., JONES, P. R. & HUGHES, R. E. (1972). The uptake of ascorbic acid by the tissues of ascorbic acid-deficient guinea-pigs. *Nutr. Metabol.*, **14**, 136–40.
JACKISCH, P. F. (1971). Vitamin C danger. *Chem. Eng. News*, **49**, 86.
JUKES, T. H. (1974). Are recommended daily allowances for vitamin C adequate? *Proc. Nat. Acad. Sci. USA*, **71**, 1949–51.
JENNER, F. A. (1973). Vitamins in schizophrenia. *Lancet*, **ii**, 787–8.
KAMM, J. J., DASHMAN, T., CONNEY, A. H. & BARNS, J. H. (1973). Protective effect of ascorbic acid on hepatotoxicity caused by sodium nitrite plus aminopyrine. *Proc. natn. Acad. Sci. USA*, **70**, 747–9.
KLAUI, H. (1974). In *Vitamin C*, ed. G. G. Birch & K. Parker, 16–30. London, Applied Science Publishers.
KOTZE, J. P., WEIGHT, M. J., KLERK, W. A. DE, MENNE, I. V. & WEIGHT, M. J. A. (1974). Effect of ascorbic acid on serum cholesterol levels and on die-away curves of ^{14}C-4-cholesterol in baboons. *South Afr. J. Lab. clin. Med.*, **20**, 34–7.
LAMDEN, M. P. & CHRYSTOWSKI, G. A. (1954). Urinary oxalate excretion by man following ascorbic acid ingestion. *Proc. Soc. exp. Biol. Med.*, **85**, 190–2.
LANG, K. VON (1965). Wirkungen sehr hoher Ascorbinsaure-Dosen. *Wissen. Veroffentlich Deutsch. Ges. Ernahrung*, **14**, 149–56.
LEWIN, S. (1974*a*). Evaluation of potential effects of high intake of ascorbic acid. *Comp. Biochem. Physiol.*, **47B**, 681–95.
LEWIN, S. (1974*b*). High intake of vitamin C in relation to adenosine 3′:5′-cyclic monophosphate concentrations and to blood sugar concentrations. *Biochem. Soc. Trans.*, **2**, 922–33.
MASEK, J. & HRUBA, F. (1969). Relationship between saturation of serum and leucocytes with vitamin C. *Int. J. vit. Res.*, **34**, 39–44.
MASEK, J. & HRUBA, F. (1974). Our experiences with metabolism of high doses of vitamin C. *Vnitrni Lek*, **20**, 670–6.
MURRAY, D. R. & HUGHES, R. E. (1976). The influence of dietary ascorbic acid on the concentration of mercury in guinea-pig tissues. *Proc. Nutr. Soc.* (in press).
NANDI, B. K., MAJUMDER, A. K., SUBRAMANIAN, N. & CHATTERJEE, I. B. (1973). Effects of large doses of vitamin C in guinea-pigs and rats. *J. Nutr.*, **103**, 1688–95.
PAULING, L. (1968). Evolution and the need for ascorbic acid. *Proc. Nat. Acad. Sci.*, **67**, 1643–8.
PAULING, L. (1970). *Vitamin C and the common cold.* San Francisco, W. H. Freeman.
PAULING, L. (1974). Are recommended daily allowances for vitamin C adequate? *Proc. Nat. Acad. Sci. USA*, **71**, 4442–6.
PRIOR, T. (1746). An authentick narrative of the success of tar-water in curing a great number and variety of distempers. Dublin & London, 1946.
RANKEN, M. D. (1974). In *Vitamin C*, ed. G. G. Birch & K. Parker, 121–35. London, Applied Science Publishers.
RHEAD, W. J. & SCHRAUZER, G. N. (1971). Risks of long-term ascorbic acid overdose. *Nutr. Rev.*, **29**, 262–4.
RINSE, J. (1973). Atherosclerosis, chemistry and nutrition. *Internat. Laboratory*, Sept./Oct., 11–22.
SCHLEGEL, J. V., PIPKIN, G. E., NISHIMURA, R. & SCHULTZ, G. N. (1970). The role of ascorbic acid in the prevention of bladder tumor formation. *J. Urol.*, **103**, 155–9.
SCHRAUZER, G. N. & RHEAD, W. J. (1973). Ascorbic acid abuse: Effects of long-term ingestion of excessive amounts on blood levels and urinary excretion. *Internat. J. Vit. Res.*, **43**, 201–11.
SMITH, L. H. (1972). Large ascorbic acid intake. *N. Engl. J. Med.*, **287**, 412–13.
SORENSEN, D. I., DEVINE, M. M. & RIVERS, J. M. (1974). *J. Nutr.*, **104**, 1041–8.
SPITTLE, C. R. (1971). Atherosclerosis and vitamin C. *Lancet*, **ii**, 1280–1.
SPITTLE, C. R. (1973). Vitamin C and deep-vein thrombosis. *Lancet*, **ii**, July 28, p. 199.
STICK, H. F., KARIM, J., KOROPATRICK, J. & LO, L. (1976). Mutagenic action of ascorbic acid. *Nature (Lond.)*, **260**, 722–4.
STONE, I. (1965). Studies of a mammalian enzyme system for producing evolutionary evidence on man. *Amer. J. phys. Anthropol.*, **23**, 83–5.
STONE, I. (1966*a*). On the genetic etiology of scurvy. *Acta Gen. Med. et Gemell*, **15**, 345–50.
STONE, I. (1966*b*). Hypoascorbemia, the genetic disease causing the human requirement for exogenous ascorbic acid. *Persp. Bio. Med.*, **10**, 133–4.
STONE, I. (1967). The genetic disease, hypoascorbemia. *Acta Gen. Med. et Gemell*, **16**, 52–62.
STONE, I. (1972). *The healing factor.* New York, Grosset & Dunlop.
STONE, I. (1974). Humans, the mammalian mutants. *Internat. Lab.*, May/June, 32–40.
TATUM, J. H., SHAW, P. E. & BERRY, R. E. (1969). Degradation products from ascorbic acid. *J. agric. Fd Chem.*, **17**, 38–40.

THEWLIS, B. H. (1974). Vitamin C in breadmaking. In *Vitamin C*, ed. G. G. Birch & K. Parker, 150–60. London, Applied Science Publishers.

TAKENOUCHI, K., ASO, K., KAWASE, K., ICHIKAWA, H. & SHIOMI, T. (1966). On the metabolites of ascorbic acid, especially oxalic acid, eliminated in urine, following administration of large amounts of ascorbic acid. *J. Vitaminol.*, **12**, 49–58.

TYRRELL, D. A. J. (1974). Vitamin C and the common cold. *Prescribers' Journal*, **14**, 21–4.

VILTER, R. W. (1967). In *The vitamins*, Vol. 1, ed. W. H. Sebrell & R. S. Harris, 501–3. New York, London, Academic Press.

WILLIAMS, R. J. & DEASON, G. (1967). Individuality in vitamin C needs. *Proc. Nat. Acad. Sci.*, **57**, 1638–41.

WILSON, C. W. M. (1974). In *Vitamin C*, ed. G. G. Birch & K. Parker, 203. London, Applied Science Publishers.

WYNGAARDEN, J. B. & ELDER, T. D. (1966). Primary hyperoxaluria and oxalosis. In *The metabolic basis of inherited disease*, 2nd edn, ed. J. B. Stanbury, J. B. Wyngaarden & D. S. Fredrickson, 189–212. New York, McGraw-Hill Book Co.

EFFECT OF HEATING TIME ON VOLATILE COMPOSITON OF CANNED PORK MEAT

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ABSTRACT

Volatiles from samples of ground pork meat, subject to 10, 20, 30 *and* 40 *min heating at* 121°*C, were isolated by trapping on Tenax GC and Porapak Q. Essences were compared on wall-coated open-tubular glass capillary columns and analysed by glass capillary gas chromatography–mass spectrometry. Low molecular weight alcohols, many with branched-chain skeletons, mercaptans and cyclic products, were among the compounds identified. The amount of volatiles present appeared to correlate well with the heating time to which the sample had been subjected.*

INTRODUCTION

While a considerable amount of work has been done on the volatile constituents of cooked beef, those of cooked pork have received little attention. Watanabe & Sato (1968, 1969) studied the incidence of lactones in meat fats, and the precursor compounds (Watanabe & Sato, 1970*a*, *b*). Mussinan & Walradt (1974) reported a large number of compounds identified as volatile constituents of pressure-cooked pork liver. The present study was concerned with the influence of heat on the volatiles of conventionally processed pork meat.

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APPARATUS AND METHODS

Meat samples

A mixture of ground pork shoulders, with 1·7% salt added, was canned at the facilities of the Eastern Utilizational Research Laboratory of the USDA in Philadelphia. Luncheon meat cans were aluminium, lacquer-coated inside, approximately 6 cm diameter × 3·5 cm, and held about 100 g of meat each. The canned samples were subjected to steam-pressure heating at 121°C for the stated periods, stored for 1 month at 4°C and then at room temperature (25°C) for approximately 1 month until used.

Essence preparation

Tenax essence: 11·5 cm of an 18-cm length of 6-mm glass tubing was packed with 60–80 mesh Tenax GC (Enka N.V., The Netherlands) between plugs of glass wool. The trap was first purged for 24 h at 120°C with purified nitrogen (Jennings *et al.*, 1974) at a flow rate of 50–70 cc/min. Two cans of meat were added to 100 ml deionised water in a 500-cc gas scrubbing cylinder and swept with purified N_2 at a flow rate of *ca.* 45 cc/min for *ca.* 15 h. The essence was recovered by backflushing to glass traps chilled with dry ice while heating the Tenax trap to 120°C for 5–6 h.

Porapak essence: This utilised the same procedure as above, except that the trapping tubes contained 11·5 cm of Porapak Q (Waters Assoc.).

Gas chromatography

A Hewlett Packard 5711A gas chromatograph, adapted to wall-coated open-tubular glass capillary columns (Jennings & Wohleb, 1974; Jennings *et al.*, 1974) and dual FID, was used for the gas chromatographic analysis. The standard ¼-in on-column injection system was converted to glass-inlet splitters similar to those reported earlier (Jennings & Adam, 1975), except that they incorporated flow-inversion characteristics (Jennings, 1975) and a commercially available adaptor that housed the expansion and splitting chambers (J. and W. Scientific, PO Box 216, Orangevale, California 95662, USA). Columns were 0·25 mm id × 45 m, coated with Carbowax 20M admixed with Ionox and benzyltriphenylphosphonium chloride (Jennings *et al.*, 1974). The detector and inlet were maintained at 250°C, and the column was programmed from 50° to 170°C at 2°/min and held. The inlet split ratio was *ca.* 1:60.

Gas chromatography–Mass spectrometry

Mass spectra were obtained on a Finnigan model 1015C with a model 6000 data system. A 0·25 mm id × 80 m Carbowax 20M wall-coated open-tubular glass capillary column served as the inlet to the mass spectrometer.

RESULTS AND DISCUSSION

Neither Porapak Q nor Tenax GC were entirely satisfactory as trapping substrates for these volatiles. In either case, volatiles of sensory significance passed through the traps and aromas could be continuously detected at the outlet. This was especially evident in preliminary trials that utilised short traps. Longer (11·5-cm) traps made this problem less acute, but did not eliminate it.

Figures 1 to 3 show chromatograms typical of Tenax essences of meat samples that had been heated at 121°C for 20, 30 and 40 min, respectively. Figure 4 shows a chromatogram of an essence from Porapak Q of a meat sample that had been heated 20 min at 121°C. The concentration of most of these volatiles appears to be directly related to the amount of heat received by the sample. Figure 5 shows total ion current 'chromatograms' from the GC–MS. The upper curve is typical of a Tenax GC essence, while the lower curve is of a Porapak Q essence.

TABLE 1

COMPOUNDS FROM CANNED PORK MEAT

Scan[a]	*Probable identity*[b]	*I*[c]
42	Acetaldehyde	821
89	Ethanol	908
130	Isobutyl mercaptan	958
160	*Sec*-butanol	992
177	*n*-propanol	1007
194	Acetylmethyl mercaptan	1024
242	*Iso*butanol	1060
310	*n*-butanol	1111
380	Hexyl mercaptan	1167
447	*n*-pentanol	1213
537	*p*-cymene isomer	1279
542	*p*-cymene isomer	1284
587	Isopropyl benzaldehyde	1309
592	2-methyl hexanol-1	1311
1140	Cyclic, aldehydic?	
1206	Cyclic, aldehydic?	
1218	Naphthalene	

[a] Relates to the total ion current chromatogram in Fig. 5.
[b] Based on mass spectral and retention data.
[c] These are rough approximations relating to the total ion current chromatogram in Fig. 5. More precise comparisons involved separate runs. (See text.)

Table 1 shows the compounds isolated. The retentions of several early compounds, as exhibited by the total ion current chromatogram (Fig. 5), are not in close agreement with the correct values. However, when the retentions of the authentic compounds were compared with those of the peaks in question under conventional gas chromatographic equipment using the WCOT glass capillary

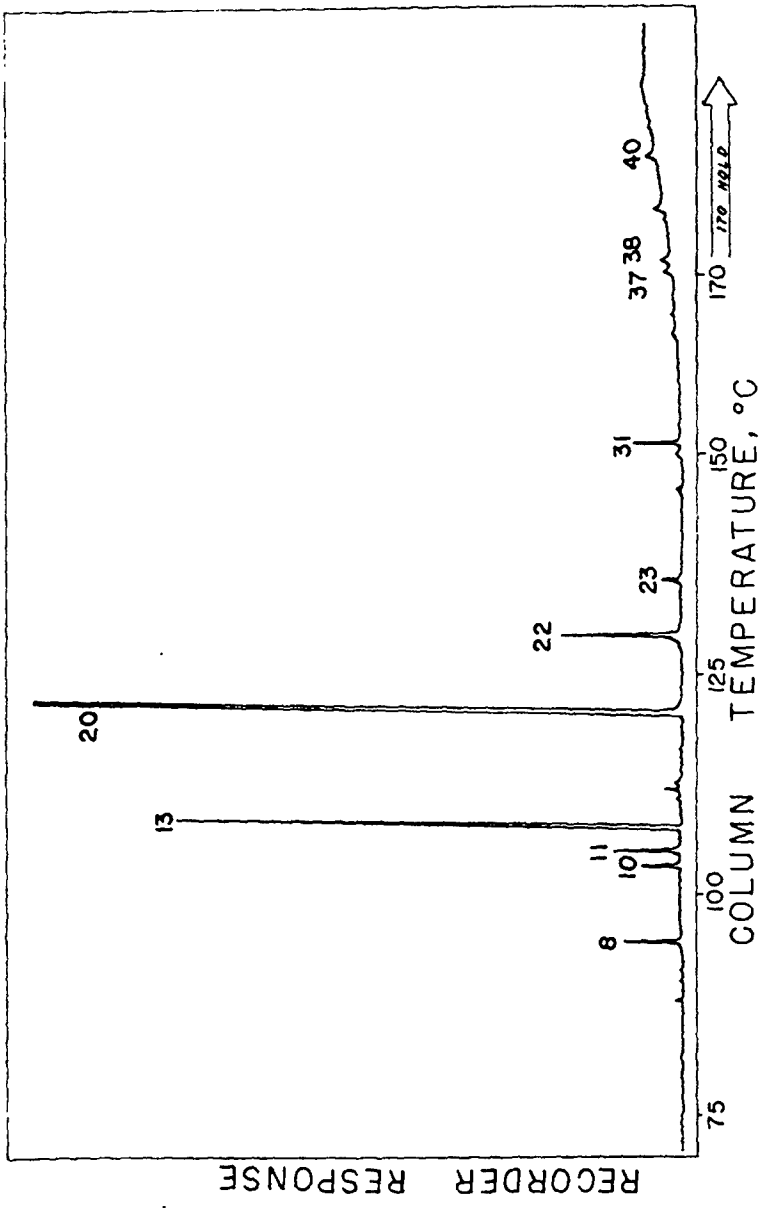

Fig. 1. Nine-microlitre injection of Tenax essence of sample heated for 20 min at 121°C. Chromatographic conditions as specified in the text.

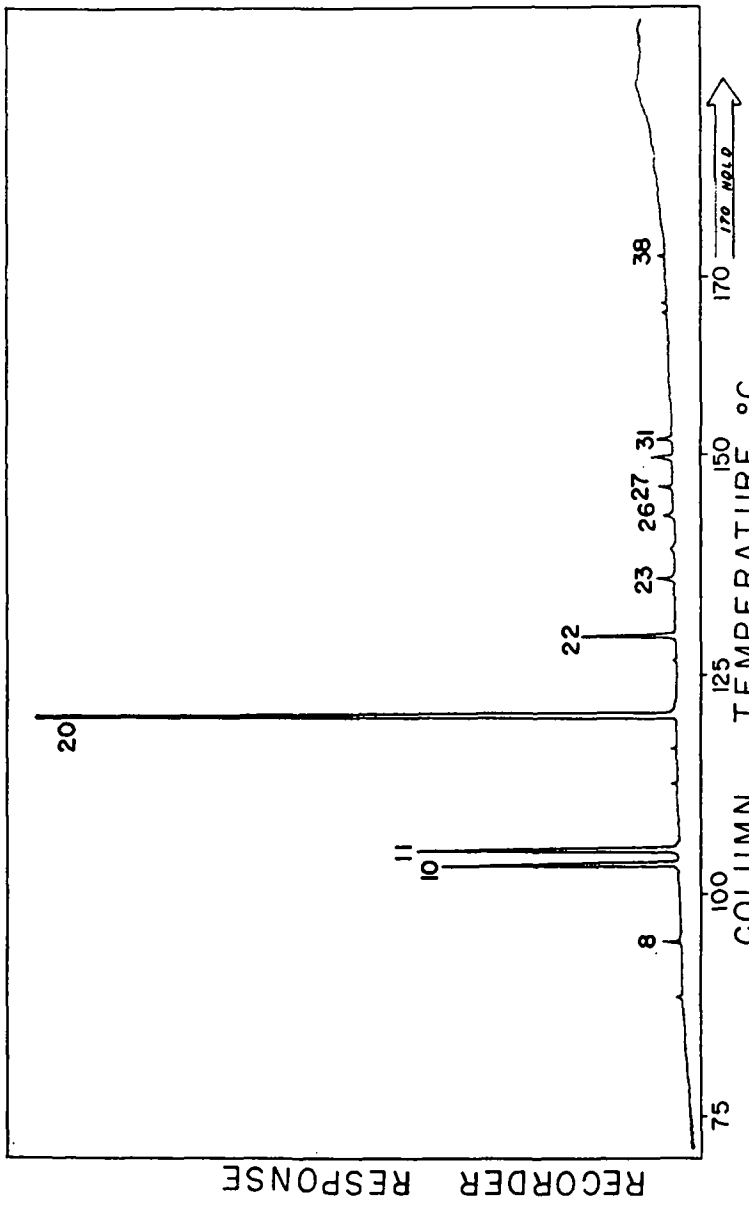

Fig. 2. Eight-microlitre injection of Tenax essence of sample heated for 30 min at 121°C. Chromatographic conditions as specified in the text.

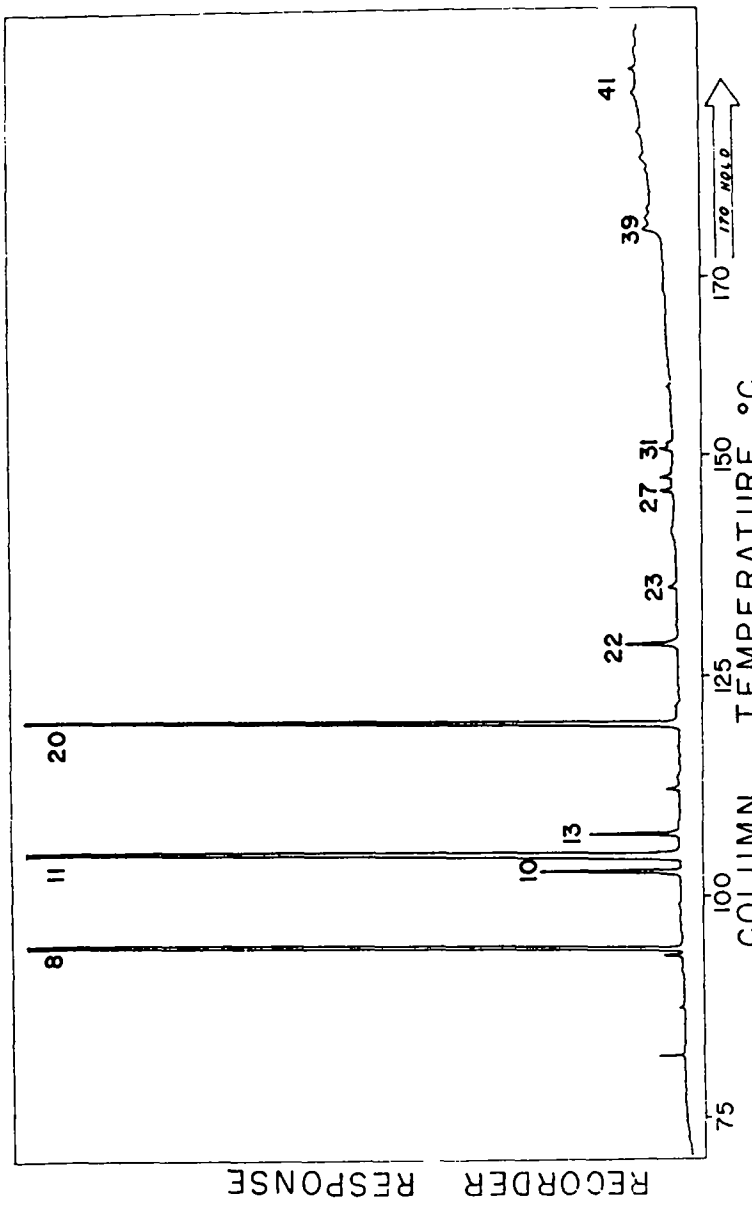

Fig. 3. Nine-microlitre injection of Tenax essence of sample heated for 40 min at 121°C. Chromatographic conditions as specified in the text.

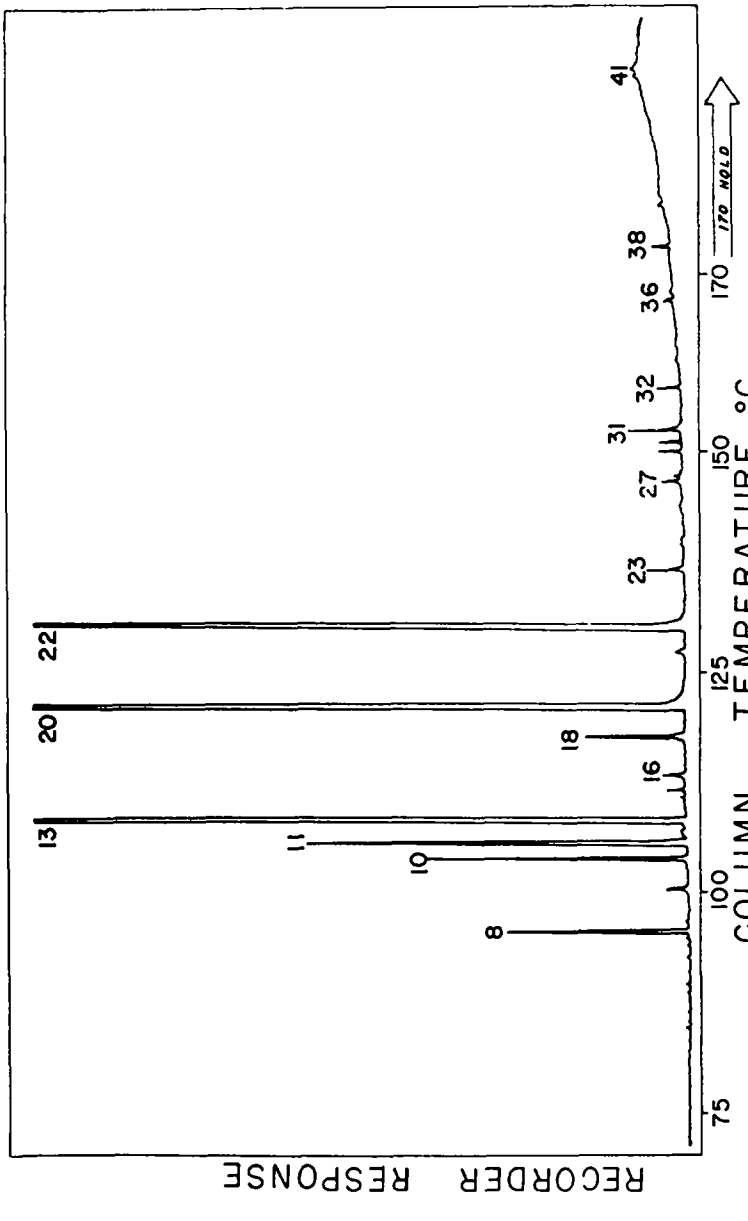

Fig. 4. Three-microlitre injection of Poropak essence of sample heated for 20 min at 121°C. Chromatographic conditions as specified in the text.

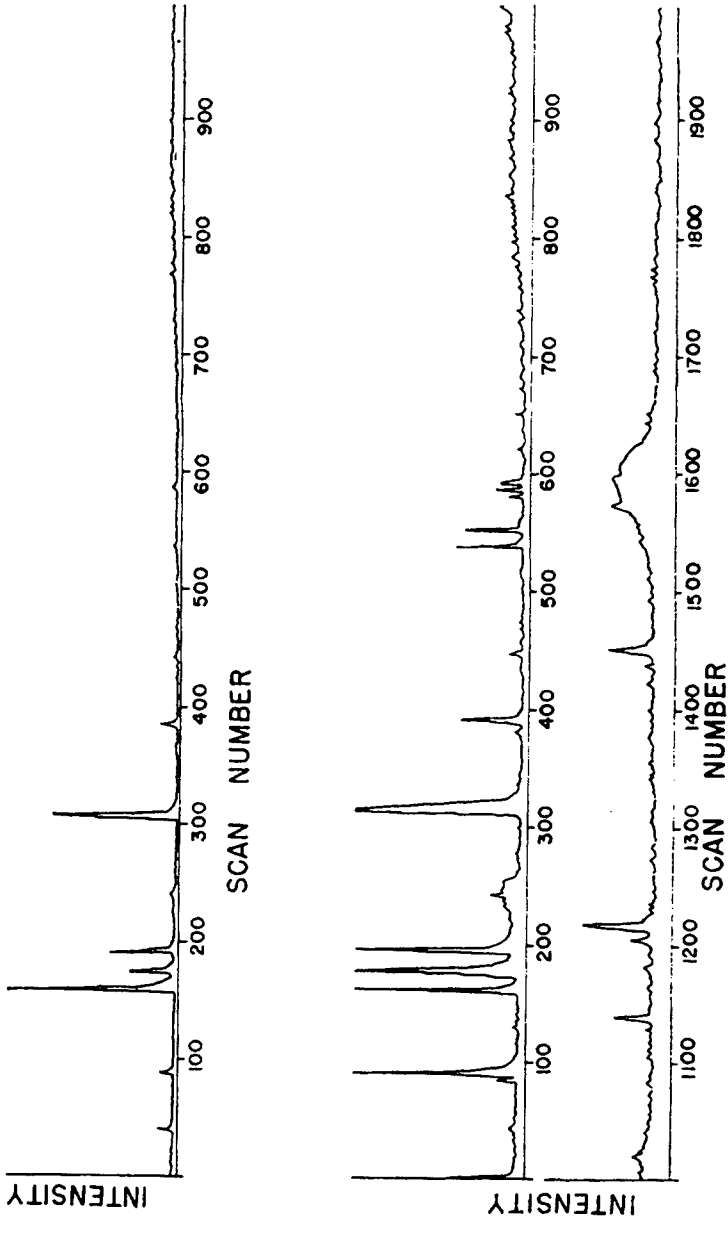

Fig. 5. Ten microlitres of Porapak essence of sample heated for 20 min at 121°C on GS–MS.

columns (*not* the GC–MS unit), the retention data showed good agreement. These comparisons were made by co-chromatographing a mixture of the sample with C-9 to C-16 *n*-paraffin hydrocarbons. Retention indices obtained at very low rates of temperature programming usually agree within $\pm 2\%$ with the isothermal Kováts indices. This observation is in agreement with that of Littlewood (1970). Variations in solid supports and liquid phases, including the presence and amount of anti-tailing additives, have at least this much influence on isothermal Kováts indices.

The compounds of significance isolated in this study are dominated by acetone, acetaldehyde, low molecular weight alcohols and isobutyl and hexyl mercaptans. The latter, which probably possess considerable sensory importance, frequently resist analysis in conventional packed and/or metal gas chromatographic systems. The alcohols are probably derived from amino acids, which could account for the many branched-chain skeletons.

The cyclic structures associated with the spectral data obtained on scans 537, 542, 587, 1140, 1206 and 1218 were at first attributed to degradation of the porous polymer, Porapak Q. However, while we have used this porous polymer extensively to prepare a wide variety of essences, we have not previously experienced such artifacts, provided the Porapak is carefully conditioned and subjected to limited heating and only after careful nitrogen purging (Jennings *et al.*, 1974). The structures of these compounds are not widely different from some that have been identified as constituents of heated beef by other workers (Herz & Chang, 1970), and the possibility that they are indeed produced by heated pork cannot be discounted at this time.

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REFERENCES

HERZ, K. O. & CHANG, S. S. (1970). *Meat flavor: Advances in food research*, **18**. New York, London, Academic Press.

JENNINGS, W. G. (1975). Glass inlet splitter for gas chromatography. *J. Chrom. Sci.*, **13**, 185–7.

JENNINGS, W. G. & ADAM, S. (1975). Gas chromatography: Elution temperature, speed of analysis and separation efficiency as influenced by rate of temperature programming and carrier gas velocity in open tubular glass capillary columns. *Anal. Biochem.*, **69**, 61–9.

JENNINGS, W. G. & WOHLEB, R. H. (1974). Routine production of the glass capillary column. *Chemi, Mikrobiol. Tech. der Lebensmittel*, **3**, 33–5.
JENNINGS, W. G., WOHLEB, R. H. & LEWIS, M. J. (1974). Isolation of volatile compounds for GLC analysis. *MBAA Tech. Quarterly*, **11**, 104–9.
JENNINGS, W. G., YABUMOTO, K. & WOHLEB, R. H. (1974). Manufacture and use of the glass open tubular column. *J. Chrom. Sci.*, **12**, 344–8.
LITTLEWOOD, A. B. (1970). *Gas Chromatography*, 2nd edn. New York, London, Academic Press.
MUSSINAN, C. J. & WALRADT, J. P. (1974). Volatile constituents of pressure-cooked pork liver. *J. Agr. Fd Chem.*, **22**, 827.
WATANABE, K. & SATO, Y. (1968). Aliphatic γ and δ lactones in meat fats. *Agr. Biol. Chem.* (Japan), **32**, 1318–24.
WATANABE, K. & SATO, Y. (1969). Lactones in the flavor of heated pork fat. *Agr. Biol. Chem.* (Japan), **33**, 242–9.
WATANABE, K. & SATO, Y. (1970*a*). Conversion of some saturated fatty acids, aldehydes and alcohols into γ and δ lactones. *Agr. Biol. Chem.* (Japan), **34**, 464–72.
WATANABE, K. & SATO, Y. (1970*b*). Volatile neutral compounds from heat-degraded pork fat and their conversion into lactones. *Agr. Biol. Chem.* (Japan), **34**, 1710–15.

A LIPOPHILIC–HYDROPHOBIC ATTRIBUTE AND COMPONENT IN THE STEREOCHEMISTRY OF SWEETNESS

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ABSTRACT

The bipartite AH,B concept of sweetness is extended to a tripartite AH,B,γ concept. This leads to the novel idea that intramolecular hydrogen bonding may, in some cases, enhance sweetness.

INTRODUCTION

By application of chemical bonding and stereochemical theory to the properties and structures of compounds, Shallenberger & Acree (1967) proposed that a bipartite chemical unit is common to the various substances that taste sweet. The common unit was described as an AH,B couple as usually used to describe either and intra- or an intermolecular hydrogen bond.

Using vicinal hydroxyl (α-glycol) groups of the sugars in the synclinal (*staggered* or *gauche*) conformation as the stereochemical model for an AH,B saporous unit established (Shallenberger & Acree, 1967) that the AH proton to B orbital distance needed to be about 3 Å for optimum sweetness. Closer approach of vicinal hydroxyls, directed by their configuration and the conformation of the pyranose and furanose ring, results in increased intramolecular hydrogen bonding and decreases their ability to elicit sweet taste. Thus, the synperiplanar (*eclipsed*) glycol conformation was viewed as being inert, and a hydroxyl group so sterically disposed as to be able to hydrogen bond the ring oxygen atom was viewed as effectively reducing the ability of an α-glycol moiety to elicit sweet taste.

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Since the varying sweetness of the sugars was attributed to the formation of an intramolecular hydrogen bond involving OH groups of an α-glycol moiety, it followed that the initial chemistry of the sweet taste sensation is a concerted intermolecular hydrogen bonding interaction between AH,B of the sweet substance and a sterically and chemically commensurate AH,B unit at the taste bud receptor site. Consequently, a second stereochemical feature of an α-glycol moiety's ability to elicit sweet taste is that the ability will diminish as the projected OH,OH angle increases beyond 60°. The antiperiplanar glycol conformation is not capable of eliciting sweet taste because it is not sterically constructed in the manner appropriate for interaction with the receptor site geometry.

The stereochemical model for the sweetness of substances, and for the initial chemistry of sweet taste, now appears to have application to such varied fields as physical chemistry, physiology, pharmacology and psychology. In addition to the previous compounds in which AH,B has been identified (amino acids, chloroform, salts of beryllium, saccharin, etc.), certain flavanones (Van Niekerk & Koeppen, 1972) and glycosides (Hodge & Inglett, 1973) have now been added.

The purpose of this paper is to review the recent findings at these and other laboratories that advance significantly the understanding of the chemistry of sweetness described above.

IDENTITY OF PRIMARY AH,B UNITS IN THE SUGARS

Examination of model compounds (glycosides and deoxy sugars) has established (Birch *et al.*, 1970*b*; Birch & Lee, 1975) that there is a primary AH,B unit for each sugar and that the hydroxyl groups at carbon atoms 3 and 4 are of special importance to sweetness for the glucopyranose structure. The OH group at carbon atom number 4 of D-glucose in the Cl conformation appears to be the principal proton donor (AH) and the oxygen atom of the OH group at carbon atom number 3 is the principal proton acceptor (B) (Fig. 1).

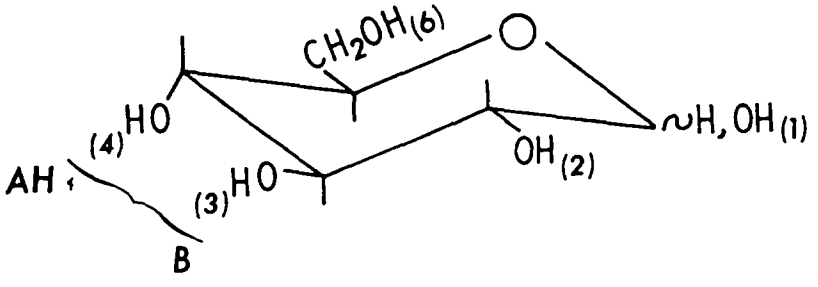

Fig. 1

Among the reasons that may be cited for this assignment are the slight sweet taste differences of the anomeric glucoses and the sweetness intensity of 2-deoxy-D-glucose. Moreover, α,α-trehalose and methyl α-D-glucoside are equally sweet when molar solutions are compared (Birch *et al.*, 1970*a*).

For D-fructose, the intuitive assignment (Shallenberger & Acree, 1967) of the anomeric OH group at carbon atom number 2 in the fructopyranosyl 1C conformation as AH and the oxygen atom of the primary alcohol group (1) as B has been confirmed (Lindley & Birch, 1975), primarily by comparing the relative sweetness and infra-red spectra of the structurally analogous compounds 2-deoxy-D-fructopyranose, methyl-β-D-fructopyranoside, β-D-arabinopyranose, methyl-β-D-arabinopyranoside and α-L-sorbopyranose (Fig. 2).

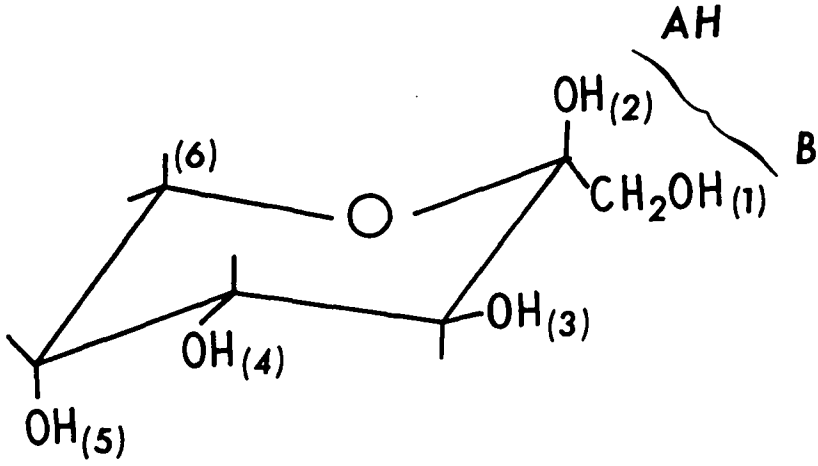

Fig. 2

Several possibilities can be offered for the inequality of gauche α-glycol groups to act as a sweet-eliciting AH,B unit. First, their stereodisposition within the sugar structure renders them unequal in their ability to act either as a proton donor or acceptor in a hydrogen bonding system due to overall molecular electronic distribution. The varying acidity of pyranose OH groups is a good analogy. Secondly, and not necessarily unrelated to the first suggestion, a third saporous unit component possibly directs that OH group whose primary function is AH and also that OH group whose function is B. Finally, *overall* molecular geometry may direct the OH group best able to *function* as AH of the compound (c) for interaction with B of the receptor site (r) and also, concertedly, B of the compound with AH of the receptor.

THE LIPOPHILIC–HYDROPHOBIC COMPONENT OF THE SAPOROUS UNIT

It was pointed out by Deutsch & Hansch (1966) that the relative sweetness of a series of closely related compounds can be correlated with their partition coefficient (π) between a polar and a non-polar solvent. Subsequently, Kier (1972) identified a third binding site in addition to the AH,B unit to explain the varying sweetness of the amino acids and substituted nitroaniline compounds. The third site (X) was viewed as being hydrophobic in nature and capable of participation in a 'dispersion' bond with an appropriate hydrophobic receptor site. The distance parameters for a compound's saporous unit (glucophore) is A of AH about 2·6 Å from B. The distance from B to the dispersion site is about 5·5 Å, and from A to X about 3·5 Å, creating an oblique planar stereogeometric arrangement. While Kier viewed the interaction between X of a sweet compound and X of a receptor site as a 'dispersion' bond, we favour the view of an hydrophobic interaction (Salem, 1964) and designate the hydrophobic site as γ.

We have now examined the sugars for a possible hydrophobic saporous function to develop more completely our understanding of the mechanism by which the sugars elicit—and vary in their ability to elicit—sweet taste. In the ensuing discussion we use the term 'lipophilicity' to indicate solubility in non-polar solvents and the term 'hydrophobicity' to indicate the attraction of non-polar units in a polar environment.

Since $OH_{(4)}$ and $OH_{(3)}$ of D-glucopyranose are now known to be AH and B, respectively, the methylene carbon atom (C-6) seems to be an unambiguous choice for a hydrophobic site or lipophilic centre. Moreover, with D-glucose in the favoured Cl conformation the geometric relation between AH,B and γ for the compound (c) describes an oblique structure with striking relation to Kier's glucophore (Figs 3(a) and (b)). Furthermore, with $OH_{(2)}$ of β-D-fructopyranose as AH and the oxygen atom of $OH_{(1)}$ as B, assignment of the ring methylene carbon atom (C-6) as γ can lead, due to the free rotation, to an identical stereogeometry for the saporous unit as just described for glucose (Fig. 4). We will subsequently demonstrate the justification for the stereogeometry of OH_1 and OH_2 as shown despite the 'ability' of C-1 to rotate freely.

There are several possible functional modes for an element of lipophilicity in a compound and for a hydrophobic component of the saporous unit. A general element of lipoid solubility may be related to a compound's ability to gain access to the receptor site and is expressed by the phenomenon of 'impact time'. The rapid but short-lived sweet sensation evoked by chloroform is an example of this probable general role of lipophilicity.

When the chemical grouping that imparts an element of lipophilicity to a compound is located at a specific point in space in relation to the AH,B unit, it may serve as a hydrophobic compliment to the compound's AH,B unit, resulting in a

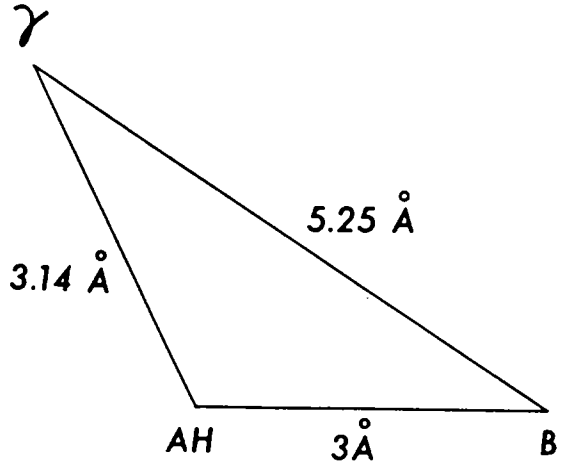

Fig. 3(a)

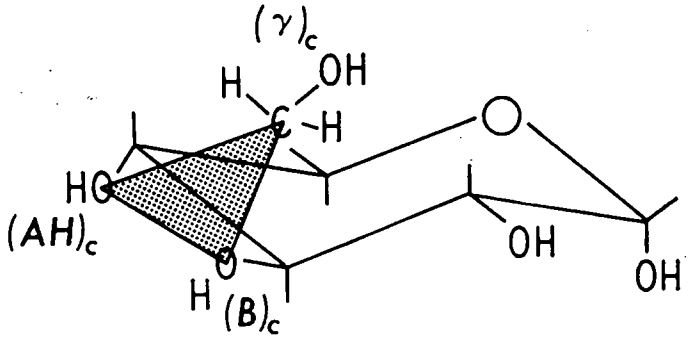

Fig. 3(b)

tripartite interaction with the receptor site to yield a more intense and prolonged sweet taste sensation.

Finally, the hydrophobic site (γ) may direct that portion of the molecule, if several choices are available, whose primary function will be AH,B, and augment the proton donor capacity of AH and the proton acceptor capacity of B. This multiple function is shown in Fig. 5 for D-glucose.

The justification for the argument just presented is the finding (Lemieux & Brewer, 1973) that the C-6 OH group of the glycopyranose structure is sterically

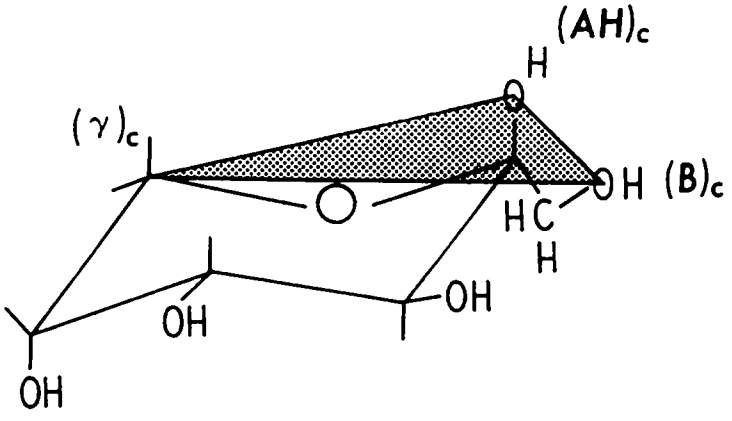

Fig. 4

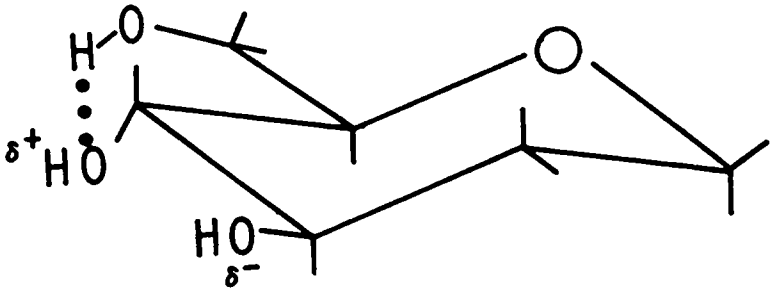

Fig. 5

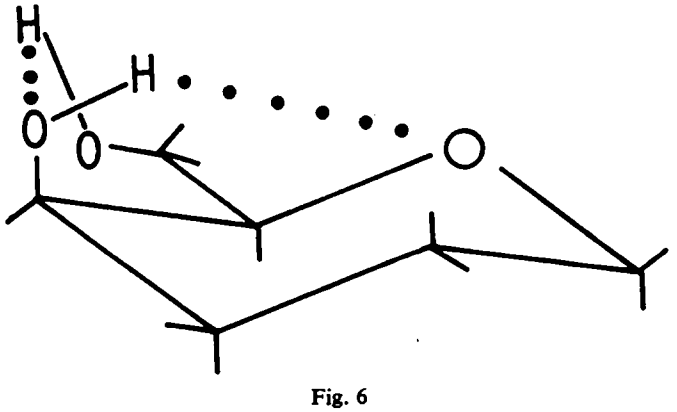

Fig. 6

disposed to bond to the C-4 OH group, particularly in a hydrophobic environment. In this sense, then, we have a case of intramolecular H-bonding actually promoting sweetness. Clearly, previous inferences to the effect that sugar sweetness varies inversely with the degree of intramolecular hydrogen bonding must be re-examined, as must evaluation of the sweetness of sugar enantiomers in view of the possible role of a third saporous unit component.

For D-galactose, such bonding serves to accentuate the ability of the primary OH group to intramolecularly bond the ring oxygen atom and, therefore, markedly lower sweetness potential (Fig. 6).

By the same token, analogous bonding in fructopyranose serves to accentuate the proton donor capacity of $OH_{(2)}$ and fixes the position of $OH_{(1)}$ in space, *i.e.* free rotation is restricted (Fig. 7).

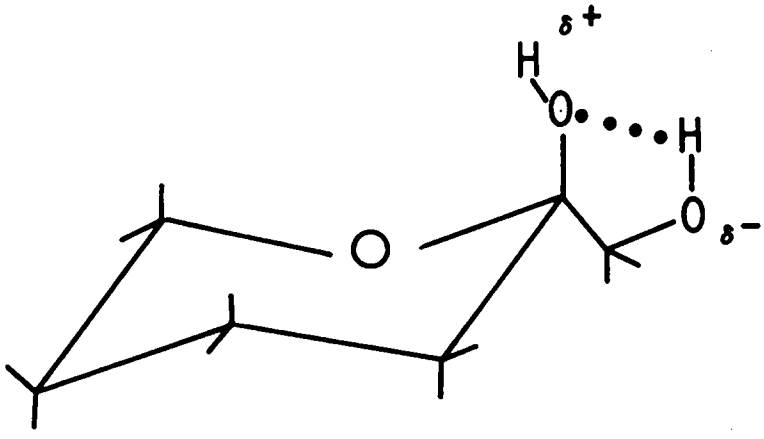

Fig. 7

By this reasoning it now seems clear that intramolecular hydrogen bonding will decrease the sweet taste potential of a compound only when that group, whose primary function is that of a proton donor, is sterically disposed to hydrogen bond elsewhere in the molecule. Otherwise, intramolecular H-bonding may be found to accentuate sweet taste.

Previous findings (Shallenberger *et al.*, 1969) indicated that there is little difference in the sweet taste of sugar enantiomers, in accordance with a bipartite saporous unit. The result seems to also be in accordance with a tripartite saporous unit. The primary AH,B and also the γ function of enantiomers can be equally positioned over a tripartite, but diastereomeric receptor site (r). As shown in Fig. 8, however, AH,B and γ of D- and L-glucose are alternately directed *away* from the receptor sites in one case and *towards* them in the other. Careful comparison of the sweetness of

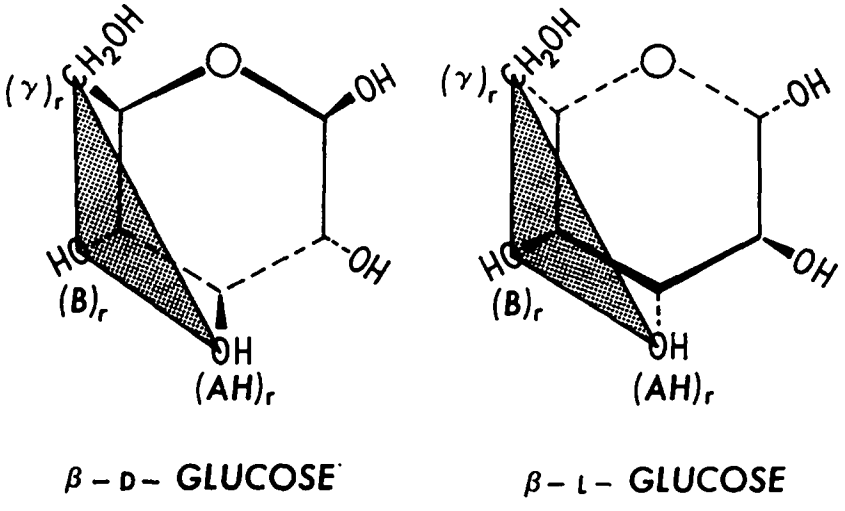

Fig. 8

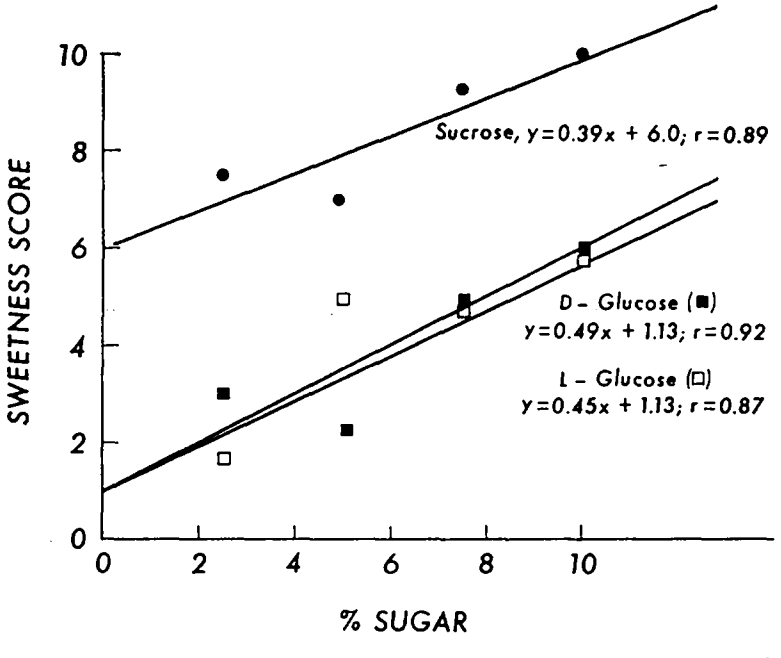

Fig. 9. Relative sweetness of D- and L-glucose and sucrose at different concentrations.

D- and L-glucose with that of sucrose at several concentrations shows (Fig. 9) that the enantiomeric saporous functions for the sugars are equal in their ability to elicit sweet taste.

REFERENCES

BIRCH, G. G., COWELL, N. D. & EYTON, D. (1970*a*). A quantitative investigation of Shallenberger's sweetness hypothesis. *J. Fd Technol.*, **5**, 277–80.

BIRCH, G. G., LEE, C. K. & ROLFE, E. (1970*b*). Organoleptic effect in sugar structures. *J. Sci. Fd Agric.*, **21**, 650–3.

BIRCH, G. G. & LEE, C. K. (1975). Structural functions of taste in the sugar series: Sensory properties of deoxy sugars. *J. Fd Sci.*, **39**, 947–9.

DEUTSCH, E. W. & HANSCH, C. (1966). Dependence of relative sweetness on hydrophobic bonding. *Nature* (*Lond.*), **211**, 75.

HODGE, J. E. & INGLETT, G. E. (1973). Structural aspects of glycosidic sweeteners containing (1 → 2) linked disaccharides. Paper presented at the Symposium on Sweeteners. 165th Meeting of the American Chemical Society, Dallas, Texas, USA.

KIER, L. B. (1972). A molecular theory of sweet taste. *J. Pharm. Sci.*, **61**, 1394–7.

LEMIEUX, R. U. & BREWER, J. T. (1973). Conformational preferences for solvated hydroxymethyl groups in hexopyranose structures. In *Advances in chemistry series, carbohydrates in solution*, ed. R. F. Gould, **117**, 121–46. Washington, D.C., American Chemical Society.

LINDLEY, M. G. & BIRCH, G. G. (1975). Structural functions of taste in the sugar series. *J. Sci. Fd Agric.*, **26**, 117–24.

SALEM, L. (1964). Intermolecular forces in biological systems. In *Electronic aspects of biochemistry, Proc. Intern. Symp. Ravello, Italy*, 293–9. New York, Academic Press, Inc.

SHALLENBERGER, R. S. & ACREE, T. E. (1967). Molecular theory of sweet taste. *Nature* (*Lond.*), **216**, 480–2.

SHALLENBERGER, R. S., ACREE, T. E. & LEE, C. Y. (1969). Sweet taste of D- and L-sugars and amino acids and the steric nature of their chemoreceptor site. *Nature* (*Lond.*), **221**, 555–6.

VAN NIEKERK, D. M. & KOEPPEN, B. H. (1972). Synthesis of 2-*O*-α-L-rhamnopyranosyl-D-galactose, a reported partial hydrolysis product of α-solanine, and some taste-eliciting flavanoid 2-*O*-α-L-rhamnopyranosyl-β-D-galactopyranosides. *Experentia*, **28**, 123–4.

NUTRIENT CHANGES IN SOME TRADITIONAL NIGERIAN FOODS DURING COOKING—PART 1: VITAMIN CHANGES

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ABSTRACT

The vitamin contents of six rural Nigerian foods were estimated before and after cooking. Losses of vitamin C in all the diets were between 40 and 100%. There were mild losses of vitamin A and beta-carotene. Of the B vitamins, the most affected were thiamine and pyridoxal. Their losses were 29–48% and 3·4–49%, respectively. The implications of these losses for the peasants are discussed.

INTRODUCTION

In many developing countries, plant products form the major foods consumed by the entire population. Animal products (which would give a more balanced diet) are, however, too expensive for a great proportion of the population, which is often poor. In Nigeria, these plant products range from starchy roots (yams, cassava, cocoyam, etc.) through seeds and grains (cowpea, groundnuts, maize, melon, locust beans, etc.) to green stems, leaves and fruits.

There are reports in the literature on the nutrient composition of some of the various plant products consumed in Nigeria (Mathieson, 1964; Oke, 1965, 1967; Munro & Bassir, 1969; Oyenuga, 1968; Abaelu, 1973). Information is, however, lacking on the nutrient composition of the combinations of plant products that form the traditional peasant diets of many parts of Nigeria, and also on the nutrient content after the foods have been cooked and are ready to be served. Cooking has been known to bring about highly complex reactions which have a direct bearing on the nutritional value of food. The variety of traditional cooking methods used

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in Nigeria has been recognised as an important factor contributing to the malnutrition and under-nutrition problems of the country (FAO/UNICEF Study Group, 1962).

The experiments reported on in this paper were therefore carried out in order to assess the effect of traditional cooking on the vitamin content of the diets of the people of the Cross River State of Nigeria.

EXPERIMENTAL

Materials and methods

Sampling and preparation of the traditional diets for analysis: The foodstuffs used for the preparation of the various traditional dishes were bought in the local markets. No reference was made to the geographical origin, storage conditions or treatment of the foodstuffs following harvest. They were quickly brought to the laboratory for cooking and analysis.

Studies were carried out on the vitamin contents of the raw and cooked forms of the following six diets commonly used in the Cross River State of Nigeria:

(a) Gari (fried cassava meal, *Manihot utilissima*) with 'afang' (*Gnetum africanum*) soup.
(b) 'Anyan ekpang' (grated cocoyam, *Xanthosama mafaffa* Schott; water yam, *Discorea alata* Linn.) and palmnut (*Elaeis guineensis*) soup.
(c) Cassava foofoo (cooked fermented cassava, *Manihot utilissima*) with 'edikang ikong' soup (thick vegetable soup—combinations, *Talinum triangulare* and *Telfaria* spp.).
(d) Pounded yam (*Discorea* spp.) with 'afia efere' (plain soup—containing small quantities of *Hibiscus esculentus*, *Allium cepa* and *Amaranthus* spp.).
(e) 'Ekpang Nkukwo' (grated cocoyam and leaves, *Xanthosoma mafaffa* Schott, with pepper (*Capsicum frustescens*) and palm oil (*Elaeis guineensis*)).
(f) 'Iwuk ukom' (plantain porridge, *Musa paradisiaca*, with *Talinum triangulare*, *Elaeis guineensis* and *Capsicum frustescens*).

The foods were all cooked as for human consumption (Umoh, 1972). The recipes and cooking methods were those traditionally used in the Cross River State of Nigeria. The proportions of the ingredients were determined by a survey conducted by us among certain peasant families in the State (Bassir & Umoh, 1973).

The cooked foods were each mixed and homogenised in bits. The bits were then pooled and finally mixed in a 'Kenwood "CHEF" domestic food mixer' (model A 700 D supplied by Kenwood Manufacturing Co. Ltd, Woking, Surrey, Great Britain). The raw forms of all the diets were similarly homogenised. Bits of the homogenised meal were then taken for vitamin analysis.

ANALYSIS OF THE FOODS

Representative samples of the cooked and raw foods were analysed for vitamin A by the Carr–Price (1926) method, and for beta-carotene by the method of Bassir (1963). Thiamine was estimated as thiochrome by the fluorometric method of the Association of Vitamin Chemists (1966). Riboflavin was also estimated fluorometrically by the method of Hodson & Norris (1939) using, in both cases, the Hitachi Perkin–Elmer fluorescence spectrophotometer (model 103). Vitamin C was estimated volumetrically using the 2,6-dichlorophenol indophenol dye method of the Association of Vitamin Chemists (1966). Pyridoxal and niacin were estimated colorimetrically by the methods of Bina *et al.* (1943) and Melnick & Field (1940), respectively, using the SP 500 spectrophotometer. The results are presented in Table 1.

RESULTS

The vitamins showed varying degrees of destruction during the traditional cooking of the different foods. The most affected were the B vitamins and ascorbic acid. Losses of vitamin A and beta-carotene varied between 11 and 43% during the cooking of the foods. Of the B vitamins, the most affected were B_1 and pyridoxal. The losses here varied between 29 and 48% for B_1 and 3·4 and 48·7% for pyridoxal. Riboflavin and niacin suffered moderate losses—between 1·2 and 30% and 5·9 and 30·3%, respectively. The losses of vitamin C varied between 40% and total destruction (100%) in some cases.

DISCUSSION

The vitamin contents of the various diets are shown in Table 1. The losses incurred might be due to some of the vitamins being thermolabile (Boas-Fixsen, 1938) and others being both thermolabile and water-soluble (Bender, 1966; Lee, 1958; Adam *et al.*, 1942). Losses of vitamin A and the pro-vitamin in cooked foods have already been reported by De (1936), Lanzing & Van Veen (1937), Maqsood *et al.* (1963) and Della Monica & McDowell (1965). The losses might be due to the high temperatures used and the presence of oxygen (Harris & Von Loesecke, 1960), and of traces of copper and iron (Bender, 1966) in the diets.

The losses of the B vitamins could in part be due to leaching into the washing water during the preparation of the vegetable prior to cooking. These results agree with those reported by Roscoe (1930), Munsell & Kifer (1932) and Langley *et al.* (1933). Actual destruction of the vitamins might also occur during cooking as a result of the possible change in the pH of the cooking medium, coupled with the

TABLE 1

VITAMINS IN THE RAW—MIXED AND COOKED DIETS

Diet		*Per 100 gm dry weight of diet*						
		Vitamin A (IU)	*Beta-carotene (IU)*	*Vitamin C (mg)*	*Vitamin B_1 (mg)*	*Vitamin B_2 (mg)*	*Niacin (mg)*	*Pyridoxal (mg)*
Gari with	Raw	104800·0 ± 125·1	22110·0 ± 111·3	24·63 ± 0·41	1·35 ± 0·11	1·11 ± 0·10	14·41 ± 0·50	15·52 ± 0·75
'afang' soup	Cooked	92955·0 ± 331	21125·0 ± 281·6	14·14 ± 0·01	0·95 ± 0·20	0·99 ± 0·06	10·05 ± 0·15	11·28 ± 0·08
'Anyang ekpang'	Raw	71650·0 ± 231·1	3099·0 ± 131·6	8·09 ± 0·01	0·39 ± 0·01	0·09 ± 0·00	3·40 ± 0·01	17·56 ± 0·83
with palmnut soup	Cooked	40990·0 ± 583·9	2483·1 ± 81·5	Trace	0·27 ± 0·03	0·08 ± 0·00	3·20 ± 0·01	9·00 ± 0·04
Cassava foofoo with	Raw	71387·0 ± 650·1	3904·3 ± 256·4	29·20 ± 1·17	0·50 ± 0·07	0·24 ± 0·02	6·87 ± 0·20	11·20 ± 0·98
'edikang ikong' soup	Cooked	55663·1 ± 128·0	3723·7 ± 135·1	17·53 ± 2·90	0·35 ± 0·04	0·18 ± 0·01	5·13 ± 0·20	9·20 ± 0·87
Pounded yam with	Raw	31385·8 ± 721·3	5111·4 ± 69·3	15·73 ± 0·05	0·65 ± 0·01	0·39 ± 0·04	10·23 ± 0·22	20·20 ± 0·09
'afia efere'	Cooked	23353·2 ± 430·0	4244·7 ± 83·0	5·12 ± 0·00	0·45 ± 0·01	0·27 ± 0·00	8·19 ± 0·11	19·51 ± 0·13
'Ekpang Nkukwo'	Raw	132930·0 ± 11·4	24823·5 ± 110·0	37·75 ± 0·43	2·35 ± 0·15	0·42 ± 0·01	9·34 ± 0·28	32·03 ± 0·43
	Cooked	109241·4 ± 266·7	22934·5 ± 222·2	12·50 ± 2·02	1·23 ± 0·07	0·33 ± 0·02	8·19 ± 0·11	29·46 ± 0·10
'Iwuk ukom'	Raw	90230·1 ± 762·1	9555·5 ± 385·0	14·90 ± 0·29	0·19 ± 0·01	0·19 ± 0·01	27·08 ± 0·43	29·98 ± 0·39
	Cooked	75127·0 ± 231·0	8888·7 ± 161·8	7·54 ± 1·02	0·12 ± 0·01	0·14 ± 0·01	22·27 ± 1·01	27·48 ± 0·27

high cooking temperature. Similar losses have been reported by Newton (1931) and by Woodring & Storvick (1960). The losses of vitamin C might be due to leaching, heat and oxygen (Bender, 1966; Boas-Fixsen, 1938), and to the presence of traces of copper and other metallic ions (Szent-Gyorgyi, 1928) in the diets.

The results, however, showed high contents of vitamin A and the pro-vitamin in the peasant diets even after significant cooking losses. This might be due to the high content of palm oil (*Elaeis guineensis*) in all the peasant diets. The area occupied by the Cross River State of Nigeria falls within the tropical rainforest zone where the oil palm trees are the most abundant economic trees. The high consumption of the palm oil probably accounts for the relatively low incidence of eye disease in this area.

There are, however, significantly low quantities of the B vitamins in all the diets. This situation was further worsened by the severe traditional processing and cooking methods which induced great losses of these vitamins. The low consumption of this group of vitamins, which serve mostly as co-enzymes, would lead to several nutritional disorders. Some clinical observations of diseased states have been reported in a number of areas within the State by Ekpo (1970). It is possible that in other areas supplementary intakes could be made through consumption of green vegetables and the abundant wild fruits and bush meat. Work is also in progress in our laboratory to estimate the blood levels of these nutrients in the peasants.

REFERENCES

ABAELU, A. (1973). Effects of Nigerian preparatory procedures on the thiamin, riboflavin and ascorbic acid content of foods. *W. Afr. J. Biol. & Appl. Chem.*, **16**, 24.

ADAM, W. B., HORNER, G. & STANWORTH, J. (1942). Changes occurring during the blanching of vegetables. *J. Soc. Chem. Ind. (Lond.)*, **61**, 96.

ASSOCIATION OF VITAMIN CHEMISTS (1966). *Methods of vitamin assay*. New York, Interscience Publishers.

BASSIR, O. (1963). In *Handbook of practical biochemistry*, 36–37. Ibadan, Nigeria, Ibadan University Press.

BASSIR, O. & UMOH, I. B. (1973). The nutritive adequacy of some Nigerian peasant diets. *Ecology of Food & Nutrition*, **2**, 297.

BENDER, A. E. (1966). Nutritional effects of food processing. *J. Fd Technol.*, **1**, 261.

BINA, A. F., THOMAS, J. M. & BROWN, E. B. (1943). The determination of vitamin B_6 (pyridoxine) in foods. *J. Biol. Chem.*, **148**, 111.

BOAS-FIXSEN, M. A. (1938). The vitamin content of human foods as affected by processes of cooking and canning. *Nutr. Abstr. & Revs*, **8**, 10.

CARR, F. H. & PRICE, E. A. (1926). Colour reactions attributed to vitamin A. *Biochem. J.*, **20**, 497.

DE, N. K. (1936). Factors affecting the carotene content of certain vegetables and foodstuffs. *Ind. J. Med. Res.*, **24**, 201.

DELLA MONICA, E. S. & MCDOWELL, P. E. (1965). Comparison of beta-carotene content of dried carrot prepared by three dehydration processes. *Food Technol. Campaign*, **19**, 1597.

EKPO, E. U. (1970). Food and health in relation to reconstruction programme in Nigeria (mimeo). Paper presented at the Annual Conference of the Nutrition Society of Nigeria held at the University of Ibadan, Ibadan, Nigeria. December, 1970.

FAO/UNICEF (1962). A draft report on the National Extension Seminar for the Federation of Nigeria. University of Ibadan, Ibadan, Nigeria.

HARRIS, R. E. & VON LOESECKE, H. (1960). In *Nutritional evaluation of food processing*. New York, Wiley.

HODSON, A. Z. & NORRIS, L. C. (1939). A fluorometric method for determining the riboflavine content of foodstuffs. *J. Biol. Chem.*, **131**, 621.

LANGLEY, D. D., RICHARDSON, J. E. & ANDES, E. J. (1933). The effect of storage and canning upon the vitamin content of carrots. *Montana Agric. Exp. Stat. Bull.*, No. 276, May.

LANZING, J. C. & VAN VEEN, A. G. (1937). Chemical composition of 'nasi tim'. *Geneesk. Tijdschr. Nederland. Indie*, **79**, 1705.

LEE, F. A. (1958). The blanching process. *Adv. Food. Res.*, **8**, 63.

MATHIESON, A. R. (1964). Protein chemistry and nutrition. *W. Afr. J. Biol. & Appl. Chem.*, **8**, 16.

MAQSOOD, A. S., HAGUE, S. A. & KHAN, A. H. (1963). Some dietary constituents and energy values of East and West Pakistan diets. *Pakistan J. Sci. Ind. Res.*, **6**, 119.

MELNICK, D. & FIELD, H., JR. (1940). Chemical determination of nicotinic acid. *J. Biol. Chem.*, **134**, 1.

MUNRO, A. & BASSIR, O. (1969). Oxalate in Nigerian vegetables. *W. Afr. J. Biol. & Appl. Chem.*, **12**, 14.

MUNSELL, H. E. & KIFER, H. B. (1932). Vitamin content of three varieties of spinach. *J. Agric. Res.*, **44**, 767.

NEWTON, C. L. (1931). Vitamin content of turnip greens, collards, cantaloupes and peaches. *Georgia Agric. Exp. Stat. Bull.*, **167**, 3.

OKE, O. L. (1965). Chemical studies on some Nigerian foodstuffs—'Lafun'. *W. Afr. J. Biol. & Appl. Chem.*, **8**, 53.

OKE, O. L. (1967). Chemical studies on some Nigerian pulses. *W. Afr. J. Biol. & Appl. Chem.*, **9**, 52.

OYENUGA, V. A. (1968). In *Nigerian foods and feedingstuffs: Their chemistry and nutritive value*, 3rd edn. Ibadan, Nigeria, Ibadan University Press.

ROSCOE, M. H. (1930). The distribution of the vitamin B complex. I. Leafy vegetables. *Biochem. J.*, **24**, 1754.

ROY, J. K. & RAO, R. K. (1963). Alkalinity of cooking water and stability of thiamine of rice. *Ind. J. Med. Res.*, **51**, 533.

SZENT-GYORGYI, A. (1928). Observations on the function of peroxidase systems and the chemistry of the adrenal cortex; a description of a new carbohydrate derivative. *Biochem. J.*, **22**, 1387.

UMOH, I. B. (1972). *Changes in the nutritive values of some Nigerian diets after cooking by certain South-Eastern Nigerian traditional methods*. Ph.D. Thesis, University of Ibadan, Ibadan, Nigeria.

WOODRING, M. J. & STORVICK, C. A. (1960). Vitamin B_6 in milk: Review of literature. *J. Assoc. Off. Agric. Chem.*, **43**, 63.

FLAVOUR EVALUATION BY MULTIPLE REGRESSION ANALYSIS OF GAS CHROMATOGRAPHIC PATTERNS IN SOY SAUCE

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ABSTRACT

The relationship between the gas chromatographic patterns and sensory tests on soy sauce aroma was investigated by modified multiple regression analysis. As a result good linear relationship between the two was observed on two different sample sets, with multiple correlation values of 0·949 *and* 0·966, *respectively. This means that the result of the sensory test on a sample can be estimated from the gas chromatographic pattern. Next, the contributing proportions were calculated on all the peaks of a gas chromatographic pattern. These values mean the quantification of the importance of each peak for the whole aroma. The calculation of the contributing proportion leads to the identification and evaluation of the components responsible for the whole aroma and accordingly the technique obtained here offers a first step toward general studies of food flavour.*

INTRODUCTION

In general, the flavour components of foods are very complicated, particularly in processed foods which are made by heating, fermentation, or ageing, etc. Any key compounds for aroma have not yet been found in these processed foods. So far, the characteristic aroma has been considered to appear as a result of the mutual interaction of many constituents. However, the authenticity of this idea has not been established because the original flavour may not be obtained by the reconstitution of each component in processed foods, as pointed out by Teranishi *et al.* (1971).

Most of the flavour investigations of foods in the past were those of the isolation and identification of the food components. The degree of the contribution of the resulting components for whole flavour was only assessed subjectively and qualitatively from its own flavour.

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The purpose of this paper is (1) to develop a method for evaluating the quality of a food flavour objectively from the gas chromatographic (GLC) patterns, (2) to determine quantitatively the degree of contribution of each GLC peak and (3) to find the standard combination of the components contributing to the characteristic flavour of the foods. Other purposes are: (1) to know the nature of the aroma as a basis of sensory evaluation by examining the accuracy of the evaluation and (2) to examine the influence of the number of constituents in samples to be compared at the same time as the sensory evaluation. For these purposes, the multiple regression analysis of the GLC patterns was examined.

Recently, statistical methods have been introduced into correlating GLC data with sensory evaluation. Stepwise discriminant analysis and two- or three-way classification discriminant analysis have been applied for potato chips by Powers & Keith (1968), for peanuts by Powers *et al.* (1971), for ginger oil by Bednarczyk & Kramer (1971) and for corn by Dravnieks *et al.* (1973), respectively. Further, Biggers *et al.* (1969) applied a new method in the flavour analysis of coffee. However, the multiple regression analysis applied for soy sauce by the authors should be more efficient for the present purpose from a logical point of view than the usual statistical methods described above, because much more information can be obtained by the former. Moreover, it is very significant that the present paper is the first report in which the degree of the contribution of each peak to aroma was determined quantitatively.

Soy sauce is used in the present paper as an example of a processed food. According to Goto (1973), over 120 kinds of aroma compound have been isolated and identified so far in soy sauce aroma, but no key compounds have been found. The statistical investigation of soy sauce flavour has been done by Saito & Tanaka (1967). They applied multiple regression analysis for non-volatile components, but did not succeed in obtaining a linear combination between the sensory test and non-volatile flavour components.

MATERIALS AND METHODS

Materials

Many different brands of two litre bottled soy sauce on the market were collected as samples in the sensory tests (from various areas in Japan). These samples can be divided into two types. One is purely fermented soy sauce and the other is a blended mixture of partly acid hydrolysed or acid hydrolysed soy sauce with a purely fermented one. The former is much better in quality than the latter.

Sensory tests

Sensory test 1: Sixty-one brands of soy sauce were used as sensory test samples. These samples consisted of purely fermented, partly acid hydrolysed and acid hydrolysed ones. The sensory test was carried out at the same time on sixty-one of these samples by the sensory test panel which consisted of 233 well trained members

of the Kikkoman Shoyu Co., Ltd. Samples were evaluated by the following standard; Extremely good, 5; Good, 4; Common, 3; Bad, 2; and Extremely bad, 1. The total of the score evaluated by each member for each item was used as a sensory score for each sample in sensory test 1.

Sensory test 2: Ten well-known brands of soy sauce (designated A, B, C, D, E, F, G, H, I, and J), were used as the samples in this test. These samples were all purely fermented ones and were divided into two groups which consisted of the group: A, B, C, D, E, and J, and the group: F, G, H, I, J, and E. The samples were collected monthly from the market and each group was evaluated by a test panel which consisted of 150 members selected arbitrarily from the members of the panel who took part in sensory test 1. The sensory test was carried out monthly and repeated five times in total on each group. On evaluation, each sample was numbered in order of preference and the total of the resulting numbers was divided by the number of the panel members. The value thus obtained, which was the mean order of preference, was used as the sensory score for each sample in sensory test 2.

Preparation of aroma concentrate and GLC analysis

Since the main purpose of this paper is to study a relationship between the sensory scores and aroma constituents in GLC analysis, it is essential to separate the aroma concentrate for GLC analysis of soy sauce, so that the aroma may be unchanged and kept in the original state before and after the separation. The aroma kept the original state organoleptically best, when the separation was carried out by the following process. Namely, soy sauce aroma was trapped in the two traps cooled by ice-water and dry ice-acetone, after being distilled at 45 °C under 15 mm Hg. The trapped distillates were mixed and then 20 ml of the mixture were extracted with 5 ml of dichloromethane. The resulting extract was treated by Whatman 1-PS in order to remove the trace of water, concentrated under a reduced pressure to an appropriate concentration, and then applied for GLC analysis under the conditions shown in Fig. 1. The gas chromatograms were recorded by JEOL JR-252A with a two pen recorder and the ratio of sensitivities for the two pens was settled as one to ten.

Multiple regression analysis

The general concept of pattern analysis is shown schematically in Fig. 2. In Fig. 2, when n different samples of soy sauce are tested organoleptically by a test panel, the resulting sensory score $Y(y_1, y_2, \ldots, y_n)$, of each sample is expressed as Matrix A. On the other hand, when these samples are analysed quantitatively by gas chromatography and the resulting chromatogram consists of m different peaks, the amount of each peak in each sample is expressed as Matrix B. The pattern analysis of the chromatograms is no other than connecting Matrix A and B with $Y = f(X)$. Multiple regression analysis is one of the pattern analysis methods and its model is generally expressed in eqn. (1):

$$Y = \alpha_1 X_1 + \alpha_2 X_2 + \cdots + \alpha_i X_i + \cdots + \alpha_m X_m + \beta \qquad (1)$$

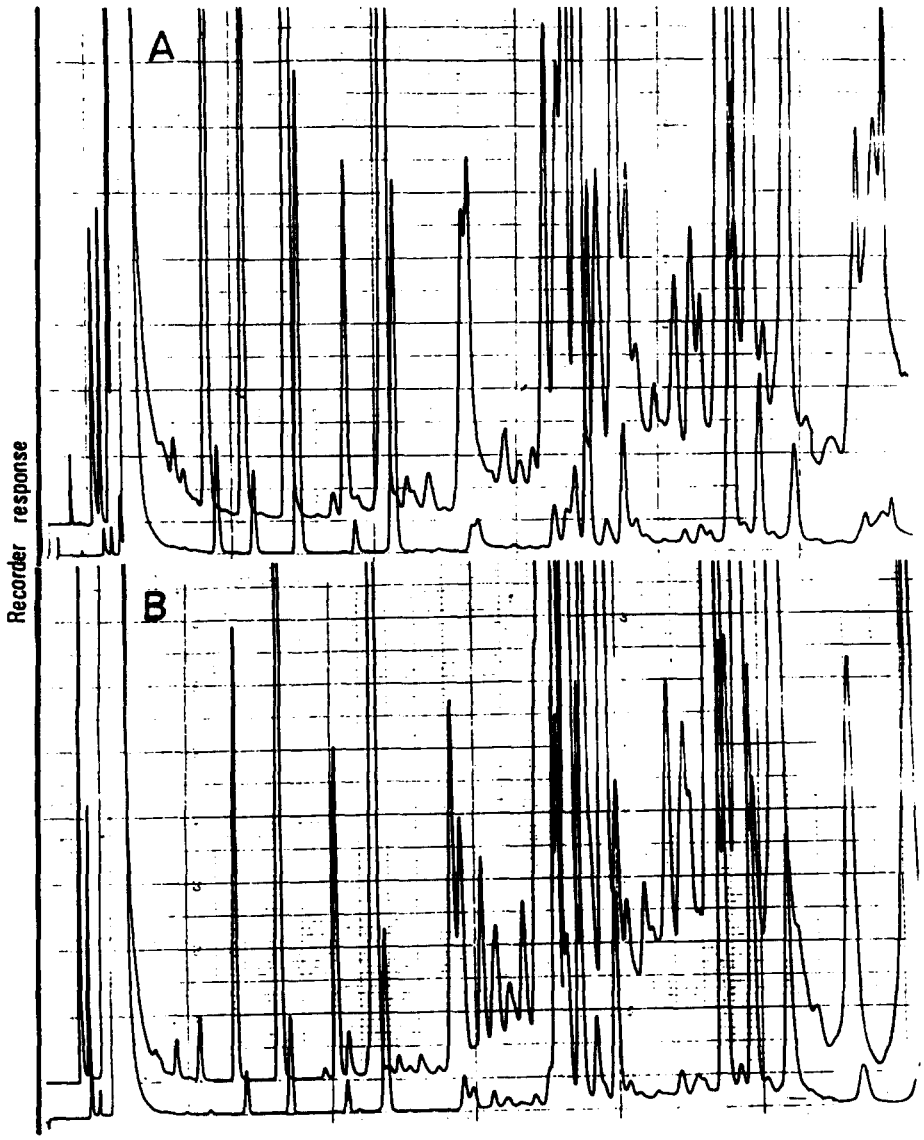

Fig. 1. Gas chromatograms of soy sauce flavour. Numbers in chromatogram D show selected peaks as independent variables. Conditions, column (glass): 20% PEG 20 M on Chromsorb W-AW-DMCS 3 mm × 2 m. Oven temperature: 50 – 200 °C. 3 °C/min., injection temperature: 240 °C, carrier gas: N_2. 40 ml/min., instrument No. JEOL JGC-1100. See text for designations.

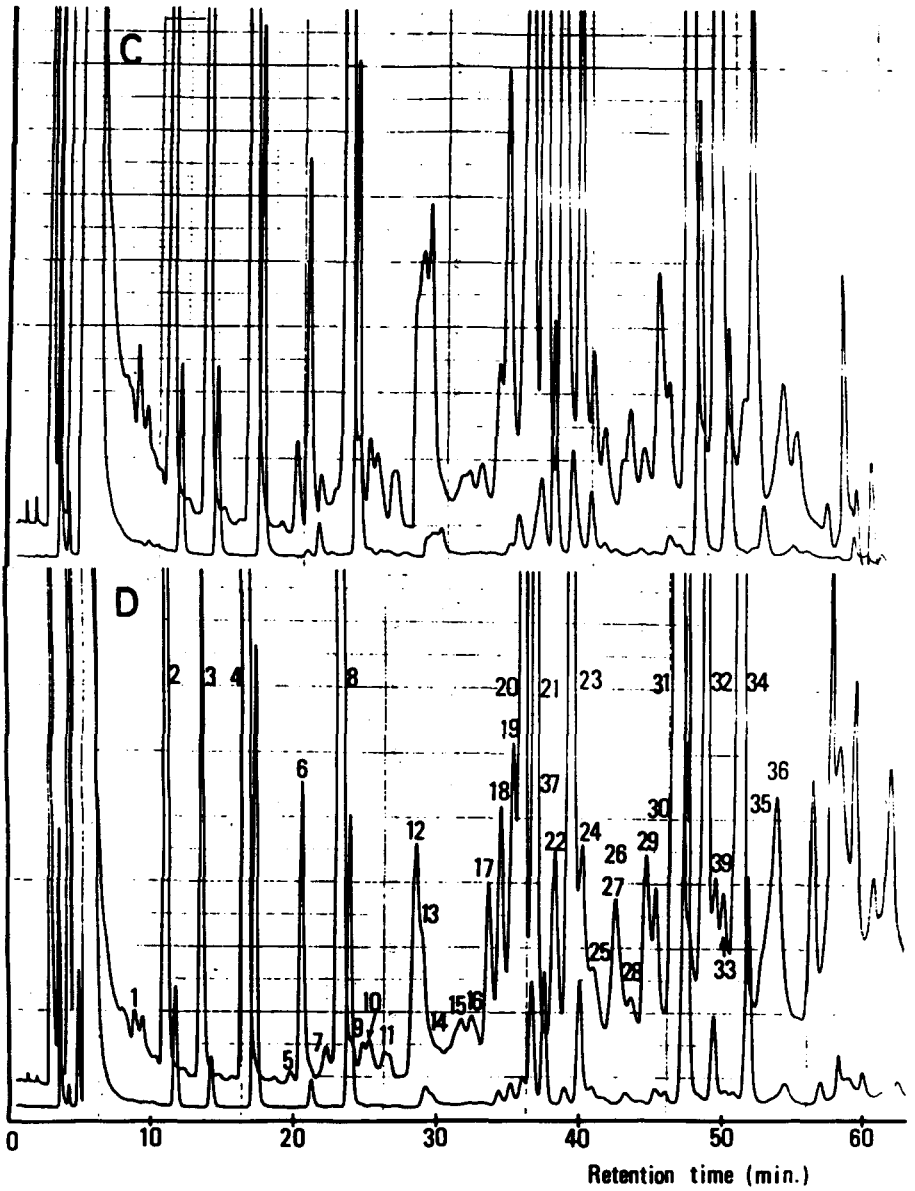
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Fig. 1—*contd.*

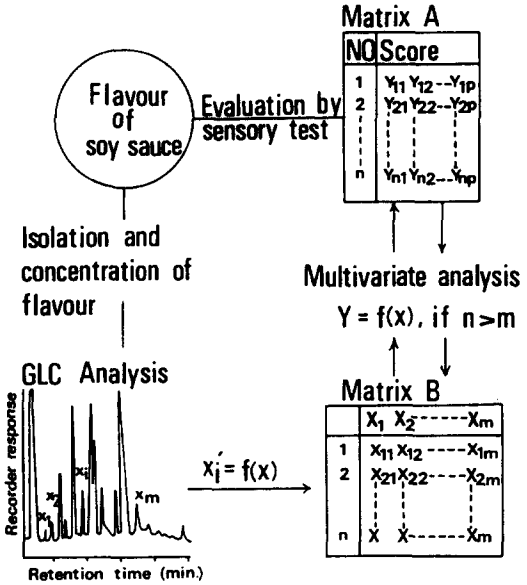

Fig. 2. A scheme for basic conception of GLC pattern analysis.

In this equation, X_i is expressed as percent of each peak height (h_i) to the total peak height of the aroma components, as shown in eqn. (2):

$$X_i = \frac{h_i}{\sum h_i} \times 100 \tag{2}$$

The reason why the absolute height was not used in X_i is that the mutual relationship of each aroma component is considered to be more important than the absolute amount of its aroma.

In eqn. (1), the dependent variables, $Y = (y_1, y_2, \ldots, y_n)$, are the sensory scores and the independent variables, $X_i = (x_{i1}, x_{i2}, \ldots, x_{im})$, are percent of the height of the ith peak for each sample of 1, 2, ..., n. When the necessary conditions for the multiple regression analysis, that is, $n > m$, are satisfied, α_i and β in the eqn. (1) can be calculated using the least squares method. These necessary conditions are satisfied in the samples of both sensory test 1 and sensory test 2, that is, $m = 39$ and $n = 61$ in the former and $m = 39$ and $n = 60$ in the latter.

Contributing proportion

The correlation coefficient between the input Y and the estimated Y, calculated by substituting the input X_i for the computed multiple regression model, is designated as multiple correlation coefficient (R). $R^2 \times 100$ is called a contributing proportion. This value means the proportion which can be explained by Y based on the multiple

regression model, to the total variation of Y. When the contributing proportion of each independent variable is P_i, the sum of P_i is equal to $R^2 \times 100$. According to Barylko-Pikielna & Metelski (1964), P_i can be calculated according to eqn. (3) where α_i is the regression coefficient, s_i the standard deviation of each independent variable, and r_{iy} the correlation coefficient between X_i and Y.

$$P_i = \frac{\alpha_i s_i r_{iy}}{\sum \alpha_i s_i r_{iy}} \times 100 R^2 \tag{3}$$

RESULTS AND DISCUSSION

Gas chromatograms and sensory scores

The GLC patterns of soy sauce are shown in Fig. 1. Chromatogram A is that of the purely fermented soy sauce, which was most liked by the panel in sensory test 1. Chromatogram B is that of the blended mixture of partly acid hydrolysed soy sauce with a purely fermented one. This chromatogram is characterised by the very high value of the peak 19 in comparison with that of the purely fermented soy sauce. Chromatograms C and D are those of purely fermented ones in sensory test 2. The former is of the most liked soy sauce, whereas the latter is one which is rather disliked by the sensory test panel.

The sensory score of the most liked soy sauce was 965, while that of the most disliked was 424, in sensory test 1. In sensory test 2, however, the sensory score of the most liked soy sauce was 1·80 and that of the most disliked 5·52. As shown in Fig. 3, it is apparent that the purely fermented soy sauce was much preferred to the blended mixtures of partly acid hydrolysed or acid hydrolysed soy sauce.

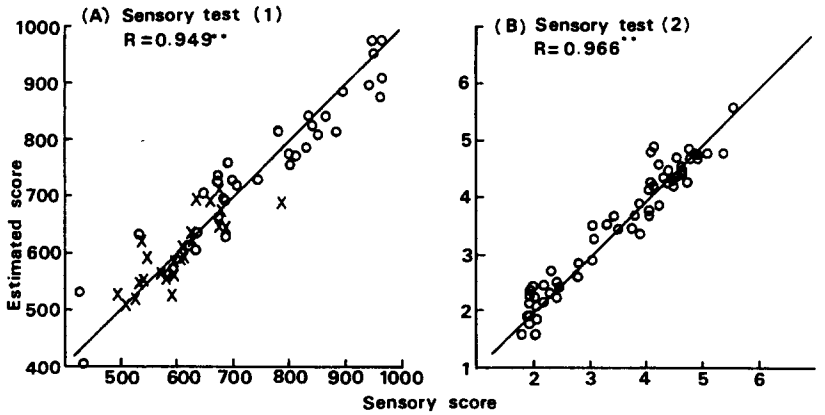

Fig. 3. Estimated score versus sensory score. Estimated scores are calculated according to the regression models shown in Fig. 5. ○: Purely fermented soy sauce; × : Acid hydrolysed or partly acid hydrolysed soy sauce. **: $P < 0{\cdot}01$.

TABLE 1
CORRELATION COEFFICIENTS OF PEAK QUANTITY (X_i) VS SENSORY SCORES (Y)

(A) Sensory test (1)

Peak No.	*r*	*Peak No.*	*r*	*Peak No.*	*r*
1	−0·116	14	−0·332**	27	−0·011
2	0·465**	15	−0·299*	28	−0·037
3	−0·140	16	−0·074	29	0·293*
4	0·553**	17	−0·002	30	0·217
5	0·115	18	0·463**	31	0·267*
6	0·470**	19	−0·465**	32	0·371**
7	0·073	20	0·275*	33	0·040
8	0·491**	21	0·434**	34	0·207
9	0·123	22	−0·154	35	−0·222
10	0·191	23	0·195	36	−0·027
11	0·095	24	−0·083	37	0·452**
12	−0·033	25	−0·256*	38	−0·060
13	0·235	26	0·150	39	−0·483**

(B) Sensory test (2)

Peak No.	*r*	*Peak No.*	*r*	*Peak No.*	*r*
1	0·068	14	0·052	27	0·105
2	−0·466**	15	0·116	28	0·184
3	−0·710**	16	0·055	29	0·052
4	−0·473**	17	0·220	30	0·269*
5	0·258*	18	−0·359**	31	0·044
6	0·016	19	0·362**	32	0·166
7	0·071	20	0·488**	33	−0·018
8	−0·538**	21	0·172	34	0·146
9	−0·290**	22	0·214	35	0·142
10	−0·574**	23	0·228	36	0·027
11	0·031	24	0·199	37	−0·568**
12	0·084	25	0·188	38	0·233
13	0·099	26	0·037	39	0·272*

*: $P < 0·05$. **: $P < 0·01$.

Correlation coefficient of each peak

The correlation coefficients of 39 peaks of the chromatograms and the sensory scores of the samples by sensory tests 1 and 2 are shown in Table 1. In the case of sensory test 1, only peak 4 showed a high correlation coefficient (0·5). In the case of sensory test 2, however, there were several peaks showing high correlation coefficients. The highest value was over 0·7 in peak 3. These peaks are all located at a region of low temperature (below 140 °C in the gas chromatograms).

The relationship between peak percent and sensory scores is shown in Fig. 4 for the three peaks having significant correlation coefficients in sensory tests 1 and 2. It is apparent that the quality of the aroma of soy sauce cannot be discriminated by only

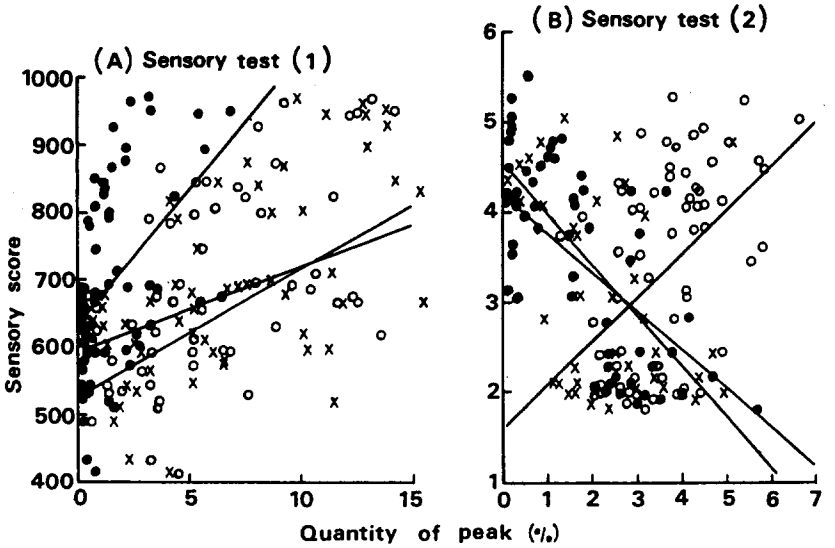

Fig. 4. Sensory scores versus quantities of peaks: (A) ●: Peak 2, $r = 0{\cdot}465^{**}$, $Y = 38{\cdot}90\,X_2 + 642{\cdot}39$. ○: Peak 4, $r = 0{\cdot}553^{**}$, $Y = 19{\cdot}12\,X_4 + 525{\cdot}50$. ×: Peak 8, $r = 0{\cdot}491^{**}$, $Y = 11{\cdot}87\,X_8 + 598{\cdot}77$. (B) ●: Peak 3, $r = -0{\cdot}710^{**}$, $Y = 0{\cdot}573\,X_3 + 4{\cdot}51$. ○: Peak 20, $r = 0{\cdot}508^{**}$, $Y = 0{\cdot}508\,X_{20} + 1{\cdot}57$. ×: Peak 37, $r = -0{\cdot}568^{**}$, $Y = -0{\cdot}415\,X_{37} + 4{\cdot}121$. **: $P < 0{\cdot}01$.

one or two peaks. This suggests that the quality of soy sauce would be discriminated as the result of integration of many gas chromatographic peaks. Therefore, we proceeded to investigate the multiple regression analysis.

Multiple regression models

The multiple regression models, computed on the basis of the samples in sensory tests 1 and 2, are shown in Fig. 5. The multiple correlation (R) and the F-value from the analysis of variance for the regression were highly significant statistically, indicating that there is a linear combination between the sensory scores and the GLC patterns of soy sauce. Further, the values of contributing proportion were over 90% and 93% on the samples in sensory tests 1 and 2, respectively. This means that over 90% and 93% of the sensory scores on the samples by sensory tests 1 and 2 could be explained from the peaks of the gas chromatograms by using the multiple regression models shown in Fig. 5.

Estimation by multiple regression model

The fact that the computed multiple regression models shown in Fig. 5 were highly significant statistically suggests the possibility of estimating the quality of unknown samples of soy sauce from GLC patterns by using these multiple regression models.

(A) *Sensory test* 1

$$Y = -29{\cdot}486X_1 - 35{\cdot}212X_2 - 41{\cdot}340X_3 - 40{\cdot}869X_4 + 139{\cdot}212X_5 + 148{\cdot}735X_6 - 385{\cdot}576X_7 - 37{\cdot}002X_8 - 269{\cdot}671X_9 + 41{\cdot}033X_{10} - 56{\cdot}268X_{11} - 97{\cdot}721X_{12} + 156{\cdot}322X_{13} - 50{\cdot}672X_{14} - 242{\cdot}231X_{15} - 145{\cdot}749X_{16} - 11{\cdot}676X_{17} - 60{\cdot}771X_{18} - 41{\cdot}104X_{19} - 66{\cdot}839X_{20} - 43{\cdot}947X_{21} - 40{\cdot}044X_{22} - 55{\cdot}329X_{23} - 64{\cdot}152X_{24} - 159{\cdot}552X_{25} + 42{\cdot}081X_{26} + 92{\cdot}221X_{27} + 121{\cdot}495X_{28} - 145{\cdot}107X_{29} - 16{\cdot}739X_{30} - 36{\cdot}368X_{31} - 26{\cdot}083X_{32} + 0{\cdot}827X_{33} - 45{\cdot}272X_{34} - 125{\cdot}424X_{35} - 102{\cdot}354X_{36} + 81{\cdot}715X_{37} - 84{\cdot}449X_{38} - 3{\cdot}130X_{39} + 4725{\cdot}547$$

(*B*) *Sensory test* 2

$$Y = -0{\cdot}665X_1 - 0{\cdot}117X_2 - 0{\cdot}351X_3 - 0{\cdot}033X_4 + 3{\cdot}308X_5 + 1{\cdot}060X_6 - 0{\cdot}011X_7 - 0{\cdot}158X_8 + 0{\cdot}365X_9 - 0{\cdot}829X_{10} - 1{\cdot}782X_{11} - 0{\cdot}354X_{12} - 0{\cdot}033X_{13} - 0{\cdot}566X_{14} + 0{\cdot}299X_{15} + 0{\cdot}734X_{16} + 0{\cdot}687X_{17} - 0{\cdot}254X_{18} + 0{\cdot}013X_{19} - 0{\cdot}069X_{20} - 0{\cdot}055X_{21} + 0{\cdot}719X_{22} + 0{\cdot}295X_{23} + 0{\cdot}359X_{24} + 0{\cdot}194X_{25} - 0{\cdot}630X_{26} + 1{\cdot}103X_{27} + 0{\cdot}788X_{28} - 0{\cdot}999X_{29} - 0{\cdot}533X_{30} - 0{\cdot}035X_{31} + 0{\cdot}038X_{32} + 0{\cdot}194X_{33} + 0{\cdot}053X_{34} + 0{\cdot}335X_{35} - 0{\cdot}015X_{36} - 0{\cdot}084X_{37} - 0{\cdot}734X_{38} + 0{\cdot}069X_{39} + 5{\cdot}740$$

	(A)	(B)
Multiple correlation (R)	0·949	0·966
Contributing proportion ($R^2 \times 100$)	90·01	93·25
Analysis of the variance for the regression (F)	4·853**	7·088**

Fig. 5. Computed multiple regression models for each sensory test. **: $P < 0{\cdot}01$.

Thirty-nine of the gas chromatographic peaks were used as independent variables in the models described previously. In order to confirm whether all the essential peaks were selected as independent variables, the residuals (e_a) defined by the equation (4), were examined:

$$e_a = Y_{\text{observed}} - Y_{\text{estimated}} \tag{4}$$

As shown in Fig. 6, the distribution of the residuals was completely random, indicating that there was no lack of necessary independent variables in these models.

Next, the sensory scores were estimated from the chromatograms of the samples in sensory tests 1 and 2 using the computed multiple regression models shown in Fig. 5. The relationship between the resulting sensory scores and the real sensory scores is shown in Fig. 3 (A) and (B). There were high correlations between the two, but it is apparent that the accuracy of the estimation is higher in the samples of sensory test 2 than in those of sensory test 1, as shown in the comparison with the distribution around the regression line in Fig. 3 (A) and (B). As a matter of course, it was additionally observed that the values of the sensory scores for fermented soy sauce are much better than those for partly acid hydrolysed and acid hydrolysed soy sauce in both the estimated and the real values.

Next, these multiple regression models, based on the samples of sensory tests 1 and 2, were applied to the estimation of the sensory scores of unknown samples of soy sauce. Unknown samples used here were all purely fermented ones, composed of ten different brands (A, B, C, D, E, F, G, H, I, and J). First, the aroma of each sample was analysed by gas chromatography and then the sensory test was carried out as follows. The samples were divided into two groups. One consisted of A, B, C, D, E,

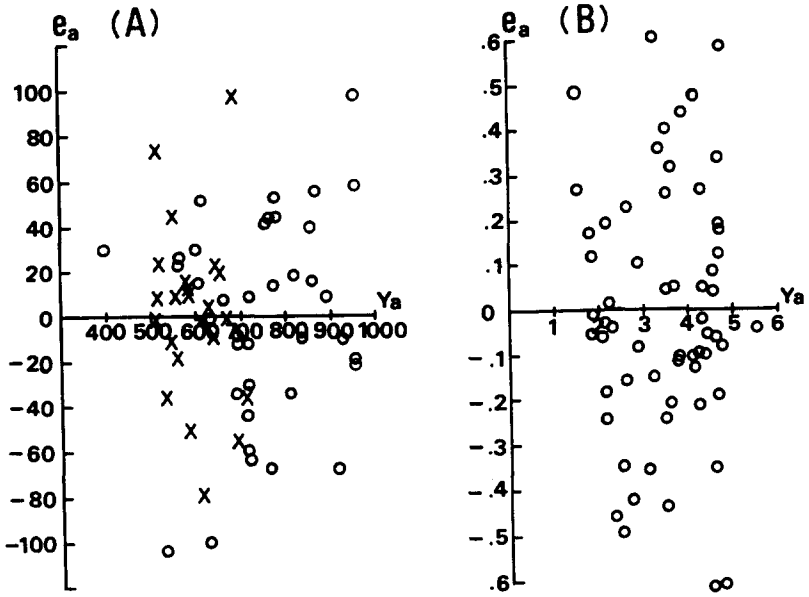

Fig. 6. Residuals plotted against estimated scores. Sensory test 1(A) Sensory test 2(B) ○: Purely fermented soy sauce. ×: Acid hydrolysed or partly acid hydrolysed soy sauce.

and J, and the other, F, G, H, I, J, and E. The sensory tests were carried out separately on each group. The evaluation was performed by numbering the order of preference. The results are shown in Fig. 7, in which the estimated orders derived from the sensory scores are plotted against the real sensory orders. According to Fig. 7 (B), high significance was observed between the real scores and the estimated scores which were calculated by the multiple regression model based on the samples of sensory test 2. The samples tested here and the samples in sensory test 2 are both of the purely fermented type which have a similar nature of aroma. Therefore, when the sensory tests on both samples are carried out on the same basis of evaluation by the sensory test panel, the high accuracy of the estimation observed in Fig. 7 (B) means that the gas chromatographic patterns of the soy sauce are recognised in the multiple regression model shown in Fig. 5 (B). On the other hand, there was no significance in the correlation coefficients between the real scores and the estimated ones which were calculated by the multiple regression model based on the samples in sensory test 1, which contain partly acid hydrolysed and acid hydrolysed soy sauce, as seen in Fig. 7 (A). In this case, however, it is very interesting that the samples were divided into the two groups of good preference and bad preference, consisting of five and seven samples, respectively. The brands belonging to the former are generally ranked as good, while those belonging to the latter are generally ranked as bad, in the usual

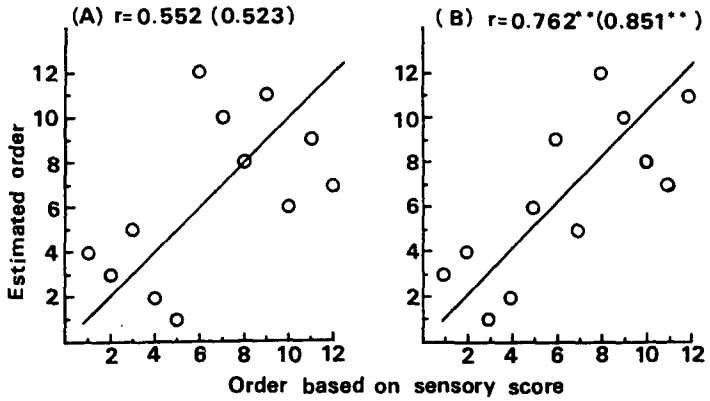

Fig. 7. Estimated orders versus evaluated orders. Estimated orders are calculated according to the regression models as shown in Fig. 5, (A) is based on the regression model of sensory test 1 and (B) is based on that of sensory test 2. Numbers in parentheses show the correlation coefficients of estimated scores and sensory scores. **: $P < 0.01$.

sensory test. In sensory test 1, the test panel members seem to have evaluated these samples simply into the categories of good and bad.

Contributing proportions of each peak as independent variables

Since the sensory scores were estimated with a high accuracy on the basis of multiple regression models, the contributing proportion of each variable was as the next step calculated from eqn. (3). The results are shown in Table 2. It is obvious that the contributing proportion of each peak in the samples of sensory test 1 differs from that of sensory test 2. In the former, peak 19 showed the very large value of contributing proportion of 36·6%, followed by peaks 4, 21, and 31 having 6·75, 4·53 and 6·33%, respectively, whereas, in the latter, there were the two major peaks having the contributing proportions of 17·63 and 12·50%, followed by many peaks having small values of several percent.

Peak 19 is a characteristic peak seen in only partly acid hydrolysed or acid hydrolysed soy sauce and sometimes attains to 80% of the whole aroma quantities of the chromatogram of partly acid hydrolysed or acid hydrolysed soy sauce. From the fact that the contributing proportion of such a peak reached 36·6%, it is assumed that the discrimination of the partly acid hydrolysed or acid hydrolysed soy sauce, by its strong characteristic odour, preceded that of the purely fermented soy sauce and as a result the discrimination of quality among purely fermented soy sauce samples, which are judged from the other standard, became rather rough, resulting in the low accuracy for the estimation of the sensory scores of the samples in sensory test 1, as shown in Fig. 3 (A). This assumption, that the evaluation is largely influenced by the simple standards good or bad, is also supported by the following facts: (1) the sensory test was performed at the same time on the 61 samples and (2) the sensory scores for

TABLE 2
CONTRIBUTING PROPORTION (P_i) OF EACH PEAK
(A) Sensory test (1)

Peak No.	P_i (%)	*Peak No.*	P_i (%)	*Peak No.*	P_i (%)
1	0·06	14	0·43	27	0·02
2	1·95	15	1·48	28	0·10
3	2·94	16	0·22	29	1·60
4	6·75	17	0·00	30	0·08
5	0·11	18	1·51	31	6·33
6	1·71	19	36·61	32	3·16
7	0·23	20	2·48	33	0·00
8	7·50	21	4·53	34	2·37
9	0·28	22	0·62	35	0·70
10	0·13	23	1·20	36	0·10
11	0·30	24	0·30	37	1·36
12	0·13	25	0·13	38	0·13
13	1·48	26	0·13	39	0·05

(B) Sensory test (2)

Peak No.	P_i (%)	*Peak No.*	P_i (%)	*Peak No.*	P_i (%)
1	0·05	14	0·27	27	1·15
2	4·42	15	0·43	28	1·99
3	17·63	16	0·33	29	1·15
4	2·73	17	2·94	30	2·68
5	6·35	18	3·13	31	0·43
6	0·32	19	1·56	32	1·45
7	0·02	20	1·87	33	0·06
8	12·50	21	1·32	34	1·05
9	0·99	22	3·59	35	1·33
10	4·23	23	3·79	36	0·02
11	1·13	24	2·05	37	3·74
12	1·11	25	0·83	38	2·92
13	0·09	26	0·37	39	0·42

the estimation of the purely fermented soy sauce were divided into two groups of good and bad, as shown in Fig. 7 (A).

On the other hand, there are no peaks which have a specially large contributing proportion, but there exist many peaks having low contributing proportions, in the samples of sensory test 2. In this case, it is assumed that precise evaluation by the standard based on many components was performed by the sensory test panel members, resulting in the high accuracy of the estimation shown in Fig. 3 (B). This assumption, based on the contributing proportions, is further supported by the following facts: (1) only six samples were evaluated at the same time in the sensory test and (2) the samples did not contain the partly acid hydrolysed or acid hydrolysed soy sauce which has a strong characteristic aroma of its own.

However, the difference of quantification between sensory tests 1 and 2 should

also be considered as a cause of the different accuracies between the two. Test 1 was carried out by the five point scaling method, whereas test (2) was done by the ordering method, because (i) it is questionable whether the preference of aroma can be expressed quantitatively by a five point scaling and (ii) the samples which are evaluated as the same and accordingly not discriminated by a five point scaling method can be discriminated by an ordering method. It is impossible, however, to evaluate the sixty-one samples at the same time by an ordering method, because of the limitation of discrimination through exhaustion. Therefore, some improvement of the sensory method would be needed for increase of accuracy in sensory test 1. In addition, the results prove that the accuracy of the sensory test is greatly influenced by the numbers to be tested at the same time. In particular, the sensory scores of the samples in small groups of ten, which contain partly acid hydrolysed and acid hydrolysed soy sauce (other than purely fermented soy sauce) were estimated with good accuracies by the multiple regression model in sensory test 2, but they could not be estimated significantly by the multiple regression model based on sensory test 1, in which sample numbers as large as sixty-one were tested at the same time, in spite of similar constitution of the samples.

The contributing proportions, the quantities, and the locations of the GLC peaks of the representative samples in sensory tests 1 and 2 are shown in Fig. 8. In the samples of sensory test 1, the peaks which show high contributing proportions are located uniformly through all temperature regions and most of these peaks are

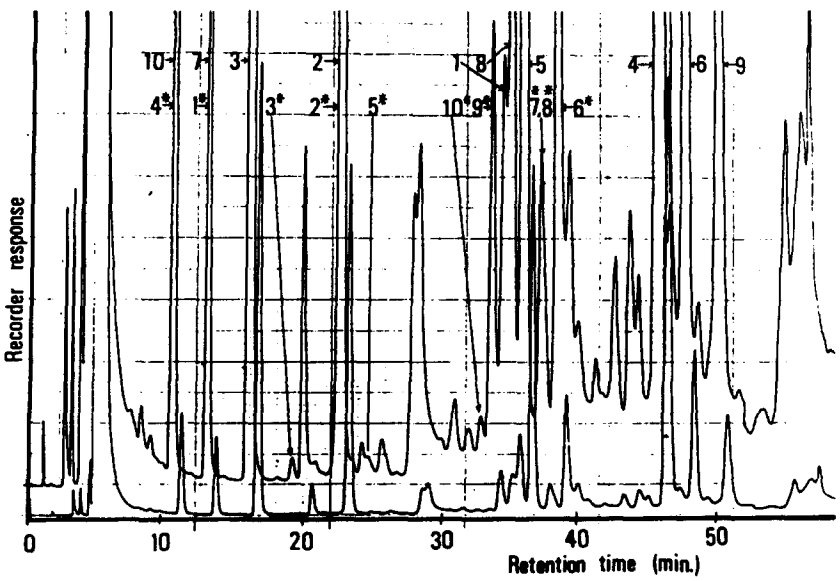

Fig. 8. GLC Peaks of high contributing proportion for sensory test 1 and sensory test 2 (*). Numbers in this figure show the order of magnitude of contributing proportion.

comparatively large. In the samples of sensory test 2, on the other hand, most of the peaks which have high contributing proportions are present at the low temperature regions and the small peaks such as peaks 3, 5 and 10 show high contributing proportions. It is very important that this is the first report in which the contributing proportion of each GLC peak for a food aroma is determined quantitatively from the multiple regression model. Hitherto, the selection of peaks responsible for the whole aroma from many GLC peaks largely consisted of trial and error. By calculating the contributing proportions, however, we can know systematically not only the kinds of peaks responsible for whole aroma, but also the degree of the contribution to whole aroma. Upon this basis, we can further proceed to elucidate the role and meaning of each peak. It sometimes occurs that an unexpected component plays an important role in whole food flavour. The determination of the contributing proportion is particularly useful in such a case. Thus, the analysis of the GLC patterns by multiple regression analysis and the determination of the contributing proportions enable us to achieve systematic improvements of food flavour as well as standardisation.

ACKNOWLEDGEMENTS

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REFERENCES

BARYLKO-PIKIELNA, N. & METELSKI, K. (1964). Determination of contribution coefficients in sensory scoring of over-all quality., *J. Food Sci.*, **29**, 109–11.

BEDNARCZYK, A. A. & KRAMER, A. (1971). Practical approach to flavour development., *Food Technol.*, **25**, 1098–107.

BIGGERS, R. E., HILTON, J. J. & GIANTURCO, M. A. (1969). Differentiation of *Coffea arabica* and *Coffea robusta* by computer evaluation of gas chromatographic profiles—Comparison of numerically derived quality predictions with organoleptic evaluations., *J. Chromatogr. Sci.*, **7**, 453–72.

DRAVNIEKS, A., REILICH, H. G. & WHITFIELD, J. (1973). Classification of corn odour by statistical analysis of gas chromatographic patterns of head space volatiles., *J. Food Sci.*, **38**, 34–9.

GOTO, T. (1973). Constituents and composition of aroma concentrate from soy sauce (Part 1)., *J. Jap. Soc. Food and Nutr.*, **26**, 135–8.

POWERS, J. J., COX, R. J., ELLAND, M. C. & ACKROYD-KELLY, P. (1971). Exposure of peanuts and peanut volatiles to light. Influence on gas chromatographic profile., *The Flavour Industry*, **2**, 87–92.

POWERS, J. J. & KEITH, E. S. (1968). Stepwise discriminant analysis of gas chromatographic data as an aid in classifying the flavour quality of foods., *J. Food Sci.*, **33**, 207–12.

SAITO, N. & TANAKA, T. (1967). Studies on sensory evaluation of soy sauce (I), *J. Ferment. Technol.*, **45**, 246–53.

TERANISHI, R., HORNSTEIN, R., ISSENBERG, P. & WICK, E. L. (1971). *Flavour research*, 16–35, New York, Marcel Dekker, Inc.

EFFECT OF MATURITY ON SOME BIOCHEMICAL CHANGES DURING RIPENING OF BANANA (*Musa sapientum* L. c. v. Lakatan)

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ABSTRACT

The starch, total sugars, titratable acids and total soluble solids of 'Lakatan' bananas picked at two different stages of maturity were determined during ripening at 23·3°C. Fruits picked at maturity stage A (between 'full' and 'full three-quarters') showed parallel trends in biochemical changes with those picked at maturity stage B (between 'full three-quarters' and 'light full three-quarters'). However, stage A fruits ripened sooner than stage B fruits. The former required 13 days to ripen, whereas the latter took more than twice as long (29 days).

Starch increased to a peak at the initial stages of ripening but decreased thereafter. Titratable acids increased, accompanied by a rapid decrease in starch, before the eating-ripe stage was reached. Total sugars and total soluble solids remained low until the final stages of ripening, when a rapid increase in these constituents was observed.

Based on the sugar-to-acid ratio, the quality of the ripened fruits was comparable.

INTRODUCTION

Bananas are grown extensively in The Philippines and are available throughout the year. Banana demand for local consumption and as an export crop is substantial. Owing to its perishable nature, the industrial value of the crop is not fully attained. The search for methods of prolonging the storage life of bananas has been the object of several studies (Murata *et al.*, 1967; Smock, 1967; Smock *et al.*, 1967).

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The perishability of bananas is the result of various physical and chemical changes which accompany the ripening process. An understanding of these changes is therefore of the utmost importance in the promotion of the industrial value of the fruit to its maximum.

In commercial practice, bananas are never allowed to ripen on the plant. They are harvested at a certain development stage and allowed to ripen in storage. It is known that the developmental stage at harvest influences, among other factors, the length of time required for subsequent ripening and the quality of the ripened fruits. Mendoza (1967) has shown that maturity has markedly affected the respiration and ripening rates of 'Lakatan' bananas. No data, however, are available on the biochemical development of the locally-grown fruit.

This study was conducted to determine (i) some biochemical changes during the ripening of 'Lakatan' bananas and (ii) how ripening is influenced by the maturity of the fruit at harvest. A somewhat similar study on 'Gross Michel' bananas has given important and useful results. Barnell (1940, 1941*a*, 1941*b*, 1943), in a series of studies on 'Gross Michel' bananas, found that the starch content of the fruits decreased as they ripened, while the total sugars increased and rose to a peak at the eating-ripe stage.

Initial studies on the biochemistry of banana indicated two distinct periods in the growth of the fruit: first, that of formation of starch when the fruit fixes its starchy reserve at the expense of reducing sugars and, second, that of maturation when the starch reserve is being transformed into soluble sugars and sucrose and the latter hydrolysed to invert sugar (Straton & Von Loesecke, 1930).

MATERIALS AND METHODS

The fruits in this study were of the local 'Lakatan' variety. The bunches were cut at two stages of maturity: stage A denotes maturity between 'full' and 'full three-quarters' and stage B maturity between 'full three-quarters' and 'light full three-quarters'. The stages of maturity were judged on the basis of 'fullness of fingers', as described by Von Loesecke (1950).

Sampling

Thirty-nine fruits from stage A and 42 fruits from stage B were each randomly divided into three lots. One fruit from each lot was immediately taken for analysis and the rest stored at 23·3°C after dipping in 5% phenolate solution for five minutes. Samples were analysed at certain time intervals until the eating-ripe stage was reached.

Sample preparation

The pulp of the fruits for analysis was separated from the skin and cut into cubes.

Part of the sample was placed in a polyethylene bag, sealed tightly and kept in the freezer at 0°C to permit later analysis. The remainder of each sample was used for analysis.

Analyses

The following determinations were performed periodically on each sample: titratable acidity, total soluble solids, starch and total sugars.

Titratable acidity was determined by titration with a 0·1N NaOH solution using a Coleman Metrion IV pH meter (AOAC, 1965). Total soluble solids were determined on the fruit slurry by reading the percent total solids directly from a hand refractometer. The starch and sugar contents were analysed using the method described by Soule & Harding (1956).

RESULTS AND DISCUSSION

The changes in starch, total sugars, titratable acids and total soluble solids during ripening at 23·3°C of 'Lakatan' bananas harvested at two different stages of maturity are shown in Figs 1 and 2. A significant increase in starch content was observed up to 3 and 9 days for stages A and B, respectively. The increase in starch during the early stages of ripening suggests a possible resynthesis from some temporary unestimated reserve, as was observed in 'Gross Michel' bananas under cold storage (Barnell, 1941*b*). A rapid decline in starch was observed for both stages after their respective peaks were reached.

Bananas from stage A and stage B showed only slight increases in sugar content 8 and 21 days after harvest, respectively, but such increases became rapid thereafter. The increase in total sugars from 8 to 11 days for stage A was 18·5% whereas an increase of 14·7% was evident from 21 to 23 days after harvest for stage B. The total sugars content of ripe bananas from maturity stage A was higher than that of maturity stage B.

Titratable acids in bananas harvested at the two stages of maturity differed slightly in their trends. Bananas harvested at stage A showed a slight decrease in titratable acids during the first three days after harvest, while the acids in bananas harvested at stage B remained essentially the same during the same period. However, both were followed by an increase to a peak, 10 and 25 days after harvest of stages A and B, respectively. A marked increase was noted from 1·47 meq (second day) to 5·63 meq (tenth day) for stage A and from 0·65 meq (sixth day) to 5·01 meq (twenty-fifth day) for stage B. After their respective peaks were attained, a rapid decline in total titratable acids occurred during the final stages of ripening.

The expected increase in total sugars which usually accompanies the decline in starch was not observed. Although there was a decline of approximately 12·0% in starch from the third to the eighth day for stage A and 6·8% from the ninth to the

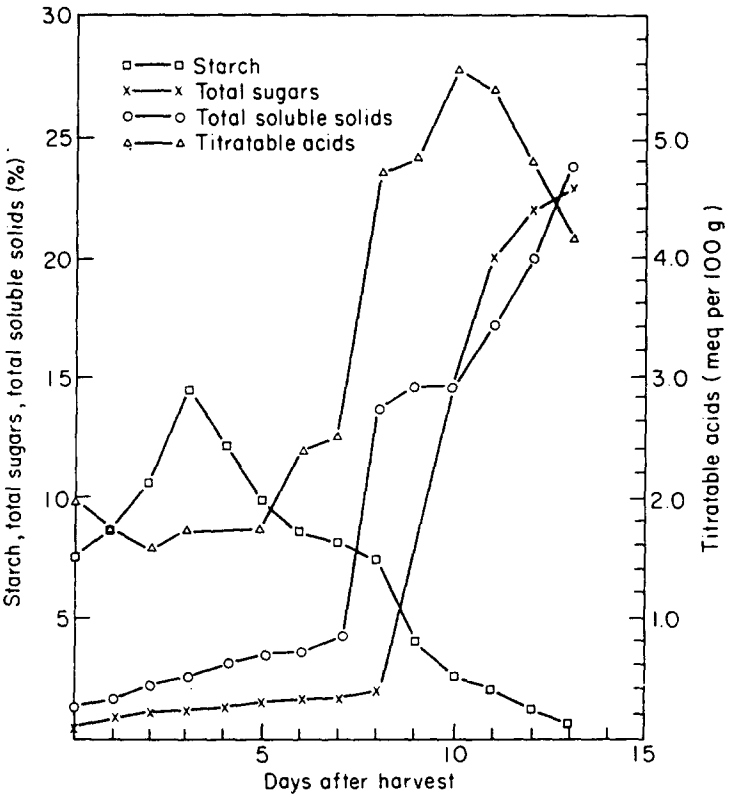

Fig. 1. Changes in starch, total sugars, total soluble solids and titratable acidity in bananas picked at maturity stage A.

twenty-first day for stage B, the increase in total sugars was only 0·9% and 0·8% for A and B, respectively, over the same period. During this time, a rapid increase in titratable acids occurred.

The data on titratable acids and starch were subjected to regression analysis to establish a relationship between them. The regression coefficient was found to be significant ($r = -0{\cdot}843$ for stage A and $r = -0{\cdot}766$ for stage B). Similarly, the regression coefficients between total sugars and titratable acids ($r = -0{\cdot}778$ for stage A and $r = -0{\cdot}840$ for stage B) and between total sugars and starch ($r = -0{\cdot}880$ for stage A and $r = -0{\cdot}789$ for stage B) were highly significant. Presumably, the sugars formed from starch hydrolysis underwent further conversion to organic acids.

When the amount of titratable acid reached 4·79 meq/100 g in fruits harvested at stage A, the sugars started to increase rapidly and there was a considerable drop in starch. This indicates that the conversion of sugars to acids had slowed down, hence

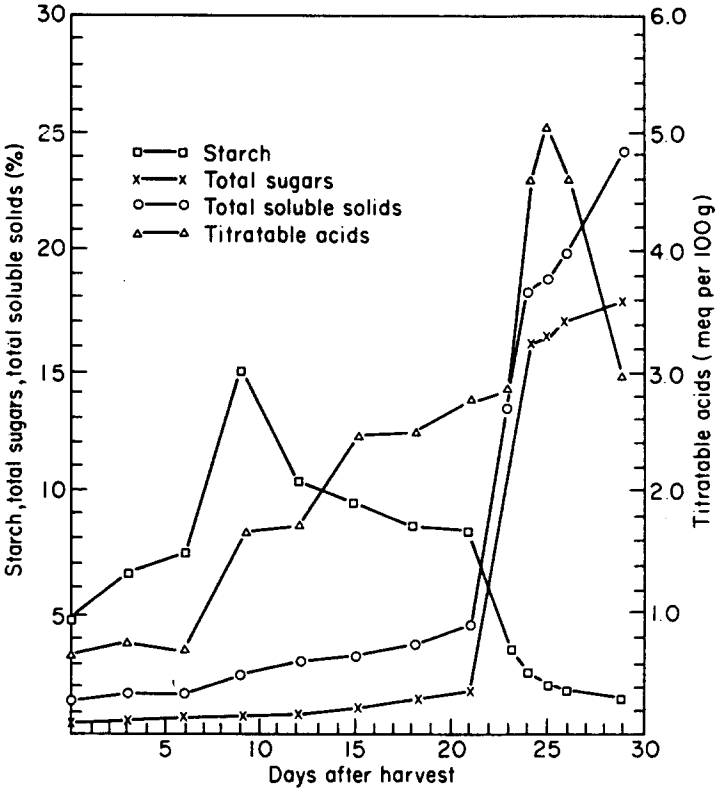

Fig. 2. Changes in starch, total sugars, total soluble solids and titratable acidity in bananas picked at maturity stage B.

the sugars started to accumulate. For stage B fruits, titratable acidity was at 2·76 meq per 100 g before the sugars started to accumulate.

The titratable acids decreased after reaching a peak which occurred after 10 and 25 days for stage A and stage B, respectively. This was accompanied by a decrease in the rate of starch hydrolysis. When the starch reserve had been depleted by continuous hydrolysis and the acids continued to undergo further metabolic transformations to carbon dioxide and water, the titratable acids became progressively less (Von Loesecke, 1950).

The increase in total soluble solids paralleled the increase in total sugars for both stages of maturity. The regression coefficients between total sugars and total soluble solids were found to be highly significant ($r = 0{\cdot}917$ for stage A and $r = 0{\cdot}992$ for stage B). This is expected inasmuch as total soluble solids are essentially sugars. Total soluble solids may therefore be used for measuring total sugars which would be a more convenient chemical maturity index during harvest.

The sugar–acid ratio has been suggested as an objective method of evaluating the eating quality of fruits. The sugar–acid ratios were calculated by dividing the percent total sugars by the percent titratable acids expressed as malic acid. The calculated values at the different stages of ripening are given in Table 1. Although bananas picked at stage A had higher total sugars and titratable acids than those picked at stage B, the sugar–acid ratios at the eating-ripe stage (10–13 days for stage A and 23–29 days for stage B) are comparable. It is evident that the eating quality based on the sugar–acid ratio of the bananas harvested at stage B is comparable to bananas harvested at stage A.

TABLE 1

THE SUGAR–ACID RATIO OF BANANAS PICKED AT TWO STAGES OF MATURITY

Stage A		*Stage B*	
Days after harvest	*Sugar–acid ratio*	*Days after harvest*	*Sugar–acid ratio*
0	0·30	0	0·66
1	0·53	3	0·67
2	0·81	6	1·16
3	0·68	9	0·51
4	1·04	12	0·62
5	1·19	15	0·61
6	0·94	18	0·75
7	0·94	21	0·76
8	0·58	23	4·08
9	2·64	24	4·82
10	3·84	25	4·86
11	5·51	26	5·45
12	6·73	29	8·92
13	8·21		

CONCLUSION

Bananas picked at two different stages of maturity showed parallel trends in biochemical changes. A difference in ripening time was, however, noted. Fruits picked at stage A (between 'full' and 'full three-quarters') ripened in 13 days, whereas those picked at stage B (between 'full three-quarters' and 'light full three-quarters') ripened in 29 days.

At the eating-ripe stage, fruits harvested at stage A had a sugar–acid ratio comparable to that of fruits harvested at stage B.

REFERENCES

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS (1965). *Official methods of analysis*, 10th edn. Washington, D.C.

BARNELL, H. R. (1940). Studies in tropical fruits. VIII. Carbohydrate metabolism of the banana fruit during development. *Ann. Botany*, **4**, 39–71.

BARNELL, H. R. (1941*a*). Studies in tropical fruits. XI. Carbohydrate metabolism of the banana fruit during ripening under tropical conditions. *Ann. Botany*, **5**, 217–48.

BARNELL, H. R. (1941*b*). Studies in tropical fruits. XIII. Carbohydrate metabolism of the banana fruit during storage at 53°F and ripening at 68°F. *Ann. Botany*, **5**, 607–46.

BARNELL, H. R. (1943). Studies in tropical fruits. XIV. Carbohydrate metabolism of the banana fruit during storage at 53°F. *Ann. Botany*, **7**, 1–22.

MENDOZA, D. B., JR. (1967). Respiration of banana fruit. *Phil. Agric.*, **51**, 747–56.

MURATA, T., KOZUKUE, N. & OGATA, K. (1967). Studies on post-harvest ripening and storage of bananas. VII. Physicochemical changes accompanying low-temperature damage to bananas. *J. Fd Sci. Technol.*, **14**, 22–7.

SMOCK, R. M. (1967). Methods of storing bananas. *Phil. Agric.*, **51**, 501–17.

SMOCK, R. M., MENDOZA, D. B. & ABILAY, R. M. (1967). Handling and storage of bananas. *Phil. Farms and Gardens*, **4**, 12–17.

SOULE, M. J., JR. & HARDING, P. L. (1956). Effects of size and date of sampling on starch, sugars, soluble solids and phenolic compounds in mangoes. *Proc. Fla. Mango Forum*, 13–18.

STRATON, F. C. & VON LOESECKE, H. W. (1930). A chemical study of different varieties of bananas during ripening. *Bull. United Fruit Co.*, No. 32.

VON LOESECKE, H. W. (1950). *Bananas*, 2nd edn. New York, Interscience Publishers, Inc.

VOLATILE COMPONENTS OF FIGS

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ABSTRACT

Volatile essences of Calimyrna, Kadota, Black Mission and Adriatic figs were prepared by passing large volumes of headspace gas through porous polymer traps at room temperature. The essences were analysed by gas chromatography, utilising wall-coated open-tubular glass capillary columns; structural elucidations were based on gas chromatography–mass spectrometry. Differences between varieties appear to be quantitative rather than qualitative, and are not noticeably greater than differences between samples of the same variety. Compounds identified included acetaldehyde, dimethyl acetal, methyl acetate, ethyl acetate, ethyl alcohol, ethyl propionate, ethyl isobutyrate, propyl acetate, methyl butyrate, isobutyl acetate, ethyl butyrate, ethyl-2-methyl butyrate, 2-methyl butyl acetate, 2-ethyl-1,2-dihydrothiophene, ethyl valerate and 3-hydroxy-2-butanone.

INTRODUCTION

Insect infestation of figs is a problem of some commercial importance to California growers. Particularly in processed figs, contamination involving principally the dried-fruit beetle and confused sap beetle has caused the rejection of a relatively large percentage of the fig crop. This has led to a situation where processed figs and fig paste are not always available, and commercial manufacturers contemplate the discontinuance of product lines such as fig bars and cookies. There are reasons to believe that the aroma volatiles of the fruit serve as insect attractants. This project was concerned with establishing the identity of these volatile constituents of fig.

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MATERIALS AND METHODS

Sample preparation

Two- to four-kilogramme batches of individual varieties of figs, picked from commercial orchards of Fresno County, were hand sorted and placed in a cylindrical glass sampling chamber 30 cm in diameter and 60 cm high. Breathing quality compressed air, further purified by passage through molecular sieves 5A and 13X, was passed through the sampling chamber at a flow rate of 60 ml/min and then through carefully prepurged traps (Jennings *et al.*, 1974*a*) consisting of 15-cm lengths of 6-mm glass tubing containing *ca.* 8 cm of a porous polymer, Porapak Q (Waters Assoc.), between glass wool plugs. After trapping at ambient temperature (25°C) for periods of 15 min to 6 h, each Porapak Q trap was reversed and back-flushed with purified nitrogen to deliver the trapped volatiles to a glass capillary tube which was flame sealed and stored at −20°C until used.

Gas chromatography

Chromatographic separations utilised a Hewlett Packard 5711 gas chromatograph fitted with flame ionisation detection. The instrument was adapted to highly linear glass inlet splitters that were a modification of an earlier reported model (Jennings, 1975) (Fig. 1), and wall-coated open-tubular (WCOT) glass capillary columns (Jennings *et al.*, 1974*b*). Columns were 0·25 mm id × 60 m, coated with Carbowax 20M admixed with 5% benzyl triphenylphosphonium chloride. The split ratio was *ca.* 1:100, and the inlet and detector were maintained at 250°C; other parameters, as reported on the individual chromatograms, were selected to achieve the desired degree of separation consistent with acceptable analysis times (Jennings & Adam, 1975).

Mass spectrometry

Identifications were based largely on mass spectra, as obtained on a Finnigan Model 1015C mass spectrometer, with the Model 6000 computerised data system. A WCOT glass capillary column served as the inlet to the mass spectrometer. Spectral identifications were confirmed by comparing Kováts Indices determined isothermally on these high-resolution columns with those of authentic samples.

RESULTS AND DISCUSSION

The amounts of essence recovered varied with the degree of ripeness exhibited by the sample; firm green figs yielded 0·5–1 μl of essence even on prolonged (6-h) trapping while, in the soft ripe stage, 15 min of trapping was sufficient to yield 2 μl of concentrated essence. In each case, the essence possessed an aroma highly typical of the starting material.

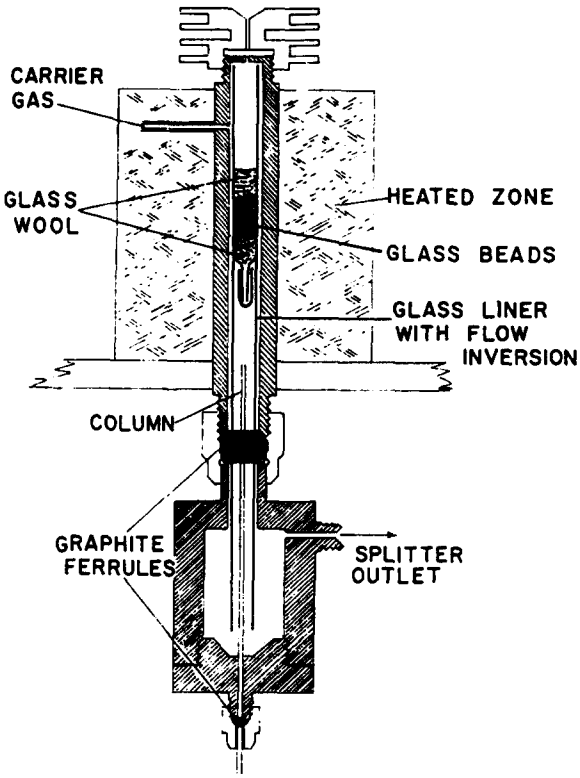

Fig. 1. Modified glass inlet splitter. Aluminium housing as shown fits the ¼-in heated inlet of the Hewlett Packard 5711 and 5720 gas chromatographs and houses a glass insert with flow inversion characteristics. The interior base of the modified Swagelok nut engages the steel circlip retainer (shown as clear circles) and converts the aluminium housing to an extension of the normal inlet; the graphite ferrule seals all three components: the glass liner, the aluminium housing and the standard ¼-in heated inlet. Normally the split gas is discharged through an activated carbon filter and thence to a needle valve (Jennings & Adam, 1975). For linearity characteristics and patent coverage, see Jennings (1975).

During the trapping process it was noticed that some odorous materials passed through the Porapak traps with relatively poor trapping efficiency; the odour of the effluent was suggestive of carbonyl compounds, probably aldehydes. The effluent gas was accordingly bubbled through a solution of dinitrophenylhydrazine and a considerable quantity of yellow dinitrophenylhydrazone precipitated. Subsequent analysis indicated that the parent compound was acetaldehyde and that no other carbonyl compounds were present.

Figure 2 shows chromatograms of Porapak essences from four varieties of fig at the full-ripe stage. Although major quantitative differences in volatile composition are clearly evident, subsequent work indicated that these differences were due less

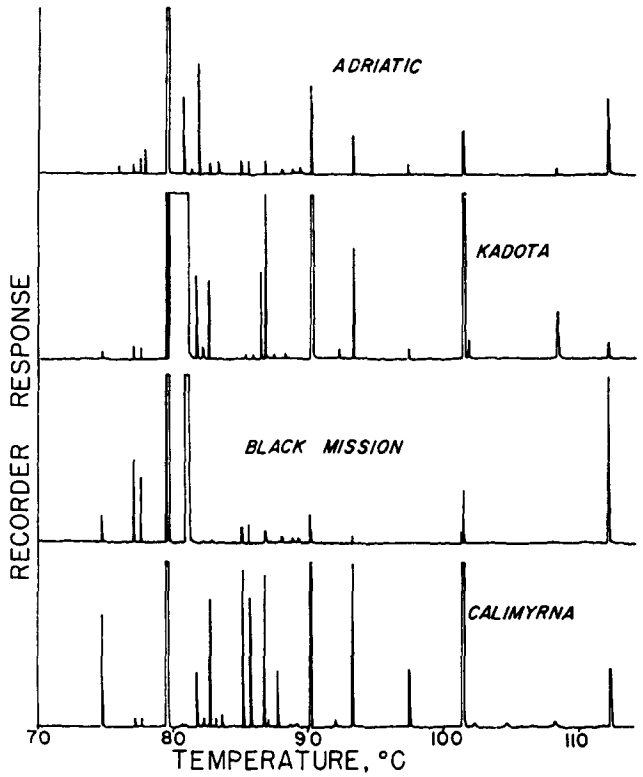

Fig. 2. Chromatograms of Porapak essences of full ripe figs. Column temperature programmed from 70° to 120° at 1°/min. Variations within a variety are at least as great as intervarietal variations; see text.

to the variety of fig than to some other factors. The fig is a highly dynamic system in which rapid and drastic changes in the concentration of individual volatiles can be demonstrated. It soon became evident that variations between the volatile compositions of different samples of the same variety were at least as great as variations between varieties; these large and variable differences suggested that external factors might be influencing volatile production (see below).

Figure 3 shows a total ion current chromatogram from the GC–MS determinations plotted as ion intensity versus scan number. Peaks represented by scans 6 and 425 are hexane and undecane, respectively, added as internal standards to aid in the correlation with analytical chromatograms. A large number of the individual spectra were typical of esters. Initial assignments involved comparison of the mass spectrum of the unknown with that of the authentic compound; Kováts Indices of the two were then compared under closely controlled separations,

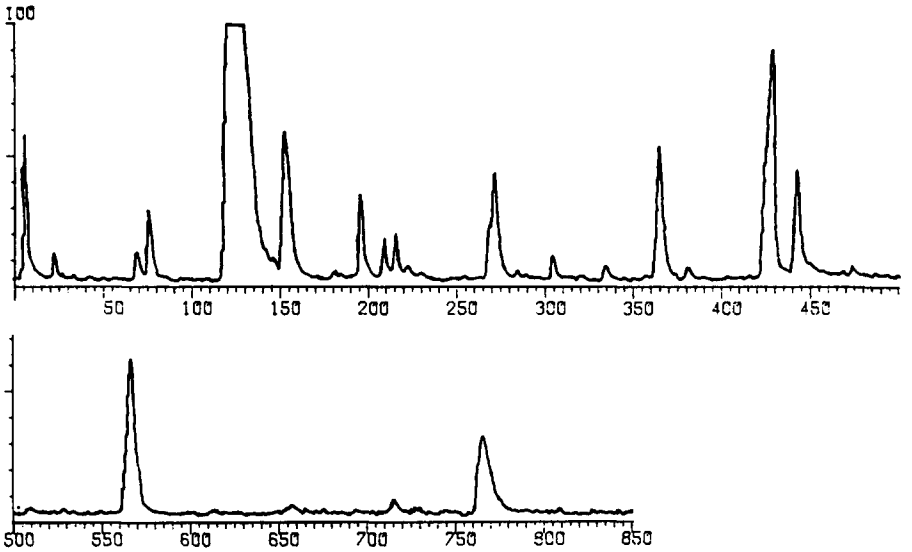

Fig. 3. Total ion current chromatogram of Porapak essence of ripe Calimyrna figs. Scans 8 and 425 represent hexane and undecane internal standards, respectively.

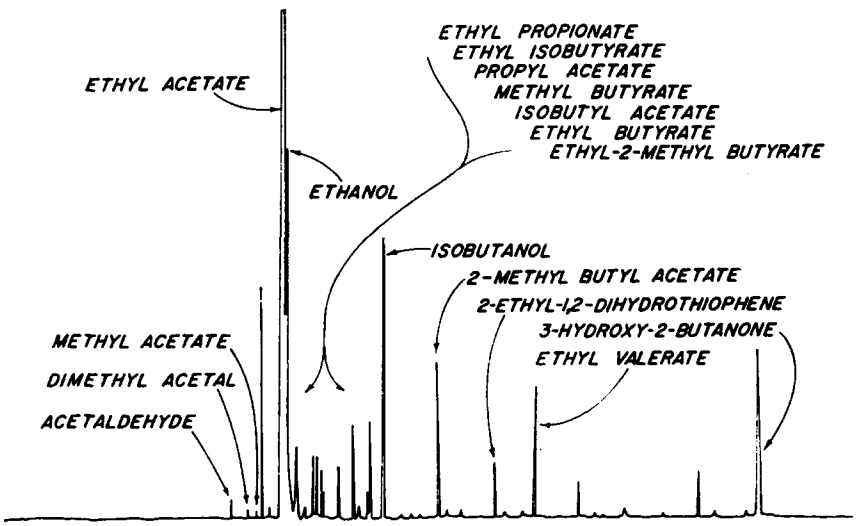

Fig. 4. Compounds isolated from Porapak essence of ripe fig.

achieving separation efficiencies of *ca.* 150,000 effective plates. Figure 4 shows the identities of the more abundant fig volatiles and Table 1 lists their Kováts Indices.

Many of these compounds are typical of almost any yeast fermentation. The fig is characterised by an ostiole, or 'eye', an opening that provides free access to the interior of the fruit. Insects entering the fruit undoubtedly inoculate the interior with variable levels of wild yeasts. As the fruit ripens, fermentation occurs and the fermentation products probably account for the high degree of intersample variability.

TABLE 1

COMPOUNDS FROM PORAPAK ESSENCES OF RIPE FIGS

Compound	*Kováts Index,* $I^{20M}_{100°}$
Acetaldehyde	695
Dimethyl acetal	710
Methyl acetate	818
Ethyl acetate	878
Ethyl alcohol	917
Ethyl propionate	952
Ethyl isobutyrate	963
Propyl acetate	969
Methyl butyrate	983
Isobutyl acetate	1008
Ethyl butyrate	1033
Ethyl-2-methyl butyrate	1053
Isobutanol	1066
Isoamyl acetate	1115
2-methyl butyl acetate	1119
2-ethyl-1,2-dihydrothiophene	*
Ethyl valerate	1133
Isobutyl isovalerate	1180
3-hydroxy-2-butanone	1290 (1293)

* No reference compound available.

The presence of 2-ethyl-1,2-dihydrothiophene, whose mass spectrum matched precisely that of the authentic compound, deserves some special comment. Because many sulphur compounds resist passage in chromatographic equipment containing packed columns or metallic components, their existence in a variety of foods has been suspected rather than known. The WCOT column in an all-glass system may demonstrate that such compounds are indeed more widely distributed than has been previously recognised.

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REFERENCES

JENNINGS, W. G. (1975). Glass inlet splitter for gas chromatography. *J. Chromatog. Sci.*, **13**, 185–7.

JENNINGS, W. G. & ADAM, S. (1975). Gas chromatography: Elution temperature, speed of analysis and separation efficiency as influenced by rate of temperature programming and carrier gas velocity in open tubular glass capillary columns. *Anal. Biochem.*, **69**, 61–9.

JENNINGS, W. G., WOHLEB, R. H. & LEWIS, M. R. (1974*a*). Isolation of volatile compounds for GLC analysis. *MBAA Tech. Quarterly*, **11**, 104–9.

JENNINGS, W. G., YABUMOTO, K. & WOHLEB, R. H. (1974*b*). Manufacture and use of the glass open tubular columns. *J. Chromatogr. Sci.*, **12**, 344–8.

NUTRITIONAL ASPECTS OF SOFT DRINKS†

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ABSTRACT

The metabolic consequences in man, both immediate and long term, of consuming soft drinks are discussed. Emphasis is placed on the role in energy and lipid metabolism of various carbohydrate constituents, on the effects on gastric emptying of the various constituents and on the biochemical implications of fructose containing drinks.

INTRODUCTION

Soft drinks are not usually considered as a source of nutrition and they provide a good example of gratification of the taste buds rather than that of the metabolising cell—except possibly for the water content of the drink. However, whether the consumer is aware of it or not, most soft drinks do contain substances that the body can metabolise and use to its advantage, the most important nutritional ingredient being the carbohydrate component. This is mainly sucrose, but recently fructose with and without glucose, glucose syrup, and perhaps even xylitol have found their way into soft drinks.

When considering the sucrose intake in the UK, the average intake of sucrose from soft drinks is over 6% of the per capita sucrose intake, which means approximately 8 g/d. Not an impressive figure, but in the heaviest using group the calculated average intake of sucrose from soft drinks is over 110 g/d or 20% of the total energy intake. As this high intake is most commonly seen in 10–15 year-olds, and in summer the value may increase to 30–35% of the energy intake, then it does seem worthwhile to consider the contribution of the nutritional, or perhaps malnutritional aspects of soft drinks.

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ACUTE EFFECTS

The effect of consuming any food can be divided into those changes which occur within an hour or two of ingestion and those changes which only become apparent days, weeks or years later.

The effect that a soft drink might have on the teeth is neither physiological nor nutritional (except to the oral bacteria) so is out of context here. However, the rate of stomach emptying is affected by the volume and composition of the soft drink. The more concentrated the sodium chloride component the slower is stomach emptying (Hunt & Knox, 1968) and the more acidic the soft drink is, the slower is the rate of gastric emptying (Hunt & Knox, 1968). In general, for a given energy content, the more dilute this is presented to the stomach, the greater the delay in stomach emptying (Hunt & Stubbs, 1975).

Recently we have been carrying out a series of experiments in which male medical and dental students were given meals containing various concentrations of three carbohydrates, namely glucose, sucrose and fructose, and examining the blood at intervals up to 90 min. Some of the more striking findings are as follows:

1. *Blood glucose*
   (a) The concentration of glucose in the drink does not significantly affect the ensuing blood glucose level at the glucose concentrations found in soft drinks.
   (b) With sucrose, the blood glucose level following ingestion is slightly lower than with glucose in the drink, but again there does not appear to be a dose related effect.
   (c) As expected, fructose in the drink does not cause a significant rise in blood glucose levels.

2. *Serum insulin*

With increasing concentrations—and therefore amounts—of glucose and sucrose drinks there is an increase in serum insulin levels.

3. *Serum fructose*

A relationship is present between concentration consumed and serum fructose levels, except, of course, for glucose.

4. *Blood lactate*

Lactate is found in increasing quantities in the blood during and just after moderate to severe exercise, and the results obtained in resting subjects are of interest in the context of exercise.

   (a) Glucose ingestion is followed by a significant fall in lactate levels, a fall that does not seem to be dose dependent.

(b) Sucrose in the drink raises blood lactate levels. In this respect sucrose simulates the effect of exercise.
(c) Only in high doses does fructose have this effect.

While discussing the acute effects of soft drinks it should be borne in mind that fructose can give rise in an adult to an osmotic diarrhoea when consumed in quantities of approximately 70 g or more and if the heaviest using group, where sucrose intake is 129 g/head/d, were to replace the sucrose with an equal weight of fructose, there is a possibility that some consumers might complain of intestinal hurry.

What is the ultimate fate of the carbohydrate in the soft drink? Most of it is broken down, after absorption, to carbon dioxide and water and the energy liberated is used by the body. Some of the carbohydrate not needed for this is converted to glycogen and stored in the liver or skeletal muscle for use between meals, and the remainder of the carbohydrate is converted to fat and stored as such in the fat depots. Soft drinkscan, therefore, like any other energy source, be stored as fat and contribute to overweight. The rate at which ingested carbohydrates are converted to serum lipid does seem to depend on the type of carbohydrate taken. More fructose seems to find its way into serum triglyceride than does glucose (Macdonald, 1968).

CHRONIC EFFECTS

Much of our knowledge of the effects of chronic ingestion of carbohydrates has been obtained from studies carried out in laboratories rather than in the field situation, and the levels of carbohydrate fed have tended to be high. However, such experiments may give a clue to the long term effect of consuming carbohydrates. It has been found that a chronic effect of consuming sucrose may be to cause an elevation of the triglyceride level in fasting blood, and this goes for fructose too. This effect is not seen in pre-menopausal women (Macdonald, 1966). The response in children is not known. Maybe even the extent or degree of hydrolysis of the glucose polymer may affect lipid metabolism (Birch & Etheridge, 1973).

Over 10 % of adult males in the UK are 'carbohydrate sensitive', that is to say their fasting level of serum triglyceride increases markedly in response to an increase in the dietary carbohydrate eaten (Stone & Dick, 1973) and it has been suggested that sucrose is more potent in this effect (Kuo & Bassett, 1965; Roberts, 1973). As this is a response that occurs more frequently as age advances it is unlikely to be of consequence to the soft drinks industry whose consumers are at a fairly young age.

It is interesting, perhaps, to speculate on the metabolic interplay between dietary carbohydrate and the oral contraceptive. As mentioned earlier, pre-menopausal women do not show the lipid response to sucrose (or fructose) that men do. There is now some evidence that suggests that one effect of the oral contraceptive is to remove

this 'immunity' from the pre-menopausal woman in her response to sucrose and fructose (Stovin & Macdonald, 1975) and an increase in the death rate from ischaemic heart disease has been reported in women on the 'pill' (Mann & Inman, 1975).

The chronic consumption of alcohol raises the triglyceride level in fasting blood, as do sucrose and fructose. Perhaps those who consume alcohol would be better advised to dilute their alcohol with a soft drink that does not contain sucrose or fructose. This is speculation, but is, perhaps, an area that needs exploring.

Another area that it would be prudent to consider is the relationship between the carbohydrate present in soft drinks and body weight. Reference was made earlier to an excess consumption of metabolisable energy of any kind leading to overweight. It is worth considering the effects on body weight of the various carbohydrates likely to be found in soft drinks, as it has been shown in rats (Allen & Leahy, 1966) and in monkeys (Brook & Noel, 1969) that, gramme for gramme, more weight is gained with sucrose as the dietary carbohydrate than with other carbohydrates. Brief studies in man would support the general premise that carbohydrates are not all equal in their effect on body weight and that sucrose is less efficiently used by men and more so by women when compared to glucose (Macdonald & Taylor, 1973). Again, though these are facts, it is not possible at this early stage to put them into perspective and work is continuing in this area.

Many nutritionists would condemn soft drinks as not only being cariogenic but full of so-called 'empty calories', i.e. an energy source that has no other nutrient with it, and it has been stated that soft drinks, like sweets, could, in theory, if taken before a meal, prevent a child from eating sufficient of the nutritionally more balanced type of food that comes with the main meal. In this country the latter is probably an exaggeration because in general we seem to have a more than adequate intake of most nutrients.

Turning finally to the future role of soft drinks in preventing disorders that are nutritional in origin, one can only guess. However, it would be useful to have a soft drink that lowered a raised serum cholesterol or a soft drink that blunted the appetite and hence assisted in the control of energy intake, or one that contained an anti-caries compound. I do not doubt that preventive medicine as applied to soft drinks is not too far in the future. It would be so nice not only to gratify the palate but at the same time to improve one's health.

REFERENCES

ALLEN, R. J. L. & LEAHY, J. S. (1966). Some effects of dietary dextrose, fructose, liquid glucose and sucrose in the adult male rat, *Brit. J. Nutr.*, **20**, 339–47.

BIRCH, G. G. & ETHERIDGE, I. J. (1973). Short-term effects of feeding rats with glucose syrup fractions and dextrose, *Brit. J. Nutr.*, **29**, 87–93.

BROOK, H. & NOEL, P. (1969). Influence of dietary liquid glucose, sucrose and fructose on body fat formation, *Nature* [*Lond.*], **222**, 87–93.

HUNT, J. N. & KNOX, M. T. (1968). Regulation of gastric emptying. In *Handbook of Physiology, Section 6, Alimentary Canal*, **4**, 1917–35. Amer. Physiol. Soc., Washington DC.

HUNT, J. N. & STUBBS, D. F. (1975). The volume and energy content of meals as determinants of gastric emptying. *J. Physiol.*, **245**, 209–25.

KUO, P. T. & BASSETT, D. R. (1965). Dietary sugar in the production of hyperglyceridemia, *Ann. intern. Med.*, **62**, 1199–212.

MACDONALD, I. (1966). Influence of fructose and glucose on serum lipid levels in men and pre- and post-menopausal women, *Am. J. clin. Nutr.*, **18**, 369–72.

MACDONALD, I. (1968). Ingested glucose and fructose in serum lipids in healthy men and after myocardial infarction, *Am. J. clin. Nutr.*, **21**, 1366–73.

MACDONALD, I. & TAYLOR, J. (1973). The effect of high-carbohydrate, low-energy diets on body-weight in man, *Proc. Nut. Soc.*, **32**, 36A.

MANN, J. I. & INMAN, W. H. W. (1975). Oral contraceptives and death from myocardial infarction, *Brit. Med. J.*, **2**, 245–8.

ROBERTS, A. A. (1973). Effects of a sucrose free diet on the serum lipid levels of men in Antarctica, *Lancet*, **1**, 1201–4.

STONE, M. C. & DICK, T. B. S. (1973). Prevalence of hyperlipoproteinaemias in a random sample of men and in patients with ischaemic heart disease, *Brit. Heart J.*, **35**, 954–61.

STOVIN, V. & MACDONALD, I. (1975). Some effects of an oral contraceptive on dietary carbohydrate–lipid interrelationships in the baboon, *Proc. Nut. Soc.*, **34**, 55A.

FOLATE—PROBLEM NUTRIENT

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ABSTRACT

Even in over-fed affluent societies, a significant proportion of the population may suffer from folate deficiency. This is of concern to the informed public and the food scientist or technologist concerned with handling and processing food that makes a contribution to the folate in our diet. In trying to understand the problem, the food scientist becomes involved in unresolved areas of physiology and nutrition, together with analytical problems and the relationship between analytical data and the physiological response to folate levels apparently in foods. This paper is an attempt, by a food scientist, to present the subject for other food scientists.

INTRODUCTION

Food folates are important to the nutritionist even in over-fed affluent societies where the young, the old, the pregnant and those with certain gastric disorders, may suffer from folate deficiency depending on their dietary habits and social status. (Folate is the term applied to a broad spectrum of substances which give rise to folacin in the body. Folacin and folic acid are respectively the more recent and original terms used to describe the various forms of the vitamin in much of the literature on this subject. The folate group is shown in Figs. 1 & 2 which are intended to show relationships between members of this group of compounds, from a nutritional (Fig. 1) and a chemical (Fig. 2) viewpoint.)

The folate activity of our food depends on several related chemical substances, some seeming more active as vitamins than others in the body and in the chemical and microbiological tests used to assay folate. We do not yet understand the relationship between laboratory analyses of folate and the activity of the various folate components in human nutrition. Consequently, although standardised

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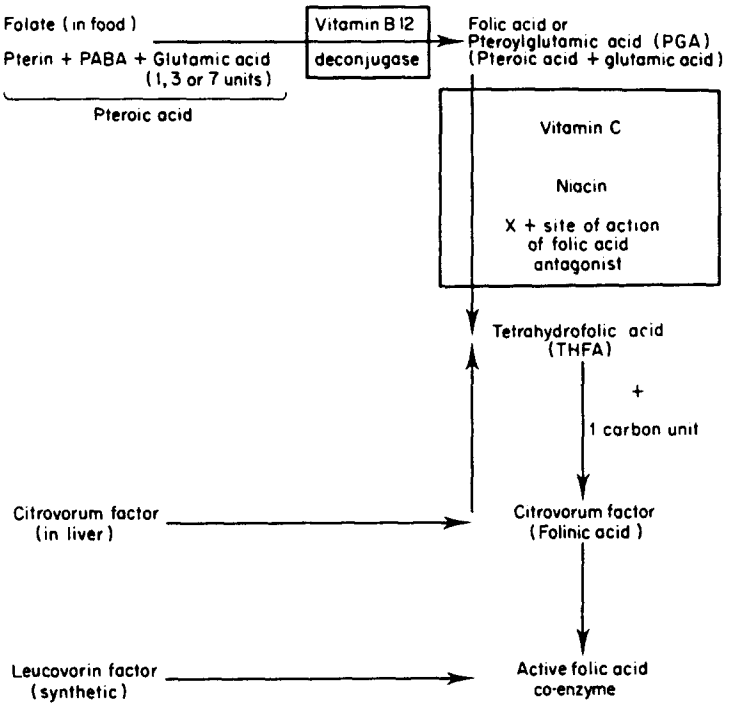

Fig. 1. Food folates—a nutritional classification.

analyses can show how the substances which react to the test procedure stand up to processing and storage, these methods tell little of the nutritional value of the folate in the foods to man.

NUTRITION AND BIOCHEMISTRY

Vegetables such as spinach contain much folate. Liver and kidney provide some and milk, poultry and eggs a little. As with several B group vitamins, folic acid comprises a group of compounds with vitamin activity. It is absorbed throughout the small intestine, where some synthesised by the human intestinal microflora is added to that ingested with the food.

Folic acid is an important co-enzyme factor, responsible for much single carbon transfer and affects many key compounds.

Haemoglobin

Folic acid performs its carbon transfer role in the formation of haem, the iron-

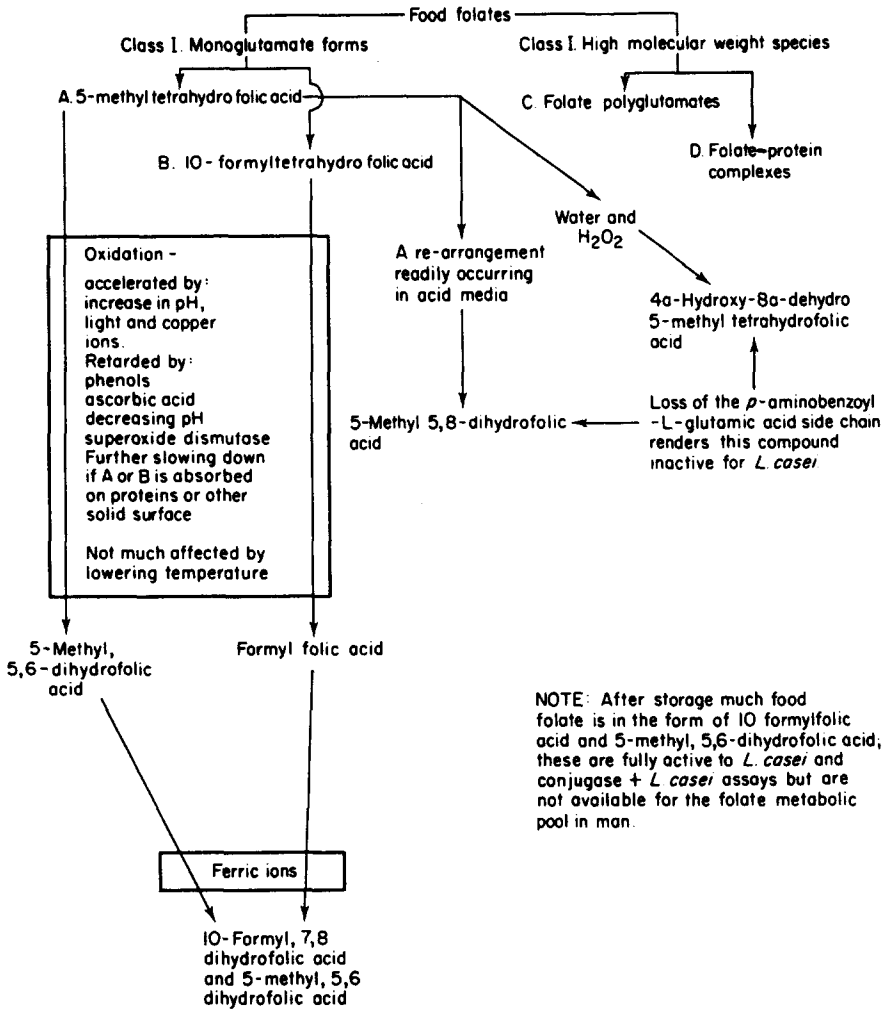

Fig. 2. Food folates—a chemical classification (Blair, private communication).

containing protein in haemoglobin. This important function relates folic acid with blood cell formation and anaemias.

Purines

Purines form part of the nucleoproteins which are essential constituents of all living cells involved in cell growth, cell division and the transmission of inherited traits. The biosynthetic pathways leading to the production of purines involve single carbon transfers.

Thymine

Folic acid participates in the reactions that synthesise thymine, an essential component of DNA.

MEDICAL SIGNIFICANCE

The relationship between anaemia and folic acid

A nutritional megaloblastic anaemia caused by simple folic acid deficiency has been clearly described. A rapid haematologic response follows treatment with folic acid alone.

Vitamin B_{12}, discovered after folic acid, proved to be the fully effective agent for both blood regeneration and for the neurologic defect associated with pernicious anaemia. The American Medical Association and the US Food and Drug Administration have recommended that not more than 400 μg of folic acid be included in non-prescription multivitamin preparations; this supply would be enough for common needs but would not mask the diagnosis of pernicious anaemia.

The need for folic acid during pregnancy

Folic acid is essential during pregnancy, for haemoglobin synthesis. Some cases of macrocytic anaemia during pregnancy have been attributed to folic acid deficiency in the diet. A case is recorded of a mother who, together with her three month old nursing infant, had megaloblastic anaemia; both responded to folic acid given to the mother alone. There are reasons, therefore, to include folic acid in the dietary supplements for pregnant women and nursing mothers.

Folic acid and gastro-intestinal disease

Folic acid is effective in treating sprue, a gastro-intestinal disease characterised by lesions, absorption defects, diarrhoea, macrocytic anaemia and general malnutrition.

Aminopterin

Aminopterin, the potent biochemical antagonist of folic acid has been used in treating malignant neoplastic disease such as leukaemia, where it can induce a temporary remission. Unfortunately the leukaemia cells seem to develop a resistance if use of the antagonist is continued.

REQUIREMENTS FOR FOLIC ACID

Studies on man suggest folic acid requirements, in terms of pure pteroylglutamic acid (PGA), of about 50 to 100 μg/day, although in pregnancy the requirement may

be 400 μg/day or more. Folic acid activity of foods is usually measured by microbiological assay and estimates of activity in man vary according to the assay organism and the assay technique; this is shown in Table 1. Thus, one method estimated a mixed diet provided about 200 μg/day of folate (compared to over 600 μg/day by another method) (Butterworth, 1968). Folate activity is reduced by cooking, especially by prolonged boiling in water, and also by pH changes in the gastro-intestinal tract (Anon, 1969).

In the United States, the National Research Council first stated requirements for folic acid in 1968; these were 400 μg/day for adults rising to 800 μg/day during pregnancy and 500 μg/day during lactation. These figures comprised total folic acid

TABLE 1
A COMPARISON OF SOME PUBLISHED VALUES FOR FOLATE IN UNCOOKED FOODS

Food	*L. casei* + *ascorbate*[a] (μg/g)	*L. casei* + *conjugase*[c] (μg/g)	*S. faecalis*[c] (μg/g)	*S. faecalis* + *conjugase*[c] (μg/g)	*McCance*†[b] (μg/g)
Apple	0·02	0·04	—	0·07	0·01
Banana	0·27	0·11	0·06	0·10	0·05
Bread					
White	0·17	0·14	—	0·16	—
Brown	0·38	0·27	—	0·36	0·20
Broccoli	0·92	0·28	—	—	—
Cabbage	2·40	0·11	0·06	0·12	0·20
Cucumber	0·14	0·09	0·04	0·08	0·06
Egg					
White	0·01	0·004	—	0·007	0·02
Yolk	1·42	0·13	—	0·13	0·22
Liver (lambs)	5·10	2·80	—	—	2·80
Milk	0·085	0·006	—	—	0·003
Mushroom	0·42	0·28	0·20	0·30	0·20

† Data derived from literature survey only. *L. casei* or *Strep. faecalis* may have been used to derive the data.
[a] Hurdle, *et al.*, 1968.
[b] McCance & Widdowson, 1960.
[c] Toepfer, *et al.*, 1951.

activity of which the average US diet contains about 600 μg/day. However, an intake of 100 μg of synthetic folacin per day provides the requirements for normal adults (Anon, 1968). The folate content of foods does not have to be stated in the Swedish voluntary nutritional labelling scheme (Declaration of Nutritional Value of Foodstuffs, a guidance elaborated by the Food Industry Group of the Federation of Swedish Industries). In the US nutritional labelling scheme for frozen 'heat and serve' dinners, a folate content is required but not specified: 'A frozen "heat and serve" dinner prepared from conventional food ingredients . . . will also contain folic acid . . . . Minimal levels for these nutrients (the group being referred to includes folic

acid) cannot be established at the present time but may be specified as additional data are obtained.' (Anon, 1973).

CHEMISTRY

Folates in foods fall into two classes:

(a) Monoglutamate folates, mainly 5-methyltetrahydrofolic acid with some 10-formyltetrahydrofolic acid.

(b) High molecular weight compounds such as folate–protein complexes and the materials usually described as folate polyglutamates.

10-formyltetrahydrofolic acid is rapidly oxidised by molecular oxygen to 10-formyl folic acid. 5-methyltetrahydrofolic acid is oxidised more slowly giving 5-methyl-5,6-dihydrofolic acid. The oxidation of either is accelerated by increasing pH, light and copper ions but is retarded by phenols, chelating agents, ascorbic acid, decreasing pH and the enzyme superoxide dismutase. The slow oxidation needs little activation energy; consequently, decreasing temperature does not slow oxidation very much.

Oxidation may be further reduced when the tetrahydrofolate is absorbed onto a surface, e.g. protein, and this effect could be further enhanced if the tetrahydrofolate is in a folded conformation. Both tetrahydrofolates react almost instantaneously with ferric ions giving 10-formyl-7,8-dihydrofolic acid and 5-methyl-5,6-dihydrofolic acid.

5-methyl-5,6-dihydrofolic acid rearranges readily, especially in acid media, to 5-methyl-5,8-dihydrofolic acid. 5-methyl-5,6-dihydrofolic acid reacts with water or hydrogen peroxide to give 4a-hydroxy-8a-dehydro-5-methyltetrahydrofolic acid. 10-formyltetrahydrofolic acid is thermodynamically less stable than 5-formyltetrahydrofolic acid and can therefore rearrange to the latter.

At high temperatures, in the absence of anti-oxidants and at neutral pH, all folates except folic acid and 5-formyltetrahydrofolic acid are oxidised to products lacking the *p*-aminobenzoyl-*l*-glutamic acid side chain.

In aqueous media tetrahydropteridines are oxidised by a free radical chain mechanism using hydroperoxy and hydroxy radicals as chain carriers. In water–organic liquid mixtures this oxidation is reduced and is stopped in non-aqueous media; the same would apply to tetrahydrofolates, so they should be very resistant to oxidation in anhydrous media.

5-methyl-5,8-dihydrofolic acid and 4a-hydroxy-8a-dehydro-5-methyltetrahydrofolic acid and pteridines formed by loss of the *p*-aminobenzoyl-*l*-glutamic acid side chain are inactive for *Lactobacillus casei* under any assay conditions. 5-methyl-5,6,-dihydrofolic acid is not active for *L. casei* but, as it is readily reduced by ascorbic acid to 5-methyl-tetrahydrofolic acid it gives a growth response in the normal *L. casei* assay (which has ascorbic acid present). Healthy

humans readily absorb folates including the high molecular weight forms. Absorption may be impaired: (a) when the jejunum is unusually alkaline, (b) in subjects taking phenytoin, barbiturates or excess alcohol, and (c) in subjects suffering from adult coeliac disease (a mild form of which may be much more common than believed) or regional enteritis.

Absorbed 10-formylfolic acid enters the folate metabolic pool extremely slowly, if at all, so it is best regarded as unavailable to man. Folic acid enters the pool rather slowly so it is only partially available. On present evidence 5-methyl-5,6-dihydrofolic acid contributes little, if at all to the folate metabolic pool. Little is known of the contribution of 5-methyl-5,8-dihydrofolic acid and 8a-dehydro-4a-hydroxy-5-methyltetrahydrofolic acid to the pool although it seems likely that this will be nil. All other folates are efficiently converted into the folate metabolic pool.

In stored foods much of the folate may be in the form of 10-formylfolic acid and 5-methyl-5,6-dihydrofolic acid. These species and their high molecular weight forms are fully active in the standard *L. casei* and conjugase + *L. casei* assays but are *not* available for the folate metabolic pool in man. Therefore diets judged by microbiological assay to be adequate in folate content may be unsatisfactory for human nutrition.

ANALYSIS

Principles

Although chemical methods are being developed for analysing folate, almost all measurements of folate activity in food depend on bacteriological assay methods which are sensitive and can detect minor differences in chemical structure.

It would be quite impracticable to monitor changes in foods during processing, storage, distribution, retail display, etc., using complex programmes of differential microbiological assays, perhaps combined with chromatographic and bioautographic techniques, to characterise the different folates. However, assay using *Lactobacillus casei* as the test organism, with and without conjugase treatment of the test samples would reveal any changes in processing or storing of those folate forms assessed by *L. casei*; the dual assay of samples before and after conjugase treatment certainly gives reproducible results.

The enzymes used to break down conjugated folate are conjugases, including a carboxypeptidase found in the pig kidney which degrades pteroylglutamate to monoglutamate and chicken pancreas extract containing a carboxypeptidase which releases a diglutamate. The optimum pH for the first conjugase is 4·5 and for the second it is 7·8.

Three microbiological assay techniques for evaluating folate have been used. These involve: *Lactobacillus casei*, *Streptococcus faecalis* and *Pediococcus cerevisiae* (= *P. pentosaceous*).

L. casei responds to some substituted and unsubstituted pteroylglutamates which do not support growth of the other two organisms; *Strep. faecalis* responds to unsubstituted PGA and formyl derivatives whereas *P. cerevisiae* requires preformed formyltetrahydrofolates. We have already stated the view that it is quite possible that diets judged to be adequate by microbiological assay may not provide sufficient available folate for adequate human nutrition. Despite these drawbacks, the microbiological assays, if interpreted with knowledge of flaws and applicability, warrant our confidence in them as the best means of routine analysis of food folate.

The only certain way of investigating the availability of forms of folate to higher animals is by direct animal tests.

Practice

In a microbiological assay, basically the same rules apply for the micro-organism as would to a reagent used in colorimetric reactions, namely:

(i) In the conditions of the test the assay organism must only respond to the compound being assayed.
(ii) The response of the organism must vary quantitatively with the concentration of the compound.

The basic procedure for a microbiological assay is the same regardless of the vitamin being assayed. The procedure can be broken down into a series of steps as follows:

(a) preparation of media for carrying and maintaining stock cultures; (b) preparation of vitamin deficient basal medium; (c) preparation of inoculum medium and inoculum culture; (d) extraction of the vitamin from the sample prior to assay; (e) setting up that assay; (f) sterilisation of the assay tubes and media; (g) inoculation with test organism; (h) incubation, and (i) measurement.

Folate assay

The fact that *L. casei*, *Strep. faecalis* and *P. cerevisiae* give different assessments of the folate activity can be used to indicate the type of folate compounds present in samples for analysis. The assay technique for each organism is the same, apart from the need for media and assay conditions appropriate for each organism.

With *L. casei* and a 22 h incubation period, the assay will detect a range of 0·1 mg/tube; for *Strep. faecalis* and a 16 h incubation period, the assay range is 0·5 mg/tube and for *P. cerevisiae* with a 16 h incubation period the assay range is 0·1 mg/tube. After the incubation, growth is usually measured by culture turbidity.

The problems of food folates and their analysis were reviewed by the MAFF Committee on Food Composition and by a special Folic Acid Panel of the Committee on Medical Aspects of Food Policy. In 1970 they concluded that assay by the use of *L. casei* in the presence of ascorbic acid, both before and after conjugase treatment, was then the most acceptable method.

Extraction

The first step in folic acid assay is to extract the sample satisfactorily. To prevent destroying the heat-labile reduced forms of folic acid, it is necessary to include ascorbic acid in the extracting medium.

Conjugase treatment

Most of the folic acid in foods is in bound or conjugated forms, e.g. folate–protein complexes, and so cannot be used by assay micro-organisms. Therefore, folic acid assays without treatment to release bound forms of the vitamin indicate smaller contents than do animal bio-assays.

The point in the procedure at which the conjugase treatment is performed is very important; foods should be incubated with the conjugase before filtration because treatment after filtration gives falsely high results.

CONCLUSIONS

Understanding folates as nutrients is a challenging problem in food chemistry, involving advanced interdisciplinary studies in organic and analytical chemistry, biochemistry, human physiology and nutrition.

REFERENCES

ANON (1968). Recommended dietary allowances. *Pub.* 1694, *Nat. Acad. Sci.*, Washington DC.

ANON (1969). Recommended intakes of nutrition for the UK. In *DHSS Reports on Public Health and the Medical Subjects* No. 120, HMSO, London.

ANON (1973). *Federal Register* **38**(49) 100.5(d) (1).

BLAIR, J. Private communication. University of Aston in Birmingham.

BUTTERWORTH, C. E. (1968). The availability of food folate. *British Journal of Haematology*, **14**, 339–43.

HURDLE, A. D. F. *et al.* (1968). *Am. J. Clin. Nutr.*, **21**(10) 1202–07.

MCCANCE, R. A. & WIDDOWSON, E. M. (1960). *Med. Res. Coun. Spec. Rep. Ser.*, No. 297, HMSO, London.

TOEPFER, E. W. *et al.* (1951). *US Dept. Agr. Handbook No.* 29.

CARBOHYDRATE/IRON COMPLEX FORMATION

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ABSTRACT

The concentrations of ferrous and ferric iron in conjunction with the concentrations of selected carbohydrates, necessary for chelation of the iron, are described together with a reappraisal of the spectral evidence for chelate formation. The formation and isolation of ferric/fructose and ferric/high fructose–glucose syrup complexes are described and an analysis of these is presented.

INTRODUCTION

The absorption of iron from the gut is a vital part of man's metabolism since the iron is required largely in the haemoglobin complex in the blood. Shortage of iron leads to anaemia. Whilst loss of iron from the body in a healthy metabolic state is only slight the converse is also true, i.e. absorption into the body is very low. In iron deficiency more is absorbed but the overall amount is still small. If excess iron is ingested a slight increase in its absorption is generally observed and some is excreted. Interest in iron/fructose absorption has recently received much attention (Davis & Deller, 1967; Bates *et al.*, 1972) because of an alleged mutual advantage in the uptake of both sugar and cation. Amine & Hegsted (1975) described an increased utilisation of iron in rats fed a diet containing lactose and less utilisation using sucrose and starch as the carbohydrate sources. Charley *et al.* (1963*a*) and Brodan *et al.* (1967) described the effects of simultaneous ingestion of carbohydrates and iron and reported that whilst fructose enhanced the uptake of iron in the body, glucose had little or no effect. This they attributed to the fact that glucose had little chelating power compared with fructose. Layrisse *et al.* (1976) described increased absorption of iron when taken in conjunction with sucrose and certain beverages and suggested that sucrose should therefore be used as an iron carrier, rather than bread products,

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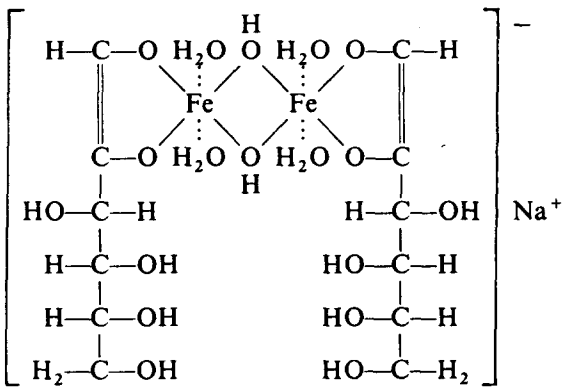
in developing countries. Angyal (1973) described the formation of chelates between sugars and metal ions and found that carbohydrates with an axial–equatorial–axial sequence of three oxygen atoms, on a six membered ring, formed a good site for complex formation with cations. Neither glucose nor fructose possesses such an arrangement, however. Charley *et al.* (1963*b*) described the preparation of these chelates using ferric and ferrous iron and fructose. They also investigated some properties of the chelate and proposed a possible structure for the chelate which they represented in the following figure:



Aasa *et al.* (1964) claim to have confirmed this structure by electron spin resonance (ESR) and nuclear magnetic resonance (NMR) studies of the chelate. It seems doubtful that such a model could exist, however, since fructose in the open chain form would be unlikely to form such a structure.

It has previously been noted that glucose syrup fractions produced by reverse osmosis contained significant amounts of inorganic matter, possibly in a chelated form (Kearsley & Birch, 1975*a*, 1975*b*). If these fractions could chelate iron salts they could be used as carriers for inorganic iron and possibly facilitate its absorption. Some initial work was accordingly directed to producing iron/glucose syrup chelates. In order to develop the techniques of chelate production, iron/glucose and iron/fructose chelates were also prepared.

MATERIALS AND METHODS

Procedure for chelation studies

The method of Charley *et al.* (1963*b*) was used throughout all the preliminary work with slight modification for each carbohydrate. Analar D-glucose, fructose and three glucose syrup fractions (15, 42 and 67 DE) produced as previously described (Kearsley & Birch, 1975*a*) were used with ferric nitrate and ferrous sulphate.

3·5M solutions of D-fructose and D-glucose and 0·1M solutions of ferric nitrate and ferrous sulphate were made up. These were mixed in pre-determined proportions and combinations such that seven dilutions of each carbohydrate (0·25, 0·5, 1·0, 1·5, 2·0, 2·5, 3·0M in the final concentration) had been mixed with seven dilutions of each iron salt (0·002, 0·005, 0·01, 0·02, 0·04, 0·06, 0·1M in the final concentration). Each solution was titrated from the acid pH to pH 10 using 2N sodium hydroxide and any precipitate noted. Any free iron, *i.e.* unchelated, would precipitate as the hydroxide as the pH was raised and thus absence of any precipitate was taken as a means of identifying total chelation of the iron by the carbohydrate.

RESULTS AND DISCUSSION

The results for the test carbohydrates and two iron salts are shown diagrammatically in Fig. 1. Each point within the figure represents each carbohydrate/iron interaction at the concentrations shown on the two axes and the respective line for each interaction the 'cut-point' between precipitation (unstable) and no precipitation (stable) at the concentrations indicated. The results show that more ferric iron can be chelated than ferrous iron by each carbohydrate and that the relative sequestering ability of each sugar for ferric and ferrous iron was fructose > fructose/glucose (1:1) > glucose > > > glucose syrup. The results indicate that glucose syrup would not make a good chelating agent for ferrous or ferric iron. No significant differences were found between the glucose syrups and the results showed a precipitate at *all* concentrations corresponding to those used for glucose and ferric/ferrous iron. (Whilst it was impossible to produce a 3M solution of a 15 DE glucose syrup—about 3·5 kg/litre—the same weight of carbohydrate as in 3M, 2·5M, etc. solutions of glucose was used, i.e. 540 g/litre, 450 g/litre, etc.) Thus it appeared that to chelate iron in reasonable concentrations, D-fructose must either be used as the carbohydrate source or at least be present as 50% of the carbohydrate source.

Recently the so-called high fructose glucose syrups (HFGS) have become commercially available and since a wide interest has been shown in these carbohydrates, they were included in the investigations.

HFGS/iron chelates

The carbohydrate composition of the syrup used in the study was about 47% glucose, 47% fructose and 6% higher saccharides. Since the syrup was composed of the monosaccharides glucose and fructose, the molecular weight was taken to be 180 and again concentrations from 0·5 to 3·0M (in the final concentration) were used. The same ferrous and ferric iron concentrations as previously used were prepared.

The results after the addition of alkali are shown in Fig. 1. Ferric/HFGS solutions show almost exactly the same trends as the results from the ferric/fructose solutions, whilst the ferrous/HFGS solutions do not possess such a wide range of chelating

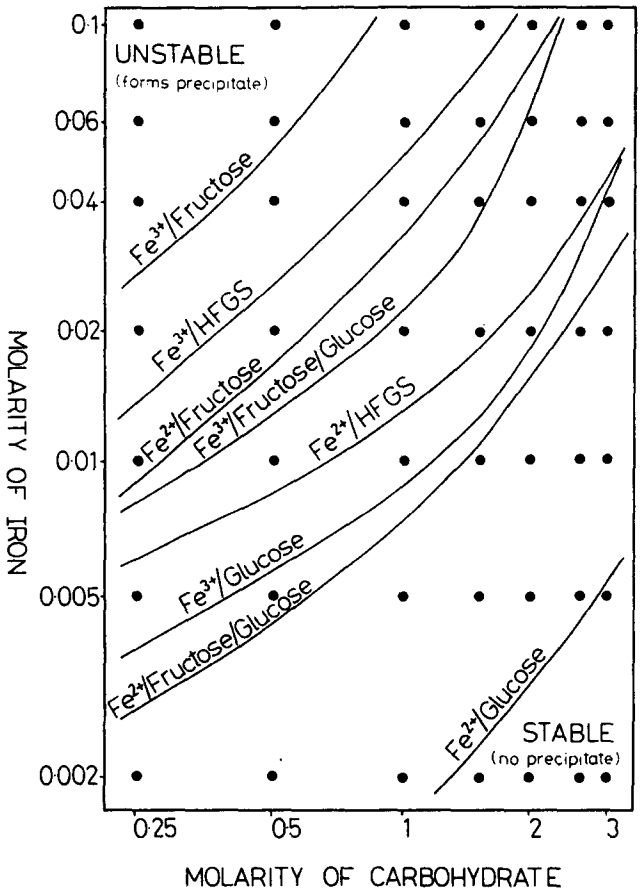


Fig. 1. Chelating concentrations of iron and carbohydrates.

concentrations as ferrous/fructose solutions and formed chelates over much the same range as ferrous/fructose/glucose solutions, as would be expected. Ferric/HFGS gave different results from ferric/fructose/glucose and this cannot easily be explained. It seems unlikely that the 6% higher saccharides of the HFGS would take part in the reaction yet some such interaction must have occurred to account for this 'extra' chelation.

Spectral evidence for chelate formation

Charley *et al.* (1963*b*) used the spectral properties of the iron/carbohydrate solutions as evidence of chelation. A characteristic shift in absorbance supposedly occurred when chelation had taken place. Figure 2 shows the results of absorbance

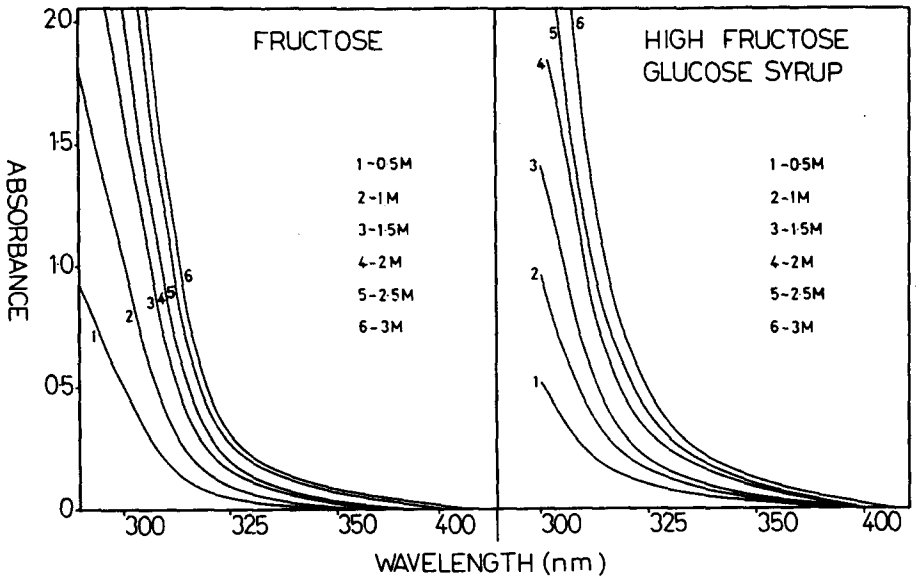


Fig. 2. Absorbance spectra of fructose and high fructose glucose syrup.

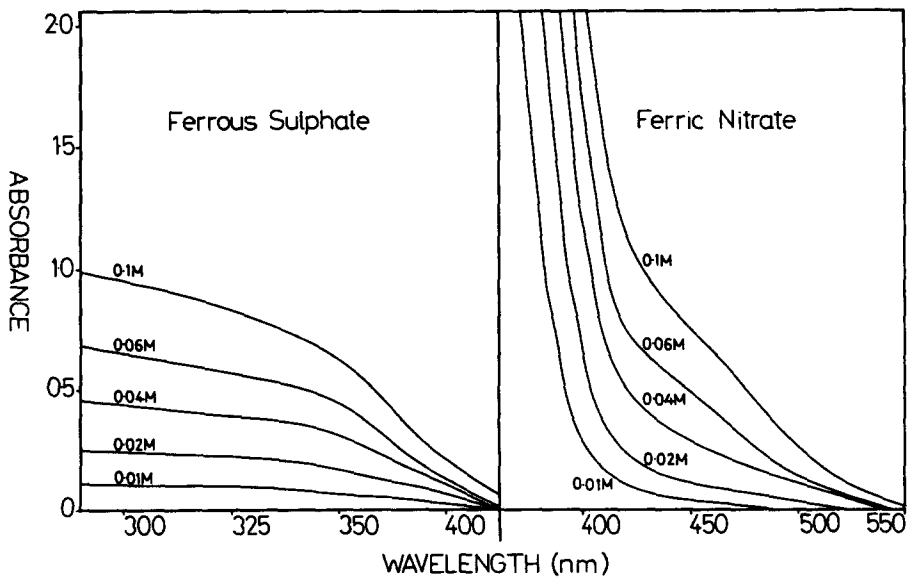


Fig. 3. Absorbance spectra of ferrous and ferric iron.

over the range 290–400 nm for fructose and HFGS and Fig. 3 the results of absorbance over the range 290–550 nm for ferrous and ferric iron. The results indicate that each produced a characteristic peak over some part of the range, and difference spectra (as used to establish chelate formation) must include all components at a particular wavelength. Figure 4 illustrates the spectra obtained with ferrous iron and fructose and Fig. 5 those with ferric iron and fructose. They are basically the same and Figs. 4(a) and 4(b) and Figs. 5(a) and 5(b) in each case are almost identical. The important difference, however, and one which is not shown by the absorbance spectrum is that in (a) in Figs. 4 and 5, total chelation of the iron had not occurred (see Fig. 1.), whilst in (b) in Figs. 4 and 5, total chelation had occurred. Thus the characteristic shift in absorbance also occurs when partial chelation has taken place and the results from such studies must be used only as an indication of chelation. Spectral changes occur according to the molar fraction of iron chelated and when an iron molecule is chelated its absorption is changed completely. Thus an increasing absorbance shift would be expected over the range of concentrations of iron and carbohydrate until at total chelation of the iron a constant shift should be observed.

Production and isolation of ferric/fructose and ferric/HFGS chelates

Since the carbohydrates chelated more ferric than ferrous iron and it is reported that when chelated, ferric iron is as well absorbed as ferrous iron, only ferric chelates were produced. An initial attempt to produce and isolate chelates by the method of Charley *et al.* (1963*b*) was unsuccessful; the method involved mixing carbohydrate and iron salt and adding alcohol to the acid solution to precipitate the chelate. Only trace amounts were precipitated, however, and the method of Barker *et al.* (1974) was then tried as an alternative.

A 3M solution of HFGS and a 3M solution of fructose were made up. A molar solution of ferric nitrate and a 5M solution of sodium hydroxide were prepared and transferred to 50 ml burettes. The ferric nitrate and sodium hydroxide were added simultaneously to the carbohydrate to maintain the pH between 8·5 and 9·0. When 50 ml ferric nitrate had been added the reaction was considered complete. At no time was any precipitate formed and it was assumed that the iron was chelated as soon as it was added to the mixture. Initially the solution was colourless but when the iron and alkali were added it changed to orange, dark brown and finally black. To the black solution, absolute alcohol was added to 80% by volume and the complex was precipitated as a yellow/brown mass which was extremely viscous and sticky. The excess liquid was decanted off and discarded. The precipitate was dissolved in about 60 ml distilled water and the precipitation was repeated. After two further precipitations a creamy-yellow, powdery precipitate remained which was filtered from the supernatant liquid and dried over P_2O_5. This powder when redissolved in water could be quantitatively recovered by the precipitation technique previously described.

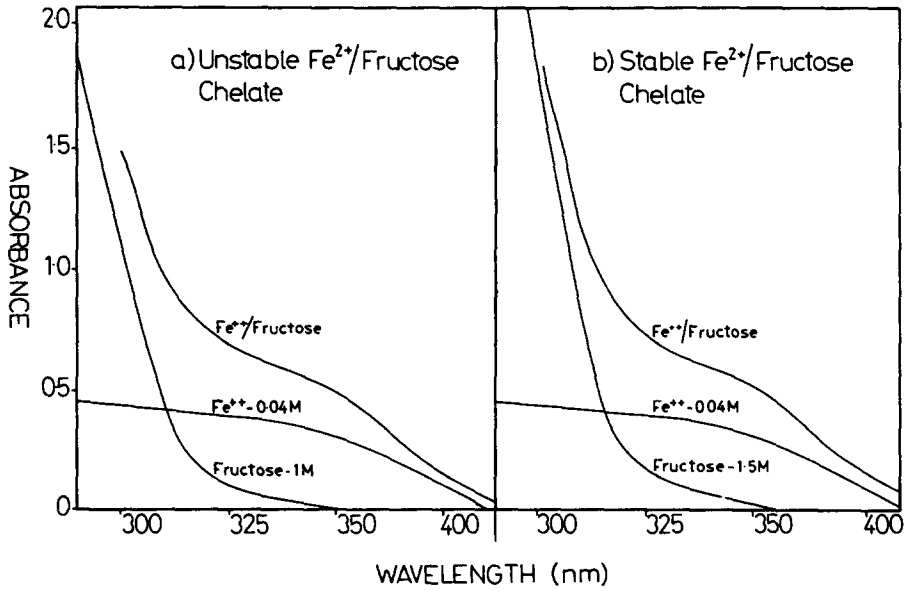


Fig. 4. Absorbance spectra of unstable and stable Fe^{2+}/fructose chelates.

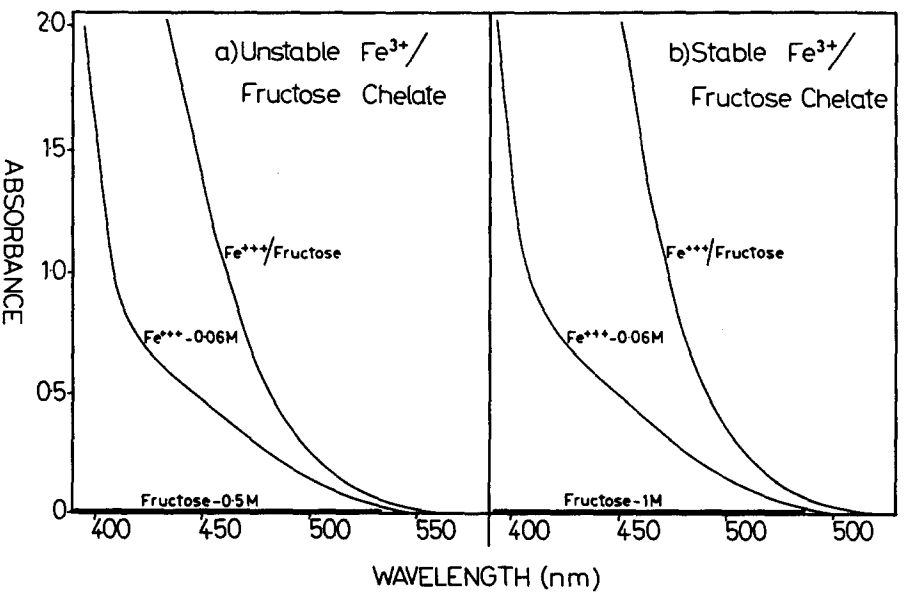


Fig. 5. Absorbance spectra of unstable and stable Fe^{3+}/fructose chelates.

Analysis of chelates

The iron content of the chelates and gustatory properties of the dried powder and aqueous solution of the chelate were considered to be of prime importance since an important outcome of the work was the possible development of a high iron, palatable drink/tablet. Sodium content, molecular weight and optical rotation were also determined.

Gustatory properties: The dried compound was only slightly salty with no metallic taste whatever. When the cream powder was placed in distilled water it dissolved easily and completely to give a dark brown solution at a concentration of 0·3%, the pH of which was 9·1. This solution had a salty and slight caramel taste but again no metallic taste.

Iron content: The iron content of each sample was determined by the Thiocyanate method (CIRF, 1963). 2 g of each was ashed at 525 °C, the ash dissolved in 10 ml of 5M hydrochloric acid and made up to 100 ml for the analysis. A red colour was produced upon the addition of potassium thiocyanate and the optical density was determined using a Unicam SP 600 Spectrometer, 5 minutes after the addition of the thiocyanate.

pH stability: The stability of the iron/fructose and iron/HFGS chelates was determined at two stages in the preparation. First, after the chelate had just been formed, i.e. before the precipitation stage, the chelate was found to be stable over the whole pH range (as judged by no formation of precipitate). Second, the stability of the isolated powdered chelate was determined and this proved to be rather different. A typical ferric hydroxide precipitate formed at about pH 7 as the pH was lowered from pH 9 (using 0·1N HCl). This remained until, at about pH 4, the precipitate redissolved and did not reform as the pH was lowered further. When the pH was raised a precipitate was again formed between pH 4–7 and no precipitate was noted over the range 7–14.

Molecular weight: The molecular weight was calculated from the depression of freezing point produced by a 5% solution of each chelate.

Sodium content: Sodium content was determined using a flame photometer and sodium chloride as standard.

Specific rotation: This was measured using a Bendix Automatic Polarimeter, using sucrose as standard and the results were compared with the specific rotation of fructose. The results of these determinations are shown in Table 1. Charley *et al.* (1963*b*) reported an iron content of 21–22%, a sodium content of 4% and a molecular weight of 594. Barker *et al.* (1974) reported similar results but slight differences in preparation technique may account for slight differences between the reported results and the results obtained above.

A sample of each chelate was also chromatographically analysed. Each chromatogram was divided into two parts. On one part the sugar(s) was located using the conventional means (CIRF, 1963) and on the other the iron was located using an acidified (HCl) thiocyanate spray. The fructose/ferric complex revealed one

TABLE 1
PROPERTIES OF FRUCTOSE AND FERRIC/FRUCTOSE AND FERRIC/HFGS CHELATES

Property	*Fructose*	*Fructose/ ferric chelate*	*HFGS/ ferric chelate*
Iron content	—	14·4%	13·5%
Molecular weight	180	571	575
Sodium content	—	5·6%	6·1%
Specific rotation	−93·4	−39·8	−44·4

spot for the carbohydrate half way down the paper and an iron 'spot' at the origin. The HFGS/ferric complex revealed glucose, fructose, maltose and maltotriose with iron again remaining at the origin. This tends to suggest the complex is not as strong as first thought since the iron and carbohydrate are so easily separated. However, to remain chelated at alkaline pH a strong chelate must initially be formed. There exists the possibility that during the development of the chromatogram the sodium salt of the chelate is disrupted by the mobile phases, thus 'freeing' the iron. Although this raises a query about the real existence of the chelate, the method used for its preparation unequivocally confirms the absence of ferric or ferrous ions. It was further demonstrated that if alcohol was added to a concentration of 80% by volume to solutions of carbohydrate and iron, separately, at the concentrations used in the experiment, no precipitate could be obtained, thus showing that both must be present, presumably as a chelate, for precipitation to occur.

ACKNOWLEDGEMENTS

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REFERENCES

AASA, R., MALMSTROM, B., SALTMAN, P. & VANGARD, T. (1964). *Biochim. biophys. Acta*, **80**, 430.
AMINE, E. K. & HEGSTED, D. M. (1975). *J. Agr. Food Chem.*, **23**(2), 204.
ANGYAL, S. J. (1973). *Pure Chem.*, **35**, 131.
BARKER, S. A., SOMERS, P. J. & STEVENSON, J. (1974). *Carbohyd. Res.*, **36**, 331.
BATES, G. W., BOYER, J., HEGENAUER, J. C. & SALTMAN, P. (1972). *Am. J. clin. Nutr.*, **25**, 983.
BRODAN, V., BRODANOVA, M., KUHN, E., KORDAC, V. & VALEK, J. (1967). *Nutr. Dieta.*, **9**, 263.
CHARLEY, P. J., STITT, C. F., SHORE, E. & SALTMAN, P. (1963*a*). *J. Lab. clin. Med.*, **3**, 397.
CHARLEY, P. J., SARKAR, B., STITT, C. F. & SALTMAN, P. (1963*b*). *Biochim. biophys. Acta*, **69**, 313.
CIRF (1963). *Standard Analytical Methods of the Member Companies*, 1st edn., Corn Industries Foundation, Washington, DC.
DAVIS, P. S. & DELLER, D. J. (1967). *GUT*, **8**, 300.
KEARSLEY, M. W. & BIRCH, G. G. (1975*a*). *J. Fd. Technol.*, **10**, 613.
KEARSLEY, M. W. & BIRCH, G. G. (1975*b*). *J. Fd. Technol.*, **10**, 625.
LAYRISSE, M., TORRES, C. M., RENZI, M., VELEZ, F. & GONZALEZ, M. (1976). *Am. J. clin. Nutr.*, **29**, 8.

DETECTION OF ADULTERATION IN CITRUS JUICE BEVERAGES†

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ABSTRACT

The detection of adulteration in citrus juices is a challenge to industry and regulatory agencies. Early work was directed towards detecting citric acid added to lemon juice. More recently, efforts have been made to develop methods of detecting adulteration in orange juice and to determine orange juice content in diluted orange beverages.

INTRODUCTION

The problem of determining juice content or authenticity of citrus-based products has been a concern of citrus processors, citrus juice users, and governmental regulatory agencies for a long time. The Fruit and Vegetable Chemistry Laboratory, United States Department of Agriculture has been actively working on this problem from 1961 to 1976 (Vandercook *et al.*, 1963; Vandercook & Rolle, 1963; Rolle & Vandercook, 1963; Vandercook & Yokoyama, 1965; Vandercook *et al.*, 1966; Vandercook & Stephenson, 1966; Vandercook & Guerrero, 1968; Vandercook & Guerrero, 1969; Vandercook, 1970; Vandercook *et al.*, 1970; Vandercook & Price, 1972; Vandercook *et al.*, 1973; Vandercook & Price, 1974; Vandercook *et al.*, 1975; Vandercook & Smolensky, 1976*a*; Vandercook & Smolensky, 1976*b*) and this paper summarises some of this work.

LEMON JUICE

Lemon juice is sold commercially on the basis of its total acidity, which is primarily citric acid. Since synthetic citric acid costs about one-fifth the price of that in lemon

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juice, the temptation exists to adulterate the juice with citric acid. A multiple regression equation for determining added citric acid in lemon juice was developed (Vandercook *et al.*, 1963; Vandercook & Rolle, 1963; Rolle & Vandercook, 1963). The determination was based on predicting citric acid concentration from an expression involving the concentrations of the total amino acids, malic acid, and total phenolics. The predicted level of citric acid was compared with the measured value, and if the difference exceeded 19·1 mEq/100 ml, the juice was considered to be outside the 99 % confidence limits. The approach was originally designed for single-strength juice, and the detection sensitivity depended upon final juice concentration.

Subsequently, by a different approach based on ratios, data were examined from 289 samples of commercial and fresh lemon juices and concentrates from California, Arizona, Florida, and Italy processed between 1959 and 1969 (Vandercook *et al.*, 1973). Ratios were used to give an expression which would be independent of concentration. The need to eliminate concentration dependence lies in the manufacturing process. To make lemon juice concentrate commercially, water is removed until the acidity reaches the desired level. Thus, a larger volume of low-acid juice or a lesser volume of a high-acid juice is required to make a particular concentrated product. The volume–concentration factor usually is not known; therefore, reconstitution is to an approximate or average value. For detection of adulteration, any approach which is based on concentrations of the juice constituents is limited to single-strength products or is made less sensitive by an unknown dilution factor.

The ratios of amino acids to total phenolics (AA:TP) and malic acid to total phenolics (MA:TP) were independent of dilution or added citric acid; citric acid to total phenolics (CA:TP) was also independent of dilution but would reflect added citric acid. The mean and standard deviation for CA:TP were 177 and 72·3, respectively. Because of this variability, the ratio by itself was of little use for the detection of adulterations with citric acid.

The two-dimensional relationships of CA:TP to AA:TP and MA:TP are shown in Figs. 1 and 2. These plots show the correlations between the variables. The standard deviations of CA:TP independent of AA:TP and MA:TP were 30·3 and 35·2, respectively. These represent highly significant reductions in the overall variability.

When the data were combined in a multiple regression expression, the variance in CA:TP was reduced even further. The multiple regression equation was CA:TP $= 10{\cdot}22 + 31{\cdot}22(\text{AA:TP}) + 6{\cdot}36(\text{MA:TP})$. The standard deviation of CA:TP, independent of AA:TP and MA:TP, was reduced to 20·8. The coefficient of determination (R^2) was 0·918, which means that 91·8 % of the variability of CA:TP was attributed to the linear relationships with AA:TP and MA:TP.

When the measured CA:TP value was divided by that value predicted from the equation, a symmetrical distribution was obtained with a mean value of 1·00 and

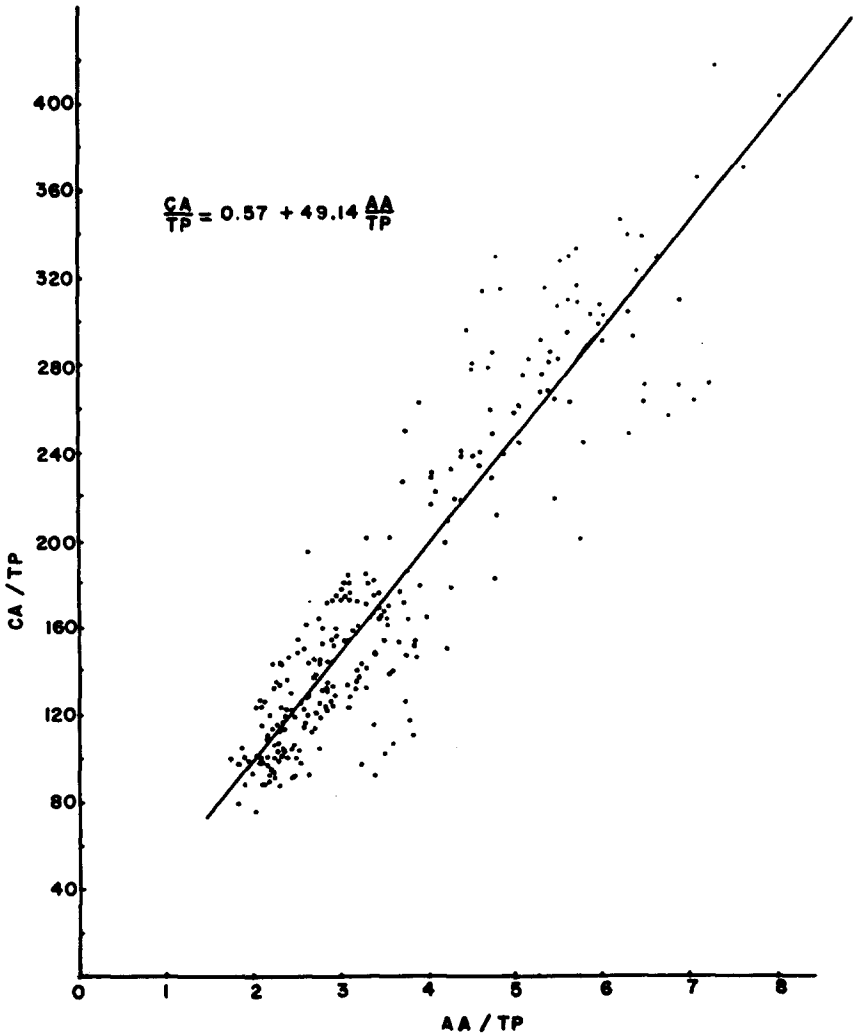

Fig. 1. Relationship between the citric acid–total phenolics ratio (CA:TP) and the amino acid–total phenolics ratio (AA:TP) in lemon juice samples.

standard deviation of 0·112. The sample distribution and the appropriate normal distribution curves are shown in Fig. 3.

The effect of added citric acid on the measured to predicted CA:TP ratio is to increase its value. On the basis of a normal distribution of the measured to predicted function, a critical value (θ) can be calculated as follows:

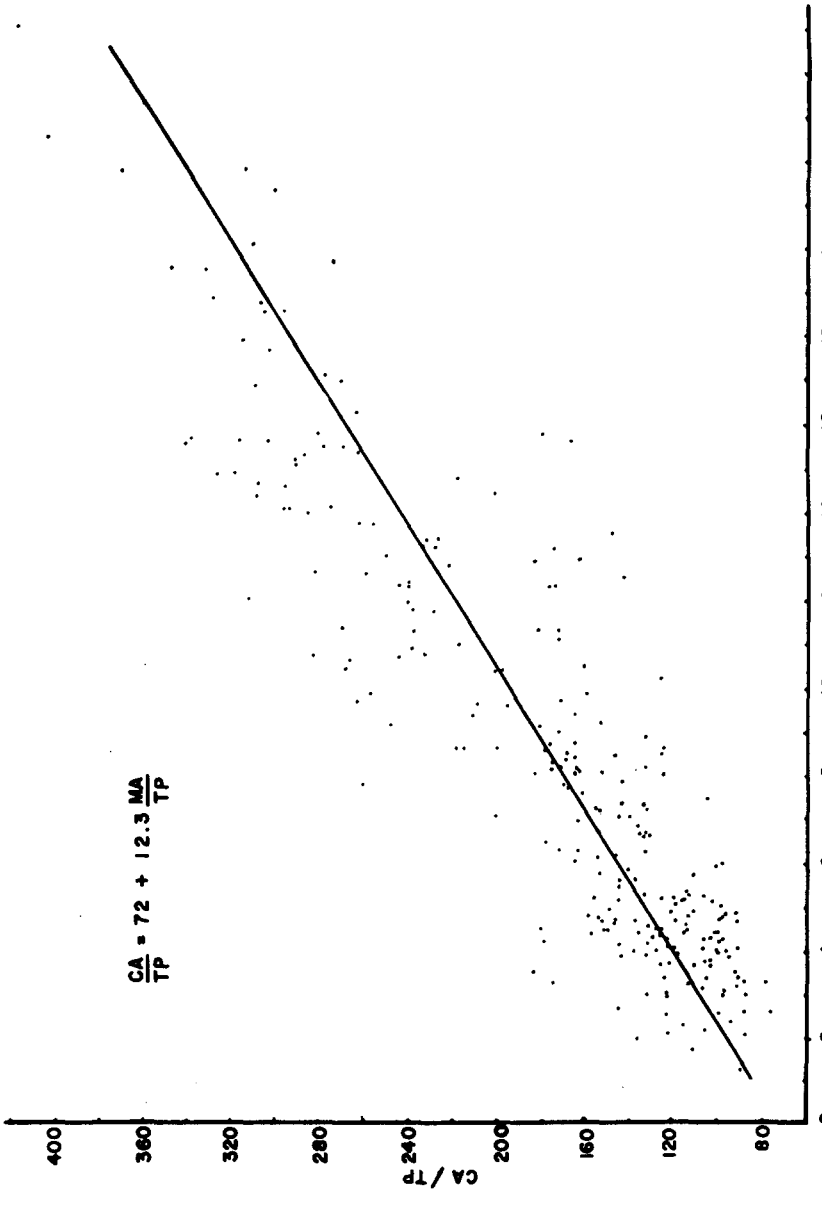

Fig. 2. Relationship between the citric acid–total phenolics ratio (CA:TP) and the malic acid–total phenolics ratio (MA:TP) in lemon juice samples.

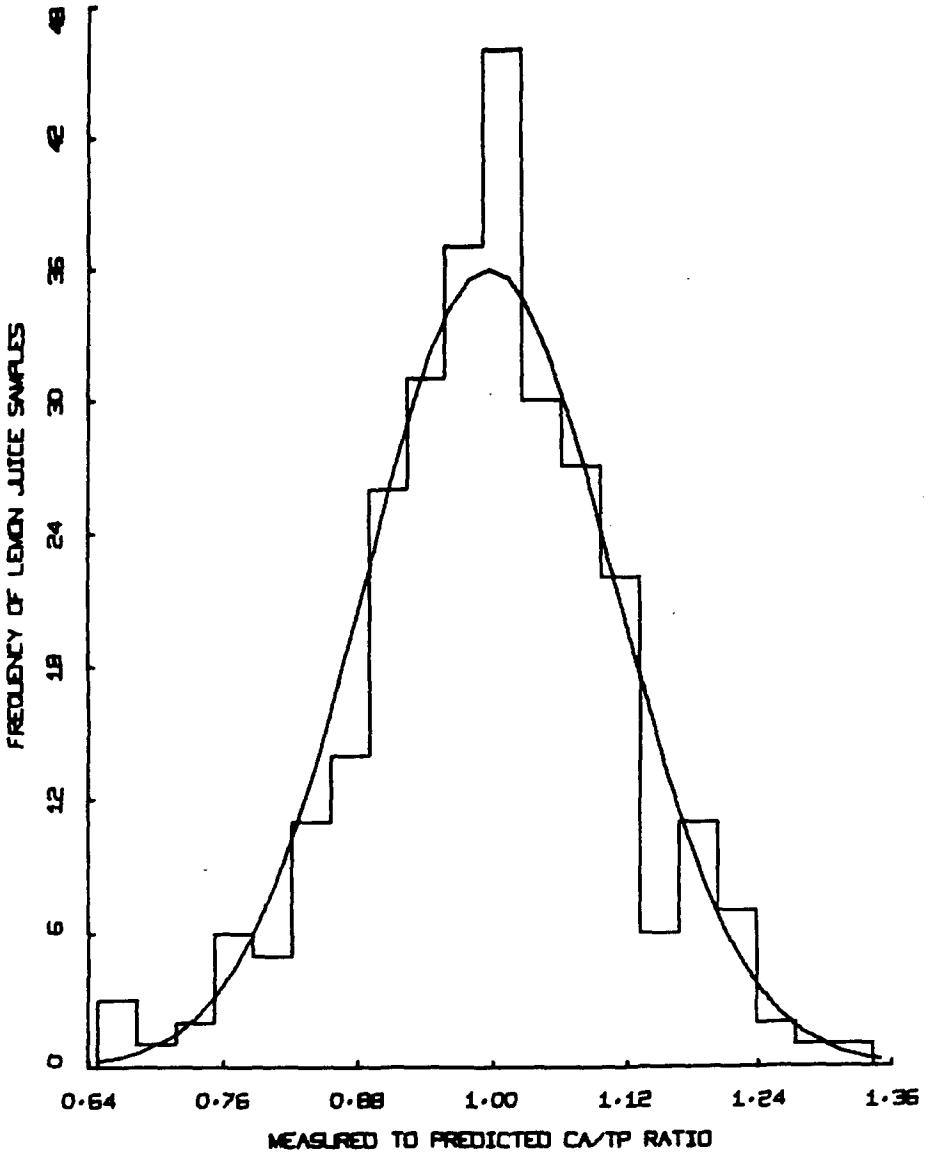

Fig. 3. Normal distribution curve and frequency distribution of measured-to-predicted (CA:TP) ratios.

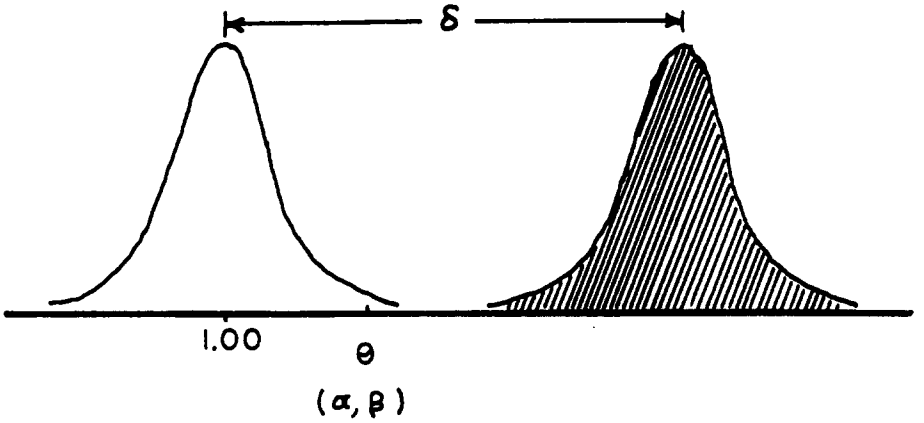

Fig. 4(a). High level of adulteration with negligible overlap of authentic and adulterated (shaded) populations.

$$\theta = 1{\cdot}00 + Z_{\alpha}(0{\cdot}112)/\sqrt{n}$$

where Z is the standard deviate for a one-tail test and α = probability of exceeding θ when the mean is 1·00. When the observed mean of n observations exceeds the critical value, then it is significantly higher than 1·00 at the α probability level.

If a group of lemon juice samples from the normal population were adulterated with citric acid, a new distribution would be obtained. It would be shifted to the right by the amount of adulteration, δ. If the adulteration is large enough, as in Fig. 4(a), there will be no appreciable overlap where the adulterated samples have values less than the critical value θ. Also there is associated with θ, a β probability that a sample

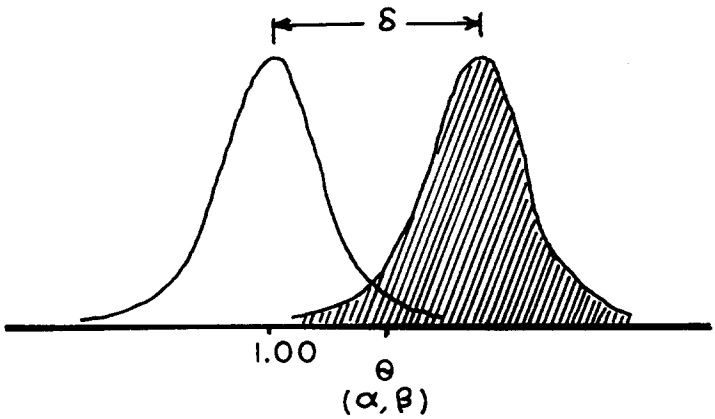

Fig. 4(b). Lower level of adulteration with appreciable overlap.

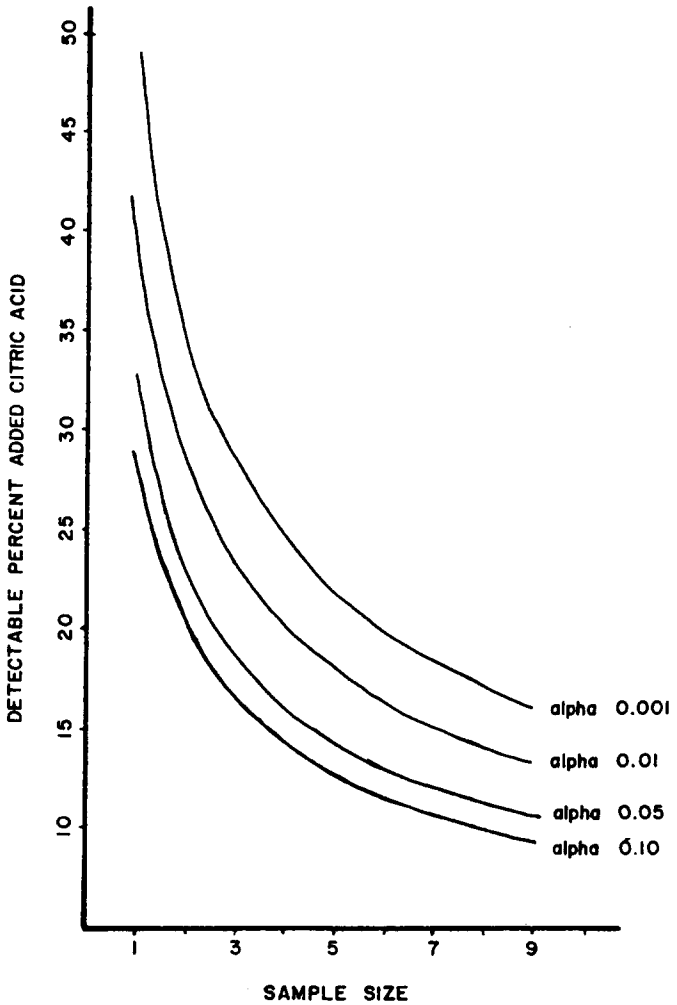

Fig. 5. Number of samples required to give 90 % probability of detecting a given percent of added citric acid with a probability α of wrongly rejecting an unadulterated juice.

from the adulterated population will be less than θ. In one sense, this is the risk of accepting an adulterated sample as authentic. In a more likely situation with a somewhat lower level of adulteration (Fig. 4(b)), the overlapping of the two distributions presents a few problems. First, a critical value θ must be chosen, which represents a compromise between the α probability of rejecting an authentic sample and the β probability of accepting an adulterated sample. The two probabilities and the level of adulteration are interrelated, and the consequences of being right or

wrong in both cases must be weighed. These are policy-type decisions based on legal, economic, and political considerations.

The relationship between the level of adulteration (δ), the two probabilities (α, β), and the number of samples analysed (n) is given by:

$$\delta = \theta + Z_{\beta}(0{\cdot}112)/\sqrt{n}$$

where θ is the critical value, as calculated above, and δ is the population mean value for the measured to predicted CA:TP ratio that gives $1 - \beta$ probability that a sample mean is significantly higher than 1·00. This is graphically illustrated in Fig. 5 where $\beta = 0{\cdot}1$, which means 90% of adulterated samples would be rejected.

ORANGE JUICE

The development of methods for determining orange juice adulteration or juice content in orange products required the analysis of many samples. An automated analytical system was designed (Vandercook *et al.*, 1975) to speed up data acquisition and also to simplify subsequent data collection for enforcement purposes. Five analyses could be made on the one manifold with only minor changes for conversion from one analysis to the next. The system was designed to measure total sugars, reducing sugars, total amino acids, total acidity and total phenolics. The sample preparation is minimal; it requires only a single strength juice free from large pulp particles.

The basic manifold (Fig. 6) was set up for the total sugar determination and was the most complex with respect to tubing and reagents. Sucrose in the juice was

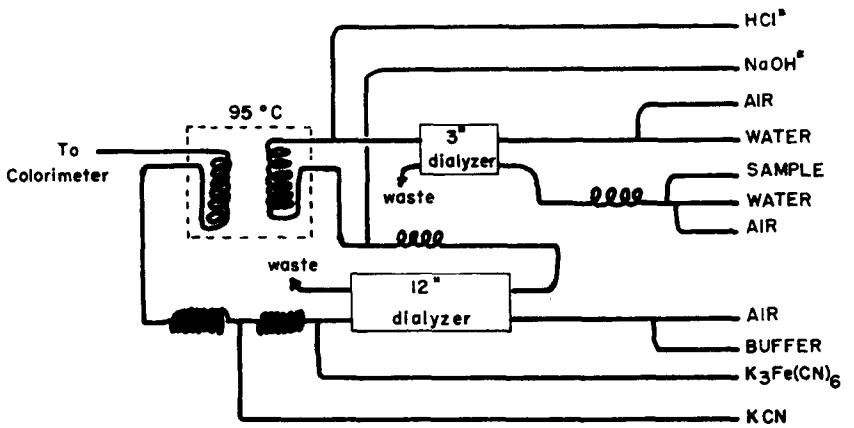

Fig. 6. Schematic flow diagram for total and reducing sugars. Reagent lines marked with asterisk are changed to water for reducing sugar determination.

hydrolysed by HCl in the first heating coil. The acid was subsequently neutralised with NaOH, and the hydrolysate was further diluted in the 12 in dialyser. The colorimetric reaction (Technicon, 1972) with $K_3Fe(CN)_6$ occurred in the second heating coil, and the final read-out was in the colorimeter. For the reducing sugars, the HCl and NaOH lines were replaced by distilled water, and the samples were run through a second time.

The total amino acids were determined by a ninhydrin colorimetric method (Technicon, 1968). The procedure was modified slightly in that absorbance was read at 480 nm, because molar absorbances for reaction products of individual amino acids are more nearly equal at that wavelength. Standards consisted of a mixture of the average composition of the major amino acids in orange juice. The changes in the manifold (Fig. 7) included those of the sample inlet block from the 3 in to the 12 in dialyser, the reagents, and the colorimeter filter.

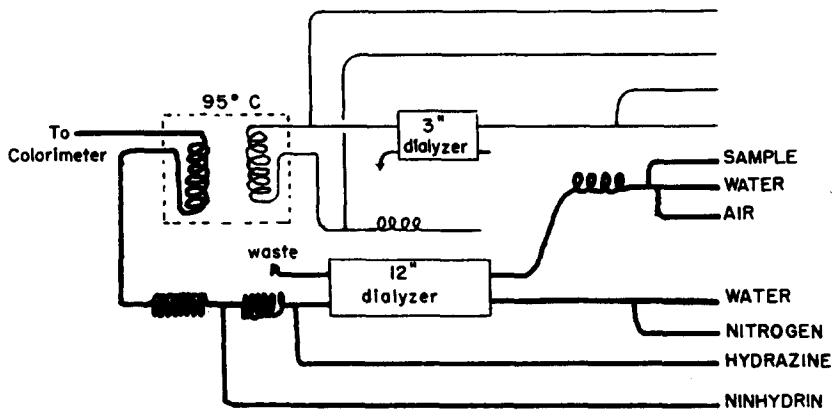

Fig. 7. Schematic flow diagram for total amino acids (heavy lines). The light lines represent unused portions of the manifold.

A total acidity procedure, with buffers and bromocresol green indicator, was adapted to orange juice (Walkley & Buchanan, 1968). The heating coil was not necessary for this measurement, so it was bypassed as shown in Fig. 8. The second diluent line to the coil after the dialyser was not needed, so it was removed from the pump and clamped shut. The colour yield was linear from 7–18 mEq/100 ml, which includes the acidity range of orange juice.

The phenolics of the juice were estimated by the coupling reaction with diazotised sulphanilic acid. They were expressed as phenol, which was used as the standard. The changes in the manifold are shown in Fig. 9. The inlet blocks for sample and reagent to the 12 in dialyser were reversed so that reagent mixing was facilitated.

Arginine and γ-aminobutyric acid were determined by a semi-automated procedure (Vandercook & Price, 1974; Vandercook *et al.*, 1975) with a short (2 × 75 mm) ion-

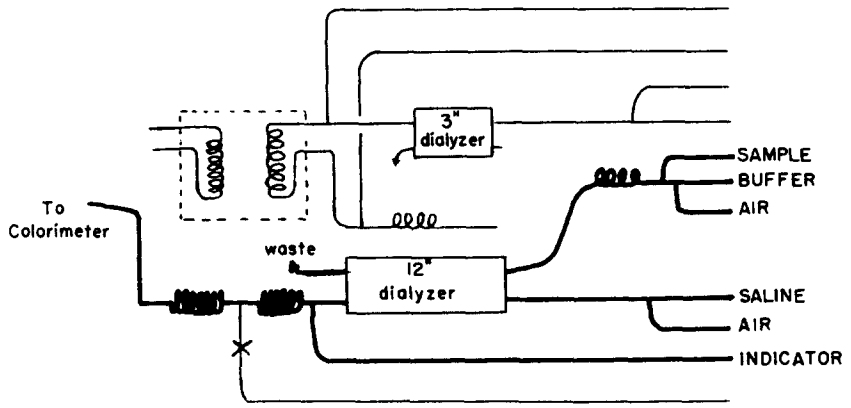

Fig. 8. Schematic flow diagram for total acidity (heavy lines). The light lines represent unused portions of the manifold.

exchange column and a lithium citrate buffer gradient from pH 4·3 to 11·3 (Fig. 10). Each determination of the two amino acids took 25 min. The resolution in the chromatogram was sufficient that added ammonium salts (to adjust the total amino acids or formol number) showed as an extraneous peak. The major acidic and neutral amino acids were not resolved with this system.

A new statistical approach for detecting adulteration in orange juice (Schatzki & Vandercook, 1976) has been developed. As with lemon juice, ratios were again used to eliminate the concentration effects of the various reconstituted products. The orange juice data obtained by the automated procedures were expressed as ratios relative to the total sugars. The resulting six ratios were transformed into logarithmic

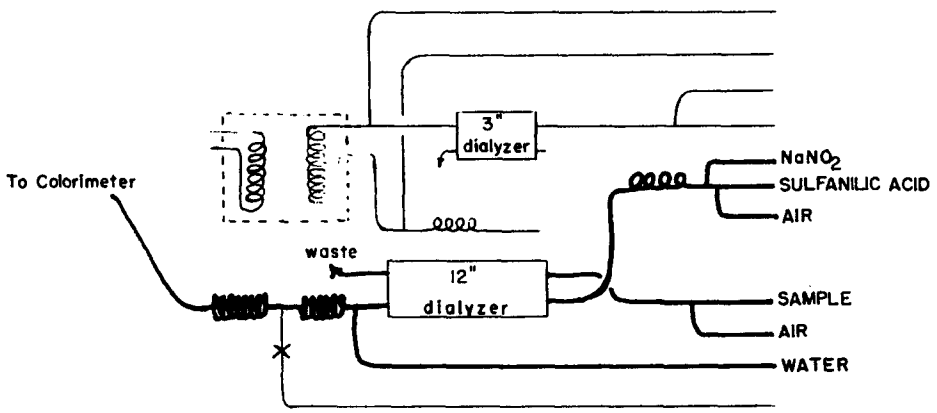

Fig. 9. Schematic flow diagram for phenolics (heavy lines). The light lines represent unused portions of the manifold.

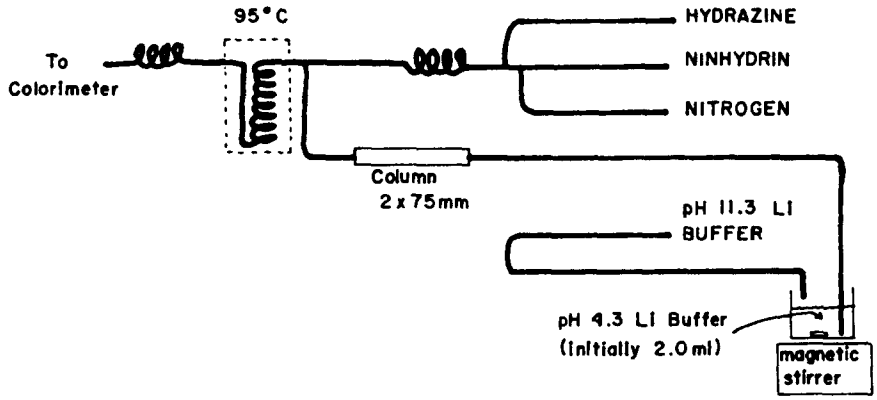

Fig. 10. Schematic diagram for γ-aminobutyric acid and arginine determination.

functions and used in a non-parametric statistical approach for classification of the authentic juices into categories based on variety and growing area. Adulteration of a particular juice with sugar and/or acid moved the sample out of its normal region but not into any other authentic region. Non-parametric statistics has the advantage of not requiring any normality assumptions or prior knowledge of the population distributions.

Three different types of adulteration were considered. The simplest was the addition of sucrose only. Next was the addition of sucrose and reducing sugars, in the proper proportion, and third was the addition of sucrose, reducing sugars, and citric acid. Figure 11 shows the effect of removing analytical parameters on our ability correctly to classify juices mathematically adulterated to the 10, 20, and 40% levels. Some ability to discriminate between authentic and adulterated juices is lost when the total acid and reducing sugars are omitted, but an adulterated mixture would very likely contain sugar and acid in the natural proportions to avoid detection and for flavour considerations. It appears that a 20% adulteration even with the appropriate mixture of sugars and acid could still be detected about 85% of the time.

MICROBIOLOGICAL ASSAY FOR ORANGE JUICE ADULTERATION DETECTION

A microbiological assay has been developed to help detect adulteration in orange juice (Vandercook & Smolensky, 1976*a* & 1976*b*). The organism *Lactobacillus plantarum* was selected for the assay because of its fastidious nature. Growth of this organism in orange juice was thought to depend upon the many minor nutrients which would be difficult to analyse individually as well as expensive and impractical

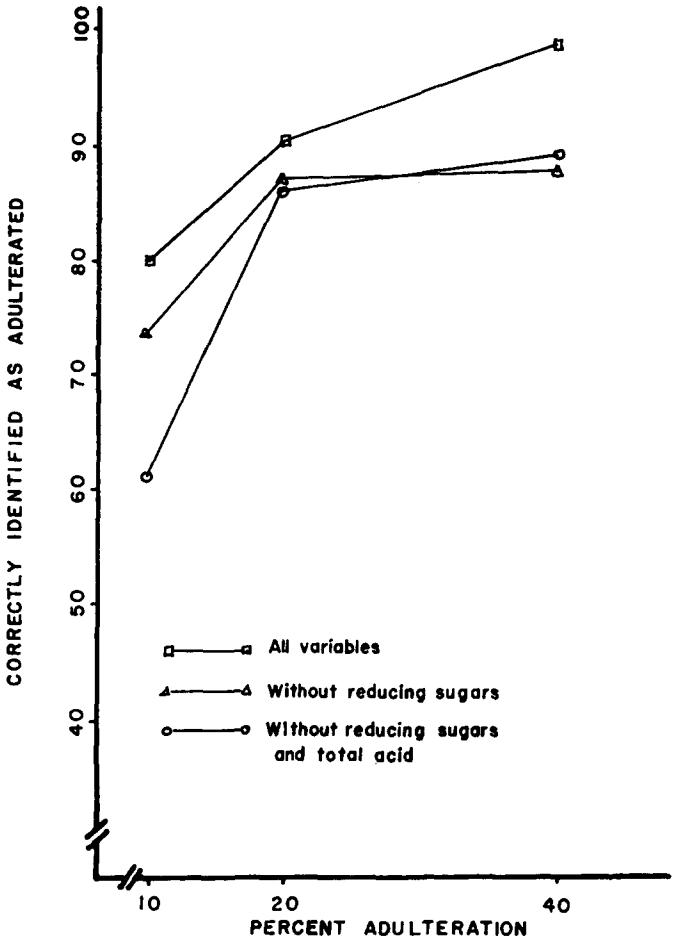

Fig. 11. The effect of removing variables from the non-parametric classification system for identifying adulterated orange juice samples.

to add. In fact, the growth of *L. plantarum* under standard assay conditions was proportional to the amount of orange juice in the assay mixture. Furthermore, as expected, imitation orange beverages did not support growth. In addition, normal levels of common beverage ingredients, such as sugar, citric acid, butylated hydroxyanisole and orange oil, did not significantly affect growth.

The assay procedure was similar to other microbiological methods. The sample was centrifuged and an aliquot of the serum was adjusted to pH 6·5 and then diluted to volume. Replicate culture tubes were sterilised then inoculated with the bacterium. After a 30-hour incubation period, the growth in the assay mixture was

determined by a turbidity measurement. The growth, in units of absorbance, was corrected to a standard dilution of 11·8° Brix.

The precision of the assay and day-to-day variations are shown in Table 1. A large batch of orange juice was prepared and the culture tubes were frozen. A set of the standards was withdrawn and run with each group of samples over a period of several weeks; the test involved two analysts. From an analysis of variance the standard deviations were 2·4% and 5·4% of the mean due to experimental error and daily variation, respectively.

Orange juices and orange concentrates (reconstituted) from Florida, California and Mexico were assayed by this procedure. Data for these samples are presented in Table 2 as absorbance values. Each value should be considered as a parameter of the particular sample. The mean and standard deviation for the 30 samples were 0·378 ± 0·092. The standard deviation was 24% of the mean and was lower than standard

TABLE 1
REPLICATION OF ASSAY FOR A COMPOSITE ORANGE JUICE BY TWO ANALYSTS ON NINE DIFFERENT DAYS

Analyst	*Measured absorbance*[a]	*Daily determination means*	*Analyst means*
A	0·337, 0·342, 0·346, 0·333, 0·337 0·325, 0·329	0·336	
A	0·333, 0·333, 0·329, 0·329, 0·329	0·331	
A	0·325, 0·316, 0·320	0·320	0·331
B	0·340, 0·340, 0·370	0·350	
B	0·340, 0·340, 0·325	0·335	
B	0·330, 0·330, 0·330	0·330	
B	0·310, 0·310, 0·320	0·313	
B	0·340, 0·330, 0·330	0·333	
B	0·340, 0·320, 0·340	0·333	0·333

Overall composite orange juice mean and standard deviation = 0·332 ± 0·011
[a] Not corrected for soluble solids.

TABLE 2
MICROBIOLOGICAL ASSAY VALUES FOR 30 DIFFERENT ORANGE JUICES FROM THREE REGIONS

Source	*Sample absorbance means*[a]	*Source mean*
California	0·456, 0·364, 0·616, 0·425, 0·411, 0·464, 0·271, 0·341, 0·413, 0·387, 0·490, 0·488	0·410
Florida	0·318, 0·314, 0·370, 0·400, 0·384, 0·350, 0·295, 0·268, 0·309, 0·253, 0·205, 0·425, 0·460	0·335
Mexico	0·355, 0·535, 0·352, 0·234, 0·372, 0·385	0·372

All samples: Mean = 0·378, Standard Deviation ± 0·092, Coefficient of Variance = 24%
[a] Absorbance adjusted to 11·8% soluble solids.

deviations for some of the juice constituents investigated previously in this laboratory (Vandercook *et al.*, 1973; Vandercook *et al.*, 1975).

Absorbance would be lower for an adulterated than for a full strength orange juice, and if the value fell below a predetermined level the product would be rejected. Assays of several samples from a given source would increase the probability that authenticity would be correctly identified. Nevertheless, because of the variability between samples, the microbiological assay probably would not be used as the only test of authenticity. It will be necessary to analyse more samples by this assay and other procedures for selection of the best combination of tests. Also, it is possible that organisms with different nutrient requirements would complement each other in a series of assay procedures for detecting adulterations. Work is continuing along these lines.

REFERENCES

ROLLE, L. A. & VANDERCOOK, C. E. (1963). Lemon juice composition. III Characterization of California–Arizona lemon juice by use of a multiple regression analysis, *J. Assoc. Off. Anal. Chem.*, **58**, 362–5.

SCHATZKI, T. F. & VANDERCOOK, C. E. (1976). Manuscript in preparation.

TECHNICON INDUSTRIAL SYSTEMS (1968). Amino acids, *Research Bulletin No.* 20, Tarrytown, New York.

TECHNICON INDUSTRIAL SYSTEMS (1972). Total and reducing sugars, *Industrial Method No.* 142–71*A*, Tarrytown, New York.

VANDERCOOK, C. E. (1970). Changes in the spectral properties of lemon juice under adverse storage conditions, *J. Fd Sci.* (Research note), **35**, 517–18.

VANDERCOOK, C. E. & GUERRERO, H. C. (1968). Effects of chemical preservatives and storage on constituents used to characterize lemon juice, *J. Assoc. Off. Anal. Chem.*, **51**, 6–10.

VANDERCOOK, C. E. & GUERRERO, H. C. (1969). Citrus juice characterization: Analysis of the phosphorus fractions, *J. Agr. Fd Chem.*, **17**, 626–8.

VANDERCOOK, C. E., GUERRERO, H. C. & PRICE, R. L. (1970). Citrus juice characterization. Identification and estimation of major phospholipids, *J. Agr. Fd Chem.*, **18**, 905–7.

VANDERCOOK, C. E., MACKEY, B. E. & PRICE, R. L. (1973). New statistical approach to evaluation of lemon juice, *J. Agr. Fd Chem.*, **21**, 681–3.

VANDERCOOK, C. E. & PRICE, R. L. (1972). The application of amino acid composition to the characterization of citrus juice, *J. Fd Sci.*, **37**, 384–6.

VANDERCOOK, C. E. & PRICE, R. L. (1974). Rapid determination of γ-aminobutyric and arginine in orange juice: application to detecting adulteration, *J. Assoc. Off. Anal. Chem.*, **57**, 124–9.

VANDERCOOK, C. E., PRICE, R. L. & HARRINGTON, C. A. (1975). Multiple automated analyses for orange juice content: determination of total sugars, reducing sugars, total amino acids and phenolics, *J. Assoc. Off. Anal. Chem.*, **58**, 482–7.

VANDERCOOK, C. E. & ROLLE, L. A. (1963). Lemon juice composition. II. Characterization of California–Arizona lemon juice by use of its polyphenolic content, *J. Assoc. Off. Anal. Chem.*, **58**, 359–62.

VANDERCOOK, C. E., ROLLE, L. A. & IKEDA, R. M. (1963). Lemon juice composition. I. Characterization of California–Arizona lemon juice by its total amino acid and *l*-malic acid content, *J. Assoc. Off. Agr. Chem.*, **46**, 353–8.

VANDERCOOK, C. E., ROLLE, L. A., POSTLMAYR, H. L. & UTTERBERG, R. A. (1966). Lemon juice composition. V. Effects of some fruit storage and processing variables on the characterization of lemon juice, *J. Fd Sci.*, **31**, 58–62.

VANDERCOOK, C. E. & STEPHENSON, R. G. (1966). Lemon juice composition. VII. Identification of the major phenolic compounds and estimation by paper chromatography, *J. Agr. Fd Chem.*, **14**, 450–4.

VANDERCOOK, C. E. & SMOLENSKY, D. C. (1976*a*). A potential microbiological assay of fruit content in orange juice products, *J. Fd Sci.*, **41**, 709–10.

VANDERCOOK, C. E. & SMOLENSKY, D. C. (1976*b*). Microbiological assay with *Lactobacillus plantarum* for detection of adulteration in orange juice, *J. Assoc. Off. Anal. Chem.*, **59**, 1375–9.

VANDERCOOK, C. E. & YOKOYAMA, H. (1965). Lemon juice composition. IV. Carotenoid and sterol content, *J. Fd Sci.*, **30**, 865–8.

WALKLEY, V. T. & BUCHANAN, J. A. (1968). Automated analysis applied to soft drinks production and quality control, *Automat. Anal. Chem. Technicon Symp.*, **2**, 339–48.

BOOK REVIEWS

Intermediate Moisture Foods. Ed. by R. Davies, G. G. Birch and K. J. Parker. London, Applied Science Publishers Ltd., 1976. xii + 306 pp. Price: £15·00.

The preservation of food by techniques such as sun-drying and salting has been known from historic times but it was only in 1957 that Scott was the first reviewer who stressed the importance of water relations and introduced the concept of water activity to microbiologists. Since that time a range of products has appeared on the market, particularly in the pet food area, which are shelf stable without refrigeration and achieve their stability by a reduction in the water activity. In the case of pet foods this can be achieved by the inclusion of polyhydric alcohols or high sugar concentrations but this results in products which are organoleptically unacceptable to man. There is now increasing interest, however, in exploiting the principles in the production of human foods and the conference held at the National College of Food Technology in 1976 was, therefore, very timely. The Editors should be commended upon producing a printed version of the papers so soon after the conference and in particular, upon the inclusion of the discussions which tend to highlight some of the important points in the papers which were presented.

Inevitably in a book which contains conference papers, there is duplication and disagreement. As an instance of duplication, the detailed and rather long formula of the ready-to-eat intermediate moisture roast beef cubes developed by Swift & Company for US Air Force is given by both Karel and by Robson. There is also some disagreement between the various authors as to the precise boundaries at which one considers a product to be of intermediate moisture content. These are, however, minor points of criticism in an otherwise excellent book which contains a reasonable balance of food technology, theoretical physics and microbiology.

The early part of the book is of value in helping the food technologist decide whether or not this should be a major area of investigation for his company. The intermediate section starts explaining the physical properties which will have to be measured in order that he may develop products which he understands. One of the major problems is, of course, the measurement of water activity and it is disappointing that only one paper is devoted to this topic and the substantial part of

Fd. Chem. (2) (1977)—
Printed in Great Britain

this paper discusses very academic techniques. The two pages devoted to instrumental techniques make it clear, however, that with all the instruments which are currently available there are great problems in achieving the stability which is necessary in a commercial operation. The paper on chemical and non-enzymic changes in intermediate moisture foods emphasises that despite the fact that a product may be microbially stable, chemical changes and particularly those which lead to the production of water, such as browning, may well be accelerated as the water activity is decreased and in certain types of foodstuffs this may be a shelf-life limiting factor.

Roughly half the book is devoted to the microbiological aspects of the storage of food, particularly with respect to food poisoning organisms. While it is understandable that the present state of knowledge is reflected in these proportions, it does indicate that physical and chemical aspects of these products need increased effort. The book contains no promise that intermediate moisture foods will suddenly appear on the human food market.

It is quite clear that the materials which can be added to food to reduce the water activity which are both safe and organoleptically acceptable are extremely limited and it appears likely from the book that the products will be produced by a dehydration–rehydration procedure which will limit the use of added chemicals.

Generally, then, this is an excellent starting point for the food technologist who is interested in exploring the potential of this new range of products and is a useful reference book for the microbiologist who has to deal with these problems.

A. W. HOLMES

Meat Science. An International Journal devoted to furthering the scientific understanding of meat. Edited by Professor R. A. Lawrie. London, Applied Science Publishers.

The stated purpose of this new Journal, the first issue of which appeared in January of this year, is 'to provide an appropriate medium for the dissemination of interdisciplinary and international knowledge on all the factors which influence the properties of meat'. The first issue contains papers from West Germany, Spain, New Zealand and Botswana as well as the UK and so can claim to be truly international. Topics are primarily oriented towards the chemistry and biochemistry of meat with reference to its processing technology, but studies on live animals related to meat production are also included.

The Journal will be of great interest to those involved in all aspects of meat production and processing, as well as to those engaged in research into muscle chemistry and biochemistry. It will undoubtedly prove a most useful medium for publications in the field of meat science and workers in other fields of food chemistry and technology might well find this a useful source of information.

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Immobilised Enzymes for Degradation (Session XV of the Third International Biodegradation Symposium). Edited by J. M. Sharpley and A. M. Kaplan. London, Applied Science Publishers, 1976. Price: £3·00.

Major symposia nowadays often cover such a wide field of interest that there is little in common between the papers presented at the different sessions. The resulting *Complete Proceedings of the Symposium* is an expensive, weighty tome in which specialists will refer only to those sections containing material specific to their area of interest.

The publishers of the *Complete Proceedings of the Third International Biodegradation Symposium* are therefore to be congratulated on their decision of grouping together papers from different sessions which have a common theme and publishing them in separate volumes. Workers in the field of immobilised enzymes will be interested in this book, but will generally have little interest in, for example, the collection of papers on 'Deterioration by Insects, Rodents, Birds and Animals'.

Although some of the volumes are substantial in themselves, this slim volume dealing with immobilised enzymes contains fewer than 50 pages, comprising only five papers. Priced at £3·00, it is clearly much cheaper than the *Complete Proceedings* which run to some 1100 pages.

The first paper gives a short review of the prospects for immobilised enzymes in biodegradation. The author points out that 'with the present state of knowledge, it is safe to say that almost any enzyme can be successfully immobilised.... The future technical problem will be the application of immobilised enzymes in an economic fashion'. This sentiment is echoed in most of the other papers, when the potential of insolubilised enzymes for such processes as the treatment of paper mill effluent and the hydrolysis of lactose in whey solutions is discussed. The economics clearly depend upon such factors as the cost of the enzyme and its support, the ease of immobilising the enzyme and its stability when used.

Successes in the UK of immobilised enzymes for industrial processes have been achieved—notably in the production of high fructose syrups—and this small collection of papers indicates some of the areas of research activity at the present time.

DAVID GREEN

Food Engineering Operations. By J. G. Brennan, J. R. Butters, N. D. Cowell and A. E. V. Lilly. London, Applied Science Publishers. Second Edition (1976). xiv + 532 pp. Price: £15·00.

The first edition of *Food Engineering Operations* filled a great need amongst teachers and students of food science and technology and those who had already been taught but who needed an *aide mémoire*. When I was a student at the National College of Food Technology engaged in the course on which this book is based, we had no

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Food Engineering Operations. By J. G. Brennan, J. R. Butters, N. D. Cowell and A. E. V. Lilly. London, Applied Science Publishers. Second Edition (1976). xiv + 532 pp. Price: £15·00.

The first edition of *Food Engineering Operations* filled a great need amongst teachers and students of food science and technology and those who had already been taught but who needed an *aide mémoire*. When I was a student at the National College of Food Technology engaged in the course on which this book is based, we had no

adequate text-book. Our information was gleaned from a variety of chemical engineering texts. But, if food engineering is a branch of chemical engineering, it is a very specialised branch, with its own peculiar problems of hygiene, raw material preparation and so on. And no chemical engineering text covers adequately these problems; indeed, no mention of 'food' is found in several of the principal texts. So here was a book that filled a very real need and was not just another text-book. Since the first edition was published in 1969, several other food engineering texts have appeared, illustrating the growth in recent years of food engineering as a scientific discipline, but *Food Engineering Operations* remains the most thorough undergraduate text.

The second edition of *Food Engineering Operations* is enlarged and is greatly improved by the adoption of SI units. Expansion by 90 pages has been necessitated by the inclusion of new sections on membrane separation and thawing and a completely new chapter on solid/liquid extraction and expression. More extended treatments of some topics have improved the clarity of these. The adoption of SI units will commend the book to those involved in teaching food engineering to students of Food Science where SI units are prevalent in other fields. In a few places where SI units are still not usual in industry the authors have retained the traditional units—a sensible scheme for a text-book for students in an applied discipline. For example, the problem of 'lethal rate', where 250 °F is still the generally accepted reference, has been overcome by including two lethal rate tables. Both are in °C, but one is based on a reference temperature of 121·11 °C (250 °F) and the other on a reference temperature of 120 °C. The book also contains a very welcome metric psychrometric chart—very rare in English—but it is a great pity that it is but 10 cm × 7 cm, which is too small for convenient use.

All operations likely to be encountered in food engineering are covered adequately in the book and are backed up by comprehensive bibliographies. The text is divided into four parts—Preliminary Operations, Conversion Operations, Preservation Operations and Ancillary Techniques—logically sub-divided into a total of nineteen chapters. There are also appendices on heat and mass transfer, psychrometry and conversion factors between SI units and common non-SI units. As an undergraduate text, *Food Engineering Operations* is almost too good. I suspect that it would be quite possible for a student to achieve a good pass in an undergraduate food engineering examination without recourse to any literature but this book. And to my mind one of the most important roles of any undergraduate course is to encourage effective use of the literature. But the references are all there for the enthusiastic student.

As a general reference book, this volume is invaluable to those unfamiliar with a particular process and who need to make a preliminary appraisal of it for a particular application. The well-worn appearance of my personal copy of the first edition bears witness to its suitability for this purpose.

The main criticism of the book is that it treats food engineering operations as an end in themselves and does not emphasise adequately that such processes are but a

means towards the end of providing wholesome food. Although it is convenient to do so for the purposes of teaching, food engineering cannot be divorced from the complementary disciplines of microbiology, chemistry, nutrition and technological economics. I do not suggest that a great deal of space should be devoted to these considerations; adequate texts on all these topics already exist. But I would like the interdependence of all these disciplines explained briefly and I would like to see some discussion of the relative technical and economic merits of various processes for achieving similar ends. It is, of course, very easy to sit in an office and criticise any book; *Food Engineering Operations* is undoubtably the best available food engineering text.

The book is well made and the matt paper of the new edition makes the text and illustrations clearer and more easy to read than did the glossy paper of the previous edition. The text is commendably free from typographical errors and all the references that I have been able to check have proved accurate.

The price of £15·00 is reasonable, though startling when compared with the original price of the earlier edition, but I suspect that students would welcome a paperback edition if this could effect a significant reduction in price.

STUART THORNE

Examination and Analysis of Starch and Starch Products and **Industrial Uses of Starch and its Derivatives.** Edited by J. A. Radley. London, Applied Science Publishers Ltd. (1976). Price: £15·00.

A balance has been produced regarding the content of both books. Sufficient detail has been included to make both books useful for reference purposes to the more academic reader whilst the more general information in the text ensures that they will be of interest to those not so inclined. Up-to-date accounts of the various subjects are provided in each case by a selection of authors and these texts are collated and contributed to by Radley himself.

The first book *Examination and Analysis of Starch and Starch Products*, carries an extremely useful two-chapter account on the microscopical examination of starch which is of fundamental interest both to universities and industrial quality control departments. Basic principles of electron microscopy are also explained and this provides a useful background to the main text. Microscopy of starch is an often neglected subject and it is gratifying to find at last a detailed account of this subject. There follow two chapters on the rheology and physical methods of characterisation of starch. An account of the analysis of raw and modified starches precedes a detailed chapter on the analysis of starch in various products. The book concludes with a section on the analysis of starch derivatives.

The second book deals, as the title suggests, with the industrial uses of starch and its derivatives. Five chapters deal with adhesives from starch, starch in food, textile

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and paper industries and miscellaneous uses of starch, reviewing in each case the traditional uses of starch in the field and emphasising the probable increasing importance of starch in the future. Finally, an important chapter on the utilisation of the by-products of starch manufacture completes an interesting book. A seemingly misplaced Chapter 3 on bread-staling is a minor criticism of the book since this appears to be out of context with the rest of the material. It is nevertheless well written and of obvious interest.

MALCOLM W. KEARSLEY

TOMATO VOLATILE COMPONENTS: EFFECT OF PROCESSING

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ABSTRACT

The effect of processing on the volatile components present in tomato products has been studied by combined gas chromatography–mass spectrometry. The conditions involved in the preparation of canned juice do not seriously affect the heavy components, while the most volatile (hexanal, hexenal, hexenols) decrease or disappear. After a prolonged treatment (of tomato paste) these components also disappear and heat-induced products are found (aromatic compounds and furan byproducts). Three compounds (furfural, linalyl acetate and 6-methyl-5-hepten-2-one) are more important. Some of the volatile compounds of the fresh fruit are present, along with heat-induced products, in water condensed from the evaporator. These results are confirmed by the study of the compounds isolated from fresh juice heated at 100 °C in a closed system. The increase of trans-2-*hexenal and decrease of* trans-2-*hexen*-1-*ol are discussed on the basis of possible pathways by which these products are formed in tomato.*

INTRODUCTION

During the last ten years the volatile components of fruits and vegetables have been extensively studied (Buttery *et al.*, 1971, Johnson *et al.*, 1971). The latter reference is a review concerning tomato, the fruit considered in this study.

Most literature concerning the effect of processing on the volatile components of tomato is less extensive and more dispersed. Nelson & Hoff (1969) have reviewed the studies published up until this time; these authors have shown that the concentrations of acetaldehyde, acetone, methanol and hexenal were modified during the processing. They confirm that methyl sulphide found by Miers (1966) is a heat induced product. Buttery *et al.* (1971) report that heating caused an increase in

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the relative amounts of α terpineol and linalol. Reymond (1971) points out that chromatograms of vacuum steam distillates of tomato pastes processed by different heat pretreatments (cold break, activated break, hot break) are different in the hexenols and hexenals regions. Seck *et al.* (1976) have observed in a commercial tomato juice the decrease or disappearance of the volatile components of the aroma of fresh tomatoes, especially *cis*-3-hexenal, and the formation of heat-induced products (furan derivatives and aromatic compounds).

The purpose of the present work is to establish these findings by studying the influence of processing on volatile tomato components under controlled conditions.

EXPERIMENTAL

Materials

Tomatoes (ROMA VF variety) were grown in an open field at the experimental station of Puyricard 13, France. Fresh juice was prepared by cold break of fruits and refining. Canned juice was obtained by hot break (80 °C), refining and pasteurisation of the cans in boiling water for 10 min. Tomato paste (28° Brix) was prepared by vacuum concentration at 63–65 cm pressure with the product at 55–60 °C in a pilot unit. Before canning the paste is heated at 94 °C and the cans immediately cooled with fresh water.

The water condensed from the concentrator unit was collected and extracted with methylene chloride.

Fresh juice was heated at 100 °C for 10 min (after thermal equilibration) in a closed system constituted by a 6 litre reactor fitted out with a reflux condenser and a mechanical stirrer. The fresh juice was frozen at −20 °C and conserved at this temperature for one to five months. The canned juice and tomato paste were stored at +4 °C for the same period.

Isolation of volatile components

The volatile components were obtained by stripping in a cyclone apparatus working under vacuum (Cobb, 1969). The extraction of 1 litre of juice or reconstituted juice (in the case of the tomato paste study) was carried out over a 2 h period at 30 °C under 80 mm pressure. The traps were cooled with ice water and liquid nitrogen successively. The condensed fractions in the two traps were extracted with methylene chloride, the organic extract was dried over a small quantity of anhydrous sodium sulphate and filtered. Most of the excess methylene chloride was then removed under a stream of nitrogen to yield a concentrate (2 ml from about 30 litres of initial product).

Analytical procedures

Analytical gas–liquid chromatography was carried out on a 10 ft × 0·25 in o.d.

stainless steel column packed with 1·5% Carbowax 20 M on Chromosorb W 60–80 mesh. The temperature was programmed from 50 to 170 °C at 2 °C per min and maintained. For preparative purposes a 10 ft × 0·5 in o.d. stainless steel column packed with 20% OV-17 on Chromosorb W, AW-DCMS was used. Fractions were collected in dry ice-methanol cooled capillary glass U-tubes.

Combined gas chromatography–mass spectrometry was carried out with a GIRDEL 3000 chromatograph coupled with a VARIAN MAT CH 5 mass spectrometer by a Watson Biemann separator. The source temperature was 250 °C and the ionising energy 70 eV. The column was the same as that used for analytical purposes. Authentic samples of chemical compounds were obtained from commercial supply houses or were synthesised by other laboratories or by ourselves using established methods.

RESULTS AND DISCUSSION

Before undertaking the study of the effect of processing on volatile tomato components, it was necessary to have a reference by studying the volatile components extracted from the fruit in the conditions utilised in the present work.

The chromatogram obtained upon separation of tomato volatile components (variety ROMA V.F.) on Carbowax 20 M 1·5% is presented in Fig. 1. Identified compounds are listed in Table 1 in order of their elution. All these products except two (carvone and citronellol) are known as volatile tomato components. Carvone (peak 47) was identified by its mass spectra (Stenhagen *et al.*, 1970) and its presence confirmed by determination of its retention time (RT). This product is probably formed by oxygenation of linalol during the crushing of the fruit or during the extraction process in the cyclone.

The peak 48–49, besides exhibiting characteristic fragments of geranial, showed the possible presence of citronellol (Cornu & Massot, 1975).

The presence of *trans*-2-hexen-1-ol tentatively identified by Seck & Crouzet (1973) was confirmed by determination of its RT. From the fractions isolated by preparative GC it was possible to detect by mass spectrometry the presence of the following compounds (Table 2): citronellyl acetate (Cornu & Massot, 1975), linalyl acetate (Stenhagen *et al.*, 1970), a product yielding a mass spectral peak at m/e:99 characteristic of some δ lactones (Urbach *et al.*, 1973) identified as δ octalactone and cuminyl alcohol (Von Sydow, 1963). The two first components have been previously described in tomato by Shah *et al.* (1969) by functional group analysis, retention time and IR spectra, and the last two are tentatively identified among the tomato volatile components for the first time.

Figure 2 shows the chromatogram obtained for the volatile components isolated from the canned juice. We can note the disappearance of the peak characteristic of hexanal while peaks of pentanol (peak 15) and of *cis*-3-hexen-1-ol (peak 28) are

considerably reduced. The quantities of *trans*-2-hexanal (peak 14) and *trans*-2-hexen-1-ol (peak 29) in the mixture are more important.

On the other hand, the furfural derivatives, especially methyl-5-acetyl-2 furan (peak 41) and furfuryl alcohol (peak 44) are more important than in the fresh juice. We can also note the appearance of ethyl benzene (peak 6) and of a trimethyl benzene (peak 20). These products are known as thermal products formed during the degradation of carbohydrates (Fragerson, 1969).

TABLE 1
IDENTIFICATION OF VOLATILE COMPONENTS ISOLATED FROM TOMATO PRODUCTS (Figs. 1 to 5)

Peak No.	*Compounds*	*Means of identification*	*Mass spectral data* m/e
1	Ethanol	RT	
2	Chloroform	MS–RT	
3	Propanol	MS–RT	
4	Toluene	MS–RT	
5	Hexanal	MS–RT	
6	Ethyl benzene	MS	
7	*p*-xylene	MS–RT	
8	*m*-xylene	MS–RT	
9	*o*-xylene	MS–RT	
10	1-pentene-3-ol	MS–RT	
11	butanol	MS–RT	
12	propyl benzene	MS	105, 119. 134, 91, 97
13	3-methyl butanol	MS–RT	
14	*trans*-2-hexenal	MS–RT	
15	pentanol	MS–RT	
16	*p*-cymene	MS–RT	
17	1-methyl-4-ethyl benzene	MS	105, 120, 77, 39, 91
18	styrene	MS–RT	104, 103, 78, 57, 77
19	acetoin	MS	
20	trimethyl benzene	MS	
21	benzyl methyl ketone	MS	134, 91, 57, 43
22	4-heptanol	MS–RT	
23	diethylbenzene	MS	105, 119, 134, 91, 97
24	2-methyl-2 pentanol	MS–RT	
25	6-methyl-5-hepten-2-one	MS–RT	
26	hexanol	MS–RT	
27	2-heptanol	MS	
28	*cis*-3-hexen-1-ol	MS–RT	
29	*trans*-2-hexen-1-ol	MS–RT	
30	2-isobutylthiazol	MS–RT	

From the fractions obtained by preparative GC we have tentatively identified the 2-methyl butenal (Table 2); this compound has been previously identified in roasted products (Kinlin *et al.*, 1972; Vitzthum *et al.*, 1975).

The chromatogram of tomato paste is given in Fig. 3. In this figure we can observe that the less volatile compounds have disappeared while many peaks are more important than in the chromatogram obtained for the fresh juice (Fig. 1).

Three peaks are particularly important: linalyl acetate (peak 37) observed in the

TABLE 1—*contd.*

Peak No.	*Compounds*	*Means of identification*	*Mass spectral data* m/e
31	2-octenal	MS–RT	
32	benzyl ethyl ketone	MS	148, 105, 43, 91, 77
33	furfural	MS–RT	
34	6-methyl-5-hepten-2-ol	MS–RT	
35	2-acetyl furan	MS–RT	
36	benzaldehyde	MS–RT	
37	linalyl acetate	MS–RT	
38	linalol	MS–RT	
39	5-methyl furfural	MS–RT	
40	*p*-tolualdehyde	MS–RT	91, 119, 120, 39, 65
41	5-methyl-2-acetyl furan	MS–RT	
42	δ butyrolactone	MS	
43	phenylacetaldehyde	MS–RT	
44	furfuryl alcohol	MS–RT	
45	α-terpineol	RT	
46	4-isopropyl phenyl methanol	MS	91, 77, 79, 132, 135, 150
47	carvone	MS–RT	82, 54, 108, 93, 58
48	geranial	MS–RT	81, 55, 82, 68, 95
49	citronellol	MS	41, 69, 65, 67, 81
50	geranyl acetone	MS	
51	geraniol	MS–RT	
52	2,4-decadienal	MS	
53	guaiacol	MS–RT	
54	benzyl alcohol	MS–RT	
55	2,6-di-methyl-2,6-undecadien-10-one	MS	
56	2-phenyl ethanol	MS–RT	
57	β-ionone	MS–RT	
58	*p*-anisaldehyde	MS–RT	
59	isopropyl anisol	MS	
60	eugenol	MS–RT	

fresh juice, furfural (peak 33) found in the compounds isolated from heated tomatoes (Buttery *et al.*, 1971) and from canned juice (Seck *et al.*, 1976) and 6-methyl-5-hepten-2-one (peak 25). Cole & Kapur (1957) have found that this last product is formed during lycopene oxidation.

TABLE 2

COMPOUNDS IDENTIFIED BY MASS SPECTROMETRY IN FRACTIONS ISOLATED BY PREPARATIVE GAS CHROMATOGRAPHY

Tomato product	*Compounds*	*Mass spectral data* m/e
Fresh juice	citronellyl acetate	43, 41, 69, 81, 28
Fresh juice	linalyl acetate	41, 93, 69, 43, 45, 136
Fresh juice	δ-octalactone	99, 42, 43, 71, 142
Fresh juice	cuminyl alcohol	150, 135, 107, 109, 91
Canned juice	2-methyl-butenal	55, 29, 84, 27, 39
Heated juice	vinyl ketone	41, 55, 70
Heated juice	2-ethyl-6-vinyl pyrazine	134, 133, 51, 52, 53

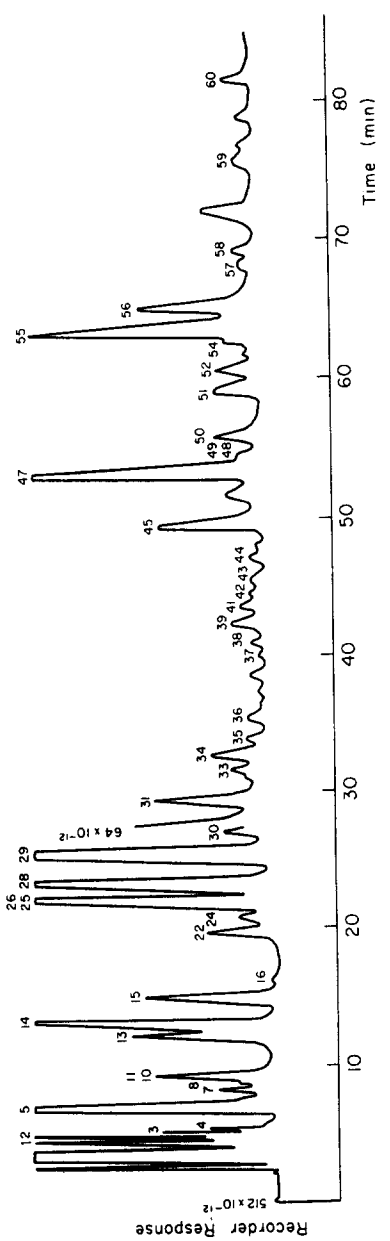

Fig. 1. Gas chromatogram of volatile components of fresh tomato juice.

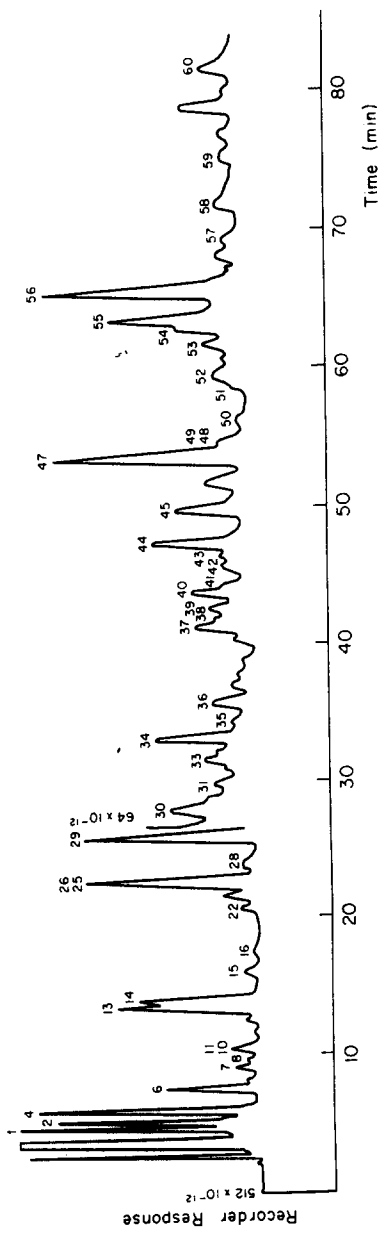

Fig. 2. Gas chromatogram of volatile components of canned tomato juice.

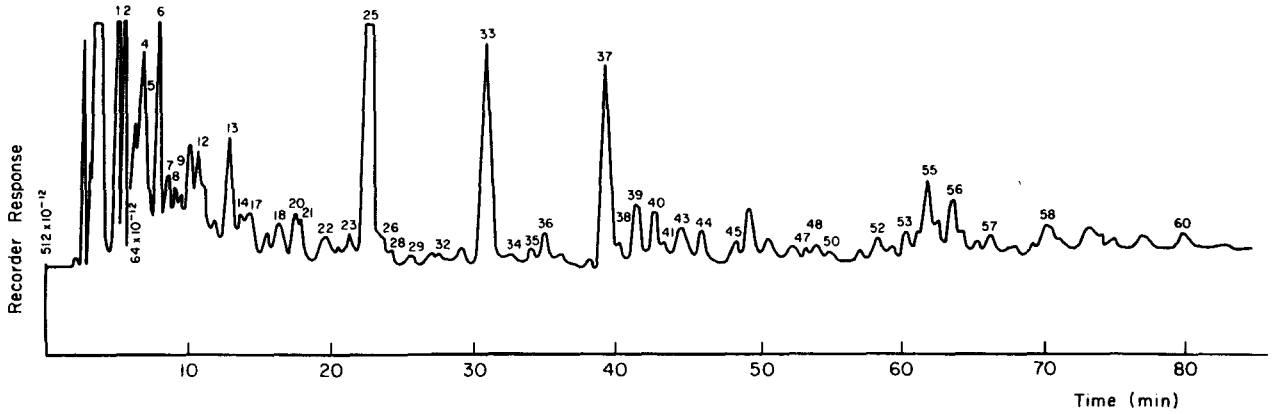

Fig. 3. Gas chromatogram of volatile components of tomato paste.

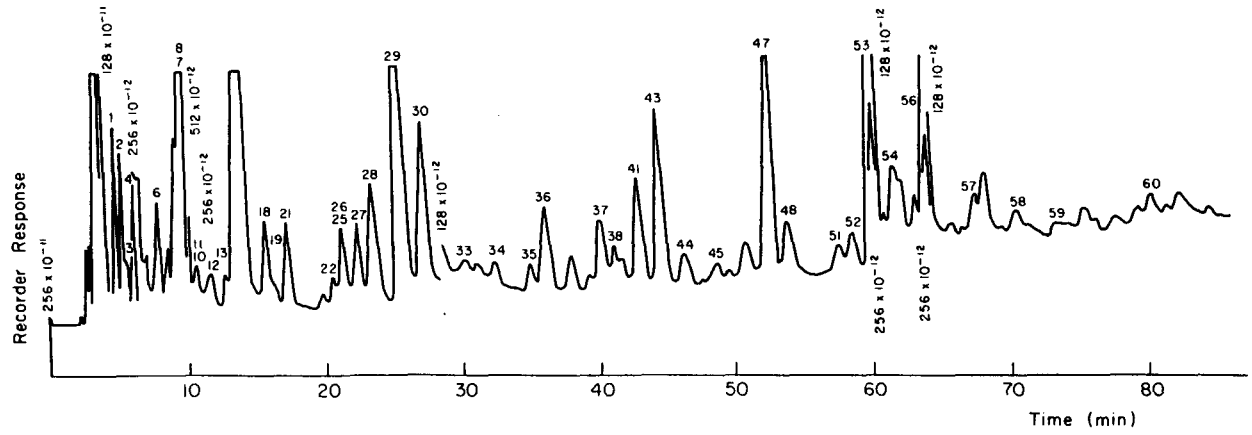

Fig. 4. Gas chromatogram of volatile components isolated from condensed water in the concentrator unit.

Mass spectral data indicate the possible presence of the following compounds: 1-methyl-4-ethyl benzene (peak 17) (Cornu & Massot, 1975); *n* propyl benzene (peak 12) (Cornu & Massot, 1975); a diethyl benzene (peak 23); benzyl ethyl ketone (peak 32) (Cornu & Massot, 1975); styrene (peak 18) (Jouret *et al.*, 1972) also identified by its RT. These products may be formed by heating during the concentration process; the styrene, also found by Jouret *et al.* (1972) during wine making, proceeds from the decarboxylation of cinnamic acid.

In condensed water (Fig. 4), we note the presence of several compounds involved in the aroma of fresh tomato: 3-methyl butanol, *trans*-2-hexenal, *cis*-3-hexen-1-ol, *trans*-2-hexen-1-ol, 2-isobutylthiazol, stripped during the concentration process. Beside these compounds we note the appearance or a marked increase of benzene derivatives: styrene already isolated from the tomato paste, toluene, *o*-, *m*- and *p*-xylene, benzaldehyde, phenylacetaldehyde and 2-phenylethanol. Furan by-products already found in tomato juice (Cobb, 1969) and especially furfural and 5-methyl-2-acetylfuran are found in this fraction.

On the other hand we have isolated from the condensed water the following compounds: a compound (peak 46) identified as an isopropyl phenyl methanol *m/e*:150 (M), *m/e*:132 (M–18), *m/e*:135 (M–15), *m/e*:91 (tropilium ion), *m/e*:77 and 79 (aromatic ring), *m/e*:43 (isopropyl) and a compound (peak 21) identified as benzyl methyl ketone, *m/e*:134(M), *m/e*:91 (tropilium ion), *m/e*:57 (CH_3–CO–CH_2^+), *m/e*:43 (CH_3–CO^+). These aromatic compounds are certainly induced by heat treatment during the concentration process.

We have tried to establish the effect of heat on the formation of volatile components by heating fresh tomato juice at 100 °C during 10 min (after thermal equilibrium) in a closed system (Karlsson-Ekstrom & Von Sydow, 1973).

Under these conditions we have found (Fig. 5) in the processed product the volatile compounds of the fresh products unchanged by the treatment. On the other hand we can see that heavy compounds are more important than in the case of fresh juice, especially furan derivatives, benzaldehyde, phenylacetaldehyde and 2,6-dimethyl-2,6-undecadien-10-one.

Among the compounds formed under these conditions we have found after fractionation by preparative chromatography a product characterised by S.M. fragments at *m/e*:41 (isopropyl), *m/e*:55 (CH_2=CH–C≡O^+), *m/e*:70

$$(CH_2{=}CH{-}\underset{\underset{O}{\|}}{C}{-}CH_2 + H)$$

tentatively identified as a vinyl ketone and 2-ethyl-6-vinylpyrazine (Friedel *et al.*, 1971) (Table 2). Pyrazines are typical heat-induced products. Ryder (1966) has previously identified two of them in tomato products as 2,6-dimethylpyrazine and 2-methylpyrazine.

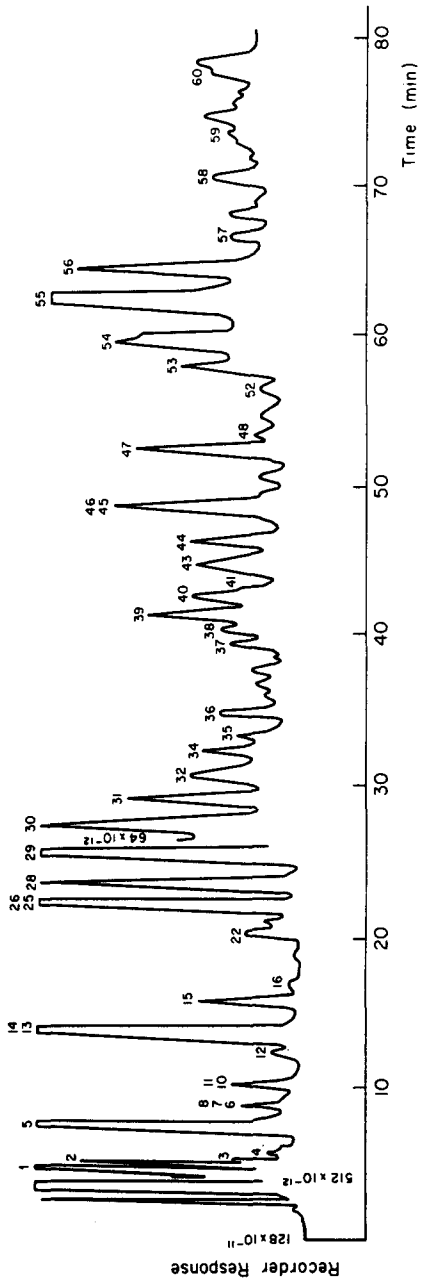

Fig. 5. Gas chromatogram of volatile components isolated from tomato juice heated at 100°C for 10 min.

The relative proportions of the most characteristic products present in fresh and heated juice were determined (Table 3). The area of each peak was estimated and the values so obtained divided by the sum of the area of all peaks present in the chromatogram (the solvent and products with lower retention times were not involved in this calculation).

TABLE 3
RELATIVE AMOUNTS OF THE MOST CHARACTERISTIC COMPOUNDS PRESENT IN FRESH AND HEATED JUICE (100 °C for 10 minutes)

Compounds	*Fresh juice*	*Heated juice*
Toluene	0·47	0·73
Hexanal	11·4	12·7
Xylenes	0·93	1·08
Trans-2-hexenal	9	19·5
Pentanol	3·8	3·25
Trimethyl benzene		
6-methyl-5-heptene-2-one	0	0·51
and *n* hexanol	9·7	11·2
Cis-3-hexenol	12·4	10·4
Trans-2-hexenol	30	21·8
2-isobutylthiazol	1·37	1·08
Furfural	0·14	0·42
2-acetyl furan	0·05	0·14
Benzaldehyde	0·07	0·30
Linalol	0·03	0·19
5-methyl furfural	0·13	0·47
5-methyl-2-acetylfuran	0·07	0·15
Phenyl acetaldehyde	0·05	0·66
Furfuryl alcohol	0·09	0·4
α-terpineol and isopropyl-4-phenyl alcohol	0·5	1·3
Carvone	2·05	0·79
2,6-dimethyl-2,6-undecadien-10-one	1·04	2·45
2-phenyl ethanol	0·44	1·02

The data obtained for the heated product confirm the qualitative findings, i.e. increase of relative amounts of aromatic and furan compounds of 2,6 dimethyl-2,6-undecadien-10-one. The increase of relative amount of terpineol and linalol reported by Buttery *et al.* (1971) is confirmed.

On the other hand we observed an increase of the amount of *trans*-2-hexenal in relation to a decrease of *trans*-2-hexen-1-ol whereas the amount of *cis*-3-hexen-1-ol is constant. This result can be explained if we consider the possible pathways by which these products are formed (Fig. 6) (Kazeniac & Hall, 1970; Jadhav *et al.*, 1972; Stone *et al.*, 1975; Sieso *et al.*, 1976).

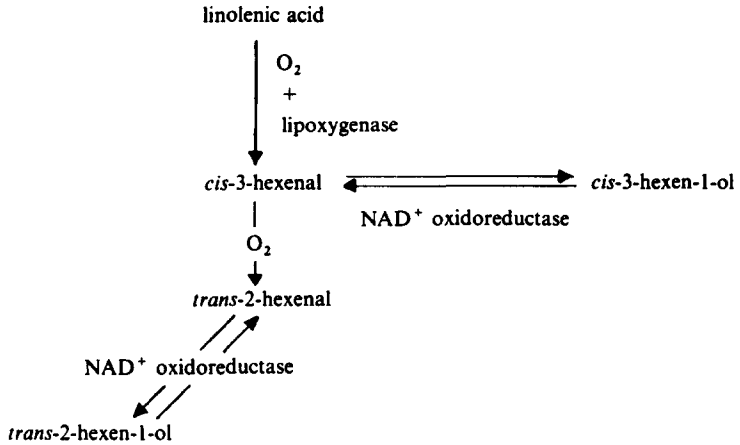

Fig. 6. Formation of hexenals and hexenols from linolenic acid.

Cis-3-hexenal formed during crushing of the fruit is in part reduced to *cis*-3-hexen-1-ol by the action of NAD^+ oxidoreductase, and in part isomerised to *trans*-2-hexenal which is in turn reduced to *trans*-2-hexen-1-ol.

We can suppose that NAD^+ oxidoreductase is always under activated form in the fresh juice (Nicolas, 1975) and that the reduction reactions occur during the stripping of volatile compounds and during the first steps of heating. Under these conditions *cis*-3-hexenal is completely reduced or isomerised. The reduction rate of *trans*-2-hexenal (lower than that of *cis*-3-hexenal) (Stone *et al.*, 1975) and the enzyme denaturation during heat increase explain the decrease of the relative amount of *trans*-2-hexen-1-ol and the increase of the relative amount of *trans*-2-hexenal in the heated product. It may be supposed that non-enzymatic oxidation at 100 °C is not a likely cause of the increase of *trans*-2-hexenal at the expense of *trans*-2-hexene-1-ol, because such a reaction would also lead to an equal decrease in the amount of *cis*-3-hexene-1-ol.

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REFERENCES

BUTTERY, R. G., SEIFFERT, R. M., GUADANI, D. G. & LING, L. C. (1971). Characterization of additional volatile components of tomato, *J. Agric. Fd Chem.*, **19**, 524–9.

COLE, E. R. & KAPUR, N. S. (1957). Stability of lycopene, I. Degradation by oxygen. II. Oxidation during heating of tomato pulp, *J. Sci. Fd Agric.*, **8**, 360–5, 366–8.

COBB, W. Y. (1969). Quantification of flavorful food components using isotope dilution, *J. Fd Sci.*, **34**, 466–9.

CORNU, A. & MASSOT, R. (1975). *Compilation of mass spectral data*, 2nd edn., Heyden, London.

FRAGERSON, I. S. (1969). Thermal degradation of carbohydrates, *J. Agric. Fd Chem.*, **17**, 747–50.

FRIEDEL, P., KRAMPL, V., RADFORD, T., RENNER, J. A., SHEPARD, F. W. & GIANTURCO, M. A. (1971). Some constituents of aroma complex of coffee, *J. Agric. Fd Chem.*, **19**, 530–2.

JADHAV, S., SINGH, B. & SALUNKHE, D. K. (1972). Metabolism of unsaturated fatty acids in tomato fruit: linoleic and linolenic acids as precursors of hexanal. *Plant and Cell. Physiol.*, **13**, 449–59.

JOHNSON, A. E., NURSTEN, H. E. & WILLIAMS, A. A. (1971). Vegetable volatiles: a survey of components identified: part II. *Chem and Ind. (London)*, **43**, 1212–24.

JOURET, C., MOUTONNET, M. & DUBOIS, P. (1972). Formation du vinylbenzène lors de la fermentation alcoolique du raisin. *Ann. Technol. Agric.*, **21**, 69–72.

KARLSSON-EKSTROM, G. & VON SYDOW, E. (1973). Aroma of black-currants. VII Influence of processing parameters on the aroma of black-currants, *Lebensm. Wiss. Technol.*, **5**, 165–9.

KAZENIAC, S. J. & HALL, R. M. (1970). Flavor chemistry of tomato volatiles, *J. Fd Sci.*, **35**, 519–30.

KINLIN, T. E., MURALIDHARA, R., PITTET, A. O., SANDERSON A. & WALDRAT, J. P. (1972). Volatile components of roasted filberts, *J. Agric. Fd Chem.*, **20**, 1021–8.

MIERS, J. C. (1966). Formation of volatile sulfur compounds in processed tomato products, *J. Agric. Fd Chem.*, **14**, 419–23.

NELSON, P. E. & HOFF, J. E. (1969). Tomato volatiles: effect of variety, processing and storage time, *J. Fd Sci.*, **34**, 53–7.

NICOLAS, M. (1975). *Alcool deshydrogénase de tomate. Purification et étude de quelques propriétés*, Thèse de Spécialité. Université de Montpellier.

REYMOND, D. (1971). Analytical evaluation of food quality, *Fd Tech.*, **25**, 78–82.

RYDER, W. S. (1966). Progress and limitations in the identification of flavor components. In *Flavor Chemistry*, ed. Gould, R. F. pp. 70–93, American Chemical Society, Washington.

SECK, S. & CROUZET, J. (1973). Constituants volatils de *Lycopersicon esculentum*, *Phytochemistry*, **12**, 2925–30.

SECK, S., CROUZET, J. & PIVA, M. T. (1976). Influence des traitements thermiques sur la nature des constituants volatils de la tomate, *Ann. Technol. Agric.*, **25**, 85–95.

SHAH, B. M., SALUNKHE, D. K. & OLSON, L. E. (1969). Effects of ripening process on chemistry of tomato volatiles, *J. Amer. Soc. Hort. Sci.*, **94**, 171–6.

SIESO, V., NICOLAS, M., SECK, S. & CROUZET, J. (1976). Constituants volatils de la tomate: mise en évidence et formation par voie enzymatique du *trans*-hexène-2-ol, *Agr. Biol. Chem.*, **40**, 2349–53.

STENHAGEN, E., ABRAHAMSON, S. & MACLAFFERTY, F. W. (1970). *Atlas of mass spectra data*, New York, Interscience Publishers.

STONE, E. J., HALL, R. M. & KAZENIAC, S. J. (1975). Formation of aldehydes and alcohols in tomato fruits from U-^{14}C-labeled linolenic and linolic acids, *J. Fd Sci.*, **40**, 1138–41.

VON SYDOW, E. (1963). Mass spectrometry of terpenes II. Monoterpene alcohols, *Act. Chem. Scand.*, **17**, 2504–12.

URBACH, C., STARK, W. & NOBUHARA, A. (1973). Low resolution mass spectra of some unsaturated δ-lactones, *Agr. Biol. Chem.*, **36**, 1217–28.

VITZTHUM, O. G., WERKHOFF, P. & HUBERT, P. J. (1975). New volatiles constituents of black tea aroma, *J. Agric. Fd Chem.*, **23**, 999–1003.

INFLUENCE OF pH AND TEMPERATURE UPON CALCIUM ACCUMULATION AND RELEASE BY BOVINE SARCOPLASMIC RETICULUM*

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ABSTRACT

Sarcoplasmic reticulum (SR) was prepared from fresh beef sternomandibularis *muscle and shown to be relatively free from contamination by lysosomes, sarcolemma and mitochondrial membranes.* Ca^{2+} *accumulation by SR from fresh and cold-shortened muscle was* 51 *and* 39 *nmoles/mg protein, respectively. The* Ca^{2+} *accumulating ability of fresh SR vesicles decreased with lowering of pH* (7·3, 6·8, 6·2, 5·5 *and* 5·0) *at all temperatures* (0, 15 *and* 38 °C). *Lowering the temperature from* 38 *to* 0 °C *at pH* 6·6 *resulted in the release of* 48% *of the total accumulated* Ca^{2+}, *whereas the corresponding value on lowering the temperature from* 38 *to* 15 °C *at the same pH was only* 12%. *Thus, low temperatures accelerate the release of* Ca^{2+} *by SR. Although simultaneously lowering pH and temperature also increased* Ca^{2+} *release by SR, the amount of* Ca^{2+} *released was less than if pH and temperature were altered independently. The findings are discussed in the light of explaining cold shortening.*

INTRODUCTION

Locker & Hagyard (1963) first demonstrated that beef muscle shortened on pre-rigor exposure to cold and that shortening was associated with decreased muscle tenderness. Although it was later shown that holding the muscle at 15 °C prevented shortening and cold-induced toughening (Marsh & Leet, 1966; Marsh *et al.*, 1968), the mechanism of cold shortening has not been unequivocally established. Weiner & Pearson (1966) reported that EDTA (ethylenediamine tetraacetic acid) injection inhibited postmortem shortening of muscle, whereas it was shown later that

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microinjections of Ca^{2+} increased pre-rigor muscle shortening and muscle toughness (Pearson *et al.*, 1973); all of which indirectly suggested that release of Ca^{2+} ions by sarcoplasmic reticulum (SR) at low temperatures may be responsible for cold shortening and the associated toughening effect on muscle. A recent report by Buege & Marsh (1975) presented evidence that the mitochondria may be directly involved in cold shortening through release of Ca^{2+} ions under postmortem anaerobic conditions, but that the SR could be indirectly involved due to a decrease in calcium uptake as a consequence of low temperatures.

The present investigation was undertaken to determine the influence of pH and temperature upon Ca^{2+} release by the SR. This was accomplished by saturating freshly isolated and purified SR vesicles with $^{45}Ca^{2+}$ ion and measuring the accumulation and release at different pH values and temperatures. Ca^{2+} accumulation and release were also measured in SR isolated from fresh (pre-rigor immediately post mortem) and from cold shortened (24 h at 0 °C) bovine *sternomandibularis* muscle.

MATERIALS AND METHODS

Preparation of sarcoplasmic reticulum (SR)

Sternomandibularis muscle was removed from beef carcasses immediately following death by exsanguination. All external fat and connective tissue were dissected from the muscle prior to use. SR vesicles were prepared immediately after trimming, or else the trimmed muscles were held at either 15 or 0 °C for 24 h prior to isolating the SR.

SR vesicles were prepared as described by Meissner & Fleisher (1971), which was carried out at 0 °C according to the diagram shown in Fig. 1. A total of 3 ml of crude SR was placed on top of a discontinuous gradient containing 5mM HEPES (0·3M sucrose and 10mM *N*-2-hydroxyethylpiperazine-*N'*-1-ethensulphonic acid) buffer (pH 7·4) with different percentages of sucrose in the different layers as shown in Fig. 2. The sucrose concentration in percent (w/w) was adjusted using a Valentine refractometer. After application of 3 ml of the crude SR, the tubes were spun for 2·5 h at 23,500 rpm in an SB-283 rotor in an IEC-preparative ultracentrifuge.

Vesicle fractions were carefully removed from the gradient with a pipette. The top 4·8 ml of the gradient was removed and discarded. The next 3·9 ml fraction was collected and diluted with 2 volumes of 5mM HEPES buffer (pH 7·4), which was added in four equal parts over a period of 30–45 min to minimise osmotic shock. The fraction was then centrifuged for 1 h at 35,000 rpm using the SB-283 rotor in the IEC-preparative ultracentrifuge. The pellet was resuspended in a solution containing 0·3M sucrose and 2·5 ml HEPES buffer and stored at 0 °C until used. Some of the resuspended SR was frozen using dry ice–acetone and stored at −20 °C for stability studies.

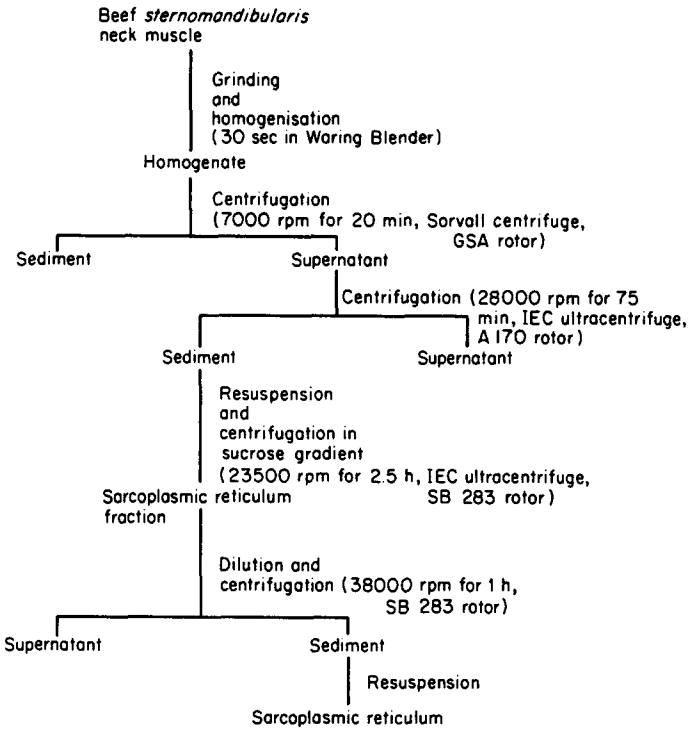

Fig. 1. Flow sheet showing procedure for isolation of sarcoplasmic reticulum.

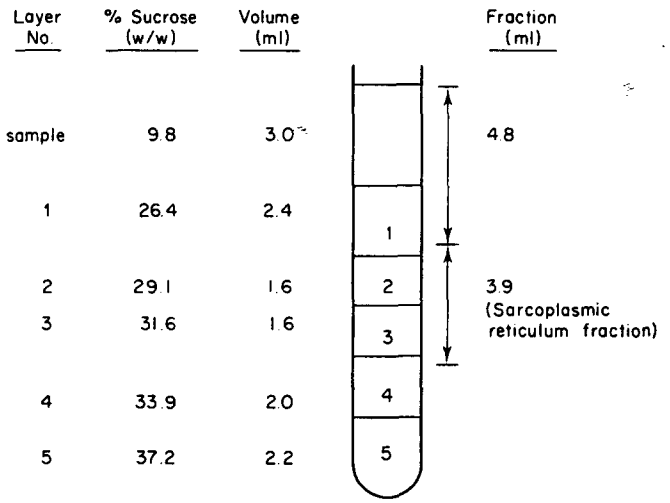

Fig. 2. Fractionation of sarcoplasmic reticulum on a sucrose gradient.

pH measurements

Fresh trimmed muscle was stored at 0 °C for pH measurements. Small portions of the stored muscle were removed after 0, 0·5, 1, 3, 5, 10 and 24 h, homogenised in 5 volumes of distilled–deionised water and the pH was measured using an expanded scale pH meter (Radiometer-type PHM-26, Copenhagen).

Ca^{2+} accumulation and Ca^{2+} release

Ca^{2+} accumulation and Ca^{2+} release were determined using a reaction mixture containing 100mM KCl, 10mM $MgCl_2$, 5mM ATP, 10mM histidine and 0·1mM $CaCl_2$. The reaction mixture was first adjusted to pH 7·3 and $^{45}CaCl_2$ was added to give a final count of 80,000 cpm/ml. Then Ca^{2+} accumulation was determined by placing 3 ml of the reaction mixture in a test tube and adding 0·1 N HCl to adjust to the desired pH (either pH 6·6, 6·2, 5·8, 5·6 or 5·0). Following temperature equilibration at 38, 15 or 0 °C, the reaction was initiated by adding 40–80 μg of SR/ml of reaction mixture. The reaction was allowed to proceed for 3 min and terminated by filtration through a Millipore filter, type GS (average pore size 0·22 μ) as described by Martonosi & Feretos (1964). Ca^{2+} accumulation was calculated from the difference in radioactivity between the reaction mixture without added SR (control) and that containing added SR. Accumulation of Ca^{2+} at each adjusted pH value was compared to that at pH 7·3.

Ca^{2+} release was determined in two tubes containing 3 ml of reaction mixture equilibrated to 38 °C. The reaction was initiated by adding 40–80 μg/ml of SR protein and allowed to continue for 3 min at 38 °C. The reaction was terminated in one tube by passing through a Millipore filter as described above, whereas the other tube was transferred from 38 °C to either a 15 or 0 °C constant temperature water-bath, to lower the temperature of the reaction mixture, and incubated for 10 min. After incubation the reaction was stopped by passing the reaction mixture through the Millipore filter. Ca^{2+} release was calculated from the amount of Ca^{2+} bound by the SR vesicles before and after changing the pH and/or temperature.

$^{45}Ca^{2+}$ radioactivity was counted on either a TRI-CARB scintillation spectrometer (Packard Instruments) or a Nuclear-Chicago, Mark I, model 6894 liquid scintillation counter after mixing the filtrates with PCS scintillation liquid (Amersham–Searle Corporation). The following counting conditions were used: (1) Packard TRI-CARB scintillation spectrometer: window setting = 50–1000 and gain = 11·5%; (2) Nuclear-Chicago Mark I; upper window setting = 9·9, lower = 0·5 and attenuator—c-550.

The concentration of $CaCl_2$ solution in the reaction mixture and the endogenous Ca^{2+} in the SR were analysed by atomic absorption spectroscopy as described by Duggan & Martonosi (1970) using a Perkin–Elmer atomic absorption spectrometer, Model 303. The instrument was calibrated with a standard Ca^{2+} solution (1–10 ppm) in the presence of 10% trichloroacetic acid and 1% of $LaCl_3$.

Protein determination

Protein concentration was determined by the method of Lowry *et al.* (1951) using 1 ml of the appropriately diluted SR preparation and comparing with a standard curve prepared from bovine serum albumen.

Enzyme assays for purity of SR

The succinate–cytochrome *c* reductase activity of the SR preparations was measured by the procedure of Tisdale (1967), which served as a marker to detect the presence of mitochondrial enzymes. Contamination of the SR preparation by the sarcolemma was monitored by the method of Mitchell & Hawthorne (1965). Acid phosphatase activity of the SR preparation was used as a marker enzyme for the presence of lysosomes using a diagnostic kit from Sigma Chemical Company.

RESULTS

Separation of SR on sucrose gradient

The density gradient profiles of SR vesicles prepared from fresh and cold-shortened muscle are shown in Fig. 3. The fresh muscle preparations usually had a broad continuous band in the upper half of the gradient and a narrower, distinct band at the interface between 33·9 and 37·2% sucrose solutions, whereas cold-shortened muscle differed only in exhibiting a marked reduction in the width of the latter band.

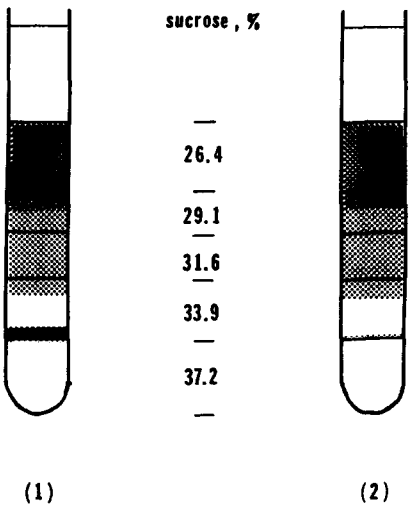

Fig. 3. Density gradient profiles of the sarcoplasmic reticulum from fresh muscle (1) and from cold-shortened muscle (2). Note the distinct differences in the width of the bands.

However, the density gradient profile for vesicles prepared from muscle held for 24 h at 15 °C exhibited the same pattern as the vesicles from cold-shortened muscle, thus indicating that cold-shortening *per se* was not responsible for the differences between vesicles prepared from fresh and cold-shortened muscle. Results suggest that the differences in the profiles are associated with post mortem holding times and may be due to autolysis.

The yield of SR vesicles was 42 ± 8 μg/g for fresh beef muscle, 25 ± 4 μg/g for cold-shortened beef muscle and 16 μg/g for beef muscle stored for 24 h at 15 °C. On the other hand, fresh rabbit *longissimus* muscle yielded 380 μg/g of SR vesicles. The much higher yield for rabbit as compared to beef muscle may be due in part to the larger amount of connective tissue in the beef *sternomandibularis* muscle, which could reduce the efficiency of removal of the SR vesicles, or perhaps be associated with more extensive development of SR in white muscle (rabbit) as compared to red beef muscle (Peachey, 1970). The higher yield of SR vesicles from cold-shortened beef muscle as compared to that of the same beef muscle held for 24 h at 15 °C may be due to a greater amount of proteolysis at the higher temperature.

Purity of SR preparations

The specific activity of succinate–cytochrome *c* reductase was 0·007 μmoles of cytochrome *c* reduced/min/mg of protein. Using the reducing rate of 0·5 μmoles/min/mg of protein for purified mitochondria (Meissner & Fleisher, 1971), the specific activity obtained in this study indicated only 1·4% contamination by mitochondria.

Acid phosphatase activity of the SR preparations was 0·015 μmoles/P_i/min/mg protein. This value may be compared with values of 0·002 to 0·005 μmoles/P_i/min/mg protein for rabbit SR reported by Meissner & Fleisher (1971). Using these values, results indicated that the SR preparations used in this study were only slightly contaminated with lysosomes.

The amount of 5′-nucleotidase activity was negligible for the preparations utilised in this investigation, indicating that there was negligible contamination by the sarcolemma.

Although an attempt was made to remove any myofibrillar protein contaminating the SR preparation by extraction with a solution containing 0·6 M KCl, 0·3 M sucrose and 10 mM histidine (pH 7·3), the extraction resulted in the inactivation of the Ca^{2+}-accumulating ability of the vesicles. Therefore, no attempt was made to purify the preparation by removal of the myofibrillar proteins.

Influence of SR protein concentration on Ca^{2+} accumulation

The relationship between protein concentration of the SR and accumulation of Ca^{2+} is shown in Fig. 4. The graph shows that the accumulation of Ca^{2+} by the SR is linear between 16 and 80 μg protein/ml of reaction mixture, although the curve did not extrapolate to zero. Since the amount of accumulated Ca^{2+} was approximately

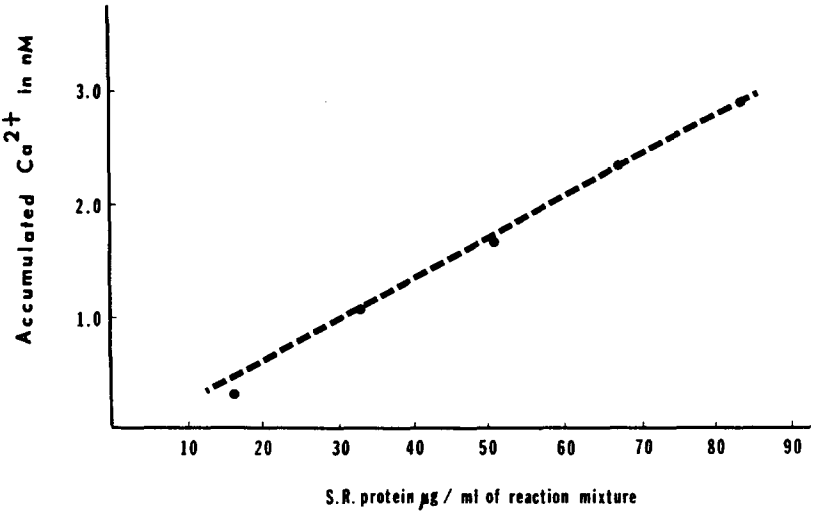

Fig. 4. Ca^{2+} accumulation by sarcoplasmic reticulum as a function of protein concentration. Carried out in HEPES buffer (pH 7·3) at 38 °C.

proportional to protein concentration between 40 and 80 µg, the measurement of Ca^{2+} accumulation and release was made within this range.

Stability of SR vesicles

A number of investigators (Ebashi & Lipmann, 1962; Muscatello *et al.*, 1962; Lee *et al.*, 1965; Eleter & Inesi, 1972) have shown that SR vesicles isolated from muscle immediately post mortem lose their activity during storage at 0 °C and neutral pH (7·0–7·4). However, the activity of isolated vesicles was fairly stable for 90 min storage at 0 °C in 0·3M sucrose and 2·5mM HEPES buffer (Fig. 5).

Figure 6 shows the stability of isolated SR at pH 7·3 in HEPES buffer. The graph shows that the vesicles lost their activity rapidly, even at −20 °C. Thus, determination of Ca^{2+}-release and Ca^{2+}-accumulation by the vesicles was measured immediately after isolation.

Ca^{2+}-accumulation by SR

The amount of endogenous Ca^{2+} bound by the SR was 16 nmoles/mg of protein for beef *sternomandibularis* muscle, which compares fairly well to a value of 35 nmoles Ca^{2+}/mg protein for rabbit muscle SR reported by Meissner *et al.* (1973) but is far different from the value of 500 nmoles Ca^{2+}/mg protein reported by Chevallier & Butow (1971) for rabbit SR. The differences observed may be associated with variations in the isolation procedures and/or differences between species.

Figure 7 shows Ca^{2+} accumulation of SR as a function of reaction time and

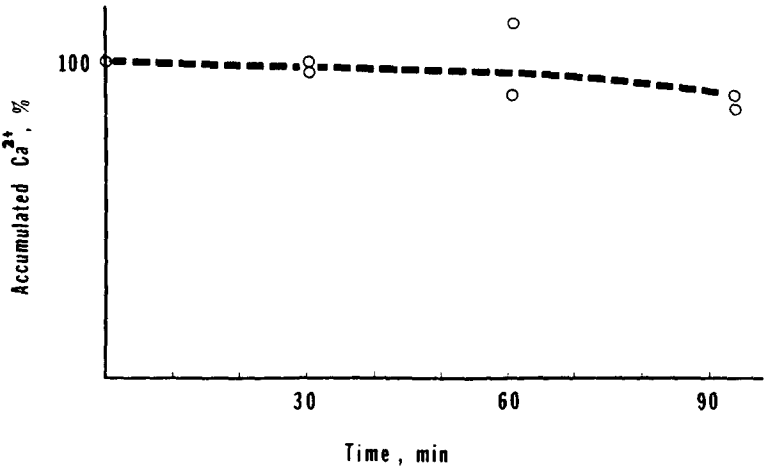

Fig. 5. Stability of sarcoplasmic reticulum vesicles at pH 7·3 (HEPES buffer) and 0 °C.

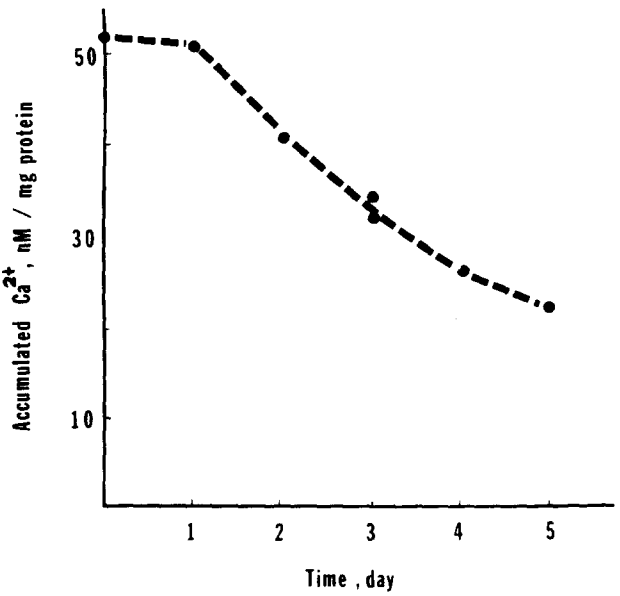

Fig. 6. Stability of sarcoplasmic reticulum vesicles at pH 7·3 (HEPES buffer) and −20 °C.

temperature. The plot shows that Ca^{2+} accumulation begins immediately upon addition of the SR, and that the SR is completely saturated within 1 min. At both 0 and 15 °C the accumulated Ca^{2+} is gradually released with the passage of time. By 20 min approximately 8 and 15 % of the accumulated Ca^{2+} was released by the SR at 15

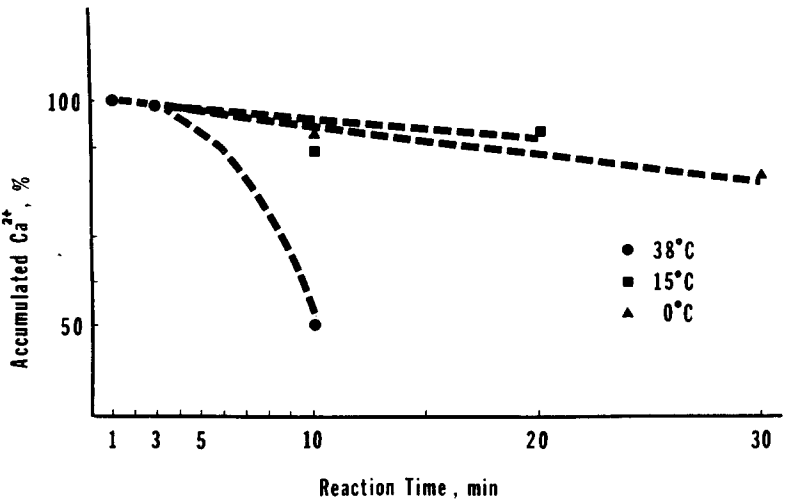

Fig. 7. Ca^{2+} accumulation of sarcoplasmic reticulum vesicles as a function of reaction time (pH 7·3).

and 0°C, respectively. At 38°C, however, the SR vesicles lost 50% of the accumulated Ca^{2+} within 10 min. This is in agreement with the findings of Inesi *et al.* (1973), who reported that raising the temperature above 35°C markedly reduced Ca^{2+} accumulation by the SR. Ca^{2+} saturated SR is extremely unstable to temperatures in the range of 30–50°C (Johnson & Inesi, 1969; Sreter, 1969), which may explain the shortening phenomenon in bovine *sternomandibularis* muscle observed at temperatures above 30°C.

TABLE 1

Ca^{2+} ACCUMULATION OF SR VESICLES FROM FRESH MUSCLE, COLD-SHORTENED MUSCLE AND MUSCLE STORED FOR 24 h AT 15°C

SR source	*Accumulated Ca^{2+} (nM/mg protein)*[a]
Fresh muscle	50·7 ± 2·6
Cold-shortened muscle	39·0 ± 1·3
Muscle stored 24 h at 15°C	0

[a] Ca^{2+} determinations were performed for 3 minutes, at pH 7·3 and 38°C. Each value represents the average of four determinations for two different muscle preparations.

Table 1 summarises the Ca^{2+} accumulation of SR vesicles prepared from fresh muscle, from cold-shortened muscle and from muscle stored 24 h at 15°C. Fresh muscle SR vesicles accumulated 50·7 nmoles of Ca^{2+}/mg of protein, whereas cold-shortened muscle SR vesicles retained about 75% of the Ca^{2+} accumulating ability of fresh SR. SR vesicles prepared from muscle stored at 15°C for 24 h completely lost their ability to bind Ca^{2+} ions. The decrease in Ca^{2+} accumulation with post

mortem time agrees with earlier reports by Greaser *et al.* (1967) and Goll *et al.* (1971).

The pH changes in beef *sternomandibularis* muscle held at 0 °C are shown in Fig. 8. During the first 3 h period, the pH declined rapidly to 6·4, and then dropped more slowly to 5·8 by 24 h. The rapid drop in pH coincident with a fast decline in temperature may accelerate shortening of muscle.

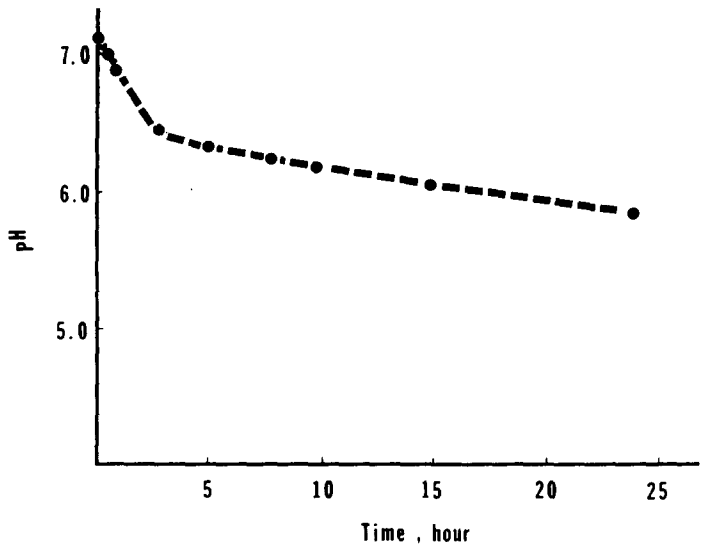

Fig. 8. Changes in pH of beef *sternomandibularis* muscle during storage at 0 °C.

Figure 9 shows the effect of pH upon Ca^{2+} accumulation by the SR at 0, 15 and 38 °C. At pH 7·3, SR vesicles accumulated only about 25 and 75 % as much Ca^{2+} at 0 and 15 °C, respectively, as at 38 °C. At pH 5·0, however, Ca^{2+} accumulation was only 10 nmoles/mg of protein at all three temperatures. As the pH was increased, the differences between different temperatures gradually widened. Maximum Ca^{2+} accumulation (50 nmoles Ca^{2+}/mg of protein) in the pH range of 5·0–7·3 occurred at the highest pH, which is equivalent to that of living muscle (Bate-Smith, 1948). On the other hand, Sreter (1969) found maximum Ca^{2+} accumulation for rabbit muscle to occur at pH of 5·6–6·5 and to be about 5-fold higher than the values for beef *sternomandibularis* in the present study.

Ca^{2+} release by SR

The effects of temperature and pH upon Ca^{2+} release by fresh bovine muscle SR is shown in Fig. 10. The graph shows that temperature did not affect the release of Ca^{2+} at physiological pH (7·3). At pH 6·6, however, about 48 % of the bound Ca^{2+} was released by lowering the temperature from 38 to 0 °C. At the same pH (6·6),

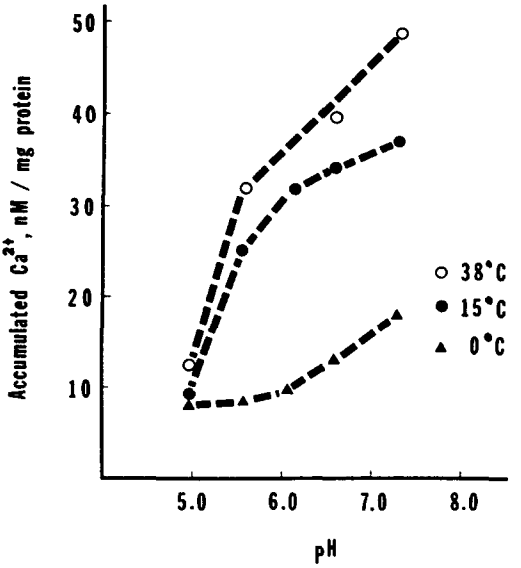

Fig. 9. Ca^{2+} accumulation of sarcoplasmic reticulum vesicles at different pH values and temperatures.

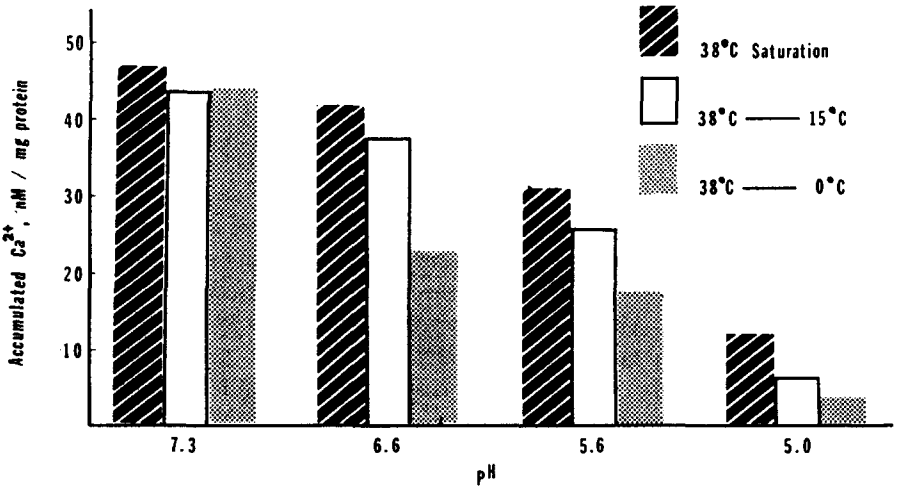

Fig. 10. Effect of pH and temperature on Ca^{2+} release from saturated sarcoplasmic reticulum vesicles.

lowering the temperature from 38 to 15 °C resulted in the release of only 12 % of the total bound Ca^{2+}. Thus, both temperature and pH influenced Ca^{2+} release from SR.

Table 2 shows the amount of Ca^{2+} released from SR vesicles on simultaneously

TABLE 2
CALCIUM RELEASE FROM SATURATED SR VESICLES ON SIMULTANEOUSLY CHANGING pH AND TEMPERATURE[a]

Final pH	*Temperature*	*Released Ca^{2+} (nM mg protein)*
6·6	38 → 0 °C	9·3 ± 6·3
5·6	38 → 0 °C	23·2 ± 0·1
5·0	38 → 0 °C	29·3 ± 5·6

[a] Saturated SR vesicles had 50·7 ± 2·6 nM Ca^{2+}/mg protein.

changing pH and temperature. Results indicate that lowering of pH and temperature simultaneously increases Ca^{2+} release from SR, and in effect was equivalent to cold-shortening. However, the amount of Ca^{2+} released from SR on simultaneously lowering pH and temperature was less than when either temperature or pH were lowered independently. Since the normal physiological changes occurring in intact muscle include a drop in both temperature and pH, the amount of Ca^{2+} accumulation and release may be less in pre-rigor post mortem muscle than was found in altering either pH or temperature alone. Nevertheless, Ca^{2+} release did occur on lowering pH and temperature together.

The amount of Ca^{2+} released on lowering the temperature from 38 to 0 °C was least at pH 6·6, but increased with each successive drop in pH. This demonstrates that SR releases more and more Ca^{2+} as the pH declines. Since there is a rather consistent drop in pH until rigor mortis occurs (Pearson *et al.*, 1973; Briskey, 1964) conditions causing maximum Ca^{2+} release (i.e. a simultaneous drop in pH and temperature) occur in pre-rigor post mortem muscle.

DISCUSSION

The results of this study indicate that both pH and temperature influence Ca^{2+} accumulation and release by bovine SR, which may be related to the phenomenon of cold shortening. Fresh SR preparations accumulated more Ca^{2+} than cold-shortened SR, which supports the hypothesis of Buege & Marsh (1975) that exposure to cold may decrease the ability of bovine SR to accumulate Ca^{2+}. Conversely, both cold temperatures and low pH values decreased Ca^{2+} accumulation and increased Ca^{2+} release by SR, which would support the theory that the leakage of Ca^{2+} by SR under such conditions is responsible for cold-shortening.

The present study indicates that simultaneously lowering the pH and temperature results in less Ca^{2+} being released by SR than when pH and temperature are lowered independently. However, conditions existing during normal chilling of pre-rigor muscle (a simultaneous drop in pH and temperature) may be sufficient to cause enough Ca^{2+} release to cause cold-shortening, as some 82% of the Ca^{2+} was released under such conditions in the present study.

Although the results of the present study do not prove whether or not cold temperatures and low pH are responsible for cold-shortening, they do show that such conditions increase Ca^{2+} release. Nevertheless, results suggest that the decreased Ca^{2+} accumulation by SR at cold temperatures and low pHs are at least contributing factors to cold-shortening. The question as to whether or not Ca^{2+} release by mitochondria (Buege & Marsh, 1975) or Ca^{2+} release by the SR is actually responsible for cold-shortening was not resolved in this study. The data obtained in this study alone are convincing for the role of SR in cold-shortening, but do not offer an explanation for the fact that red muscles shorten much less under aerobic than under anaerobic conditions (Buege & Marsh, 1975). Further work will be required to resolve the relative roles of the SR and mitochondria in cold-shortening of pre-rigor post mortem muscle.

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REFERENCES

BATE-SMITH, E. C. (1948). The physiology and chemistry of rigor mortis with special reference to the aging of beef. *Adv. Rood Res.*, **1**, 1–38.

BRISKEY, E. J. (1964). Etiological status and associated studies of pale, soft, exudative porcine musculature. *Adv. Food Res.*, **13**, 89–178.

BUEGE, D. R. & MARSH, B. B. (1975). Mitochondrial calcium and postmortem muscle shortening. *Biochem. Biophys. Res. Commun.*, **65**, 478–82.

CHEVALLIER, J. & BUTOW, R. A. (1971). Calcium binding to the sarcoplasmic reticulum of rabbit skeletal muscle. *Biochemistry*, **10**, 2733–7.

DUGGAN, P. F. & MARTONOSI, A. (1970). Sarcoplasmic reticulum. IX. The permeability of sarcoplasmic reticulum membranes. *J. Gen. Physiol.*, **56**, 147–53.

EBASHI, S. & LIPMANN, F. (1962). Adenosine-triphosphate-linked concentration of calcium ions in a particulate fraction of rabbit muscle. *J. Cell Biol.*, **14**, 389–97.

ELETER, S. & INESI, G. (1972). Phase changes in the lipid moieties of sarcoplasmic reticulum membranes induced by temperature and protein conformational changes. *Biochim. Biophys. Acta*, **290**, 178–85.

GOLL, D. E. STROMER, M. H., ROBSON, R. M., TEMPLE, J., EASON, B. A. & BUSCH, W. A. (1971). Tryptic digestion of muscle components simulates many of the changes caused by postmortem storage. *J. Anim. Sci.*, **33**, 963–82.

GREASER, M. L., CASSENS, R. G. & HOEKSTRA, W. G. (1967). Changes in oxalate-stimulated calcium accumulation in particulate fractions from postmortem muscle. *J. Agr. Food Chem.*, **15**, 1112–17.

INESI, G., BLANCHET, S. & WILLIAMS, D. (1973). *Organization of Energy Transducing Membranes*, pp. 93–136. M. Nakano and L. Packer, eds., University Park Press, Baltimore, Maryland.

JOHNSON, P. N. & INESI, G. (1969). The effect of methyl-xanthines and local anesthetics on fragmented sarcoplasmic reticulum. *J. Pharm. Expt. Ther.*, **169**, 308–12.

LEE, K. S., TANAKA, K. & YU, D. H. (1965). Studies on adenosine triphosphatase, calcium uptake and relaxing activity of the microsomal granules from skeletal muscle. *J. Physiol.*, **179**, 456–64.

LOCKER, R. H. & HAGYARD, C. J. (1963). A cold shortening effect in beef muscles. *J. Sci. Food Agr.*, **14**, 787–95.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**, 265–75.

MARSH, B. B. & LEET, N. G. (1966). Studies on meat tenderness. III. Effect of cold shortening on tenderness. *J. Food Sci.*, **31**, 450–9.

MARSH, B. B., WOODHAMS, P. R. & LEET, N. G. (1968). Studies on meat tenderness. V. The effects on tenderness of carcass cooling and freezing before the completion of rigor mortis. *J. Food Sci.*, **33**, 12–18.

MARTONOSI, A. & FERETOS, R. (1964). Sarcoplasmic reticulum. I. The uptake of Ca^{2+} by sarcoplasmic reticulum fragments. *J. Biol. Chem.*, **239**, 648–58.

MEISSNER, G. & FLEISHER, S. (1971). Characterization of sarcoplasmic reticulum from skeletal muscle. *Biochim. Biophys. Acta*, **241**, 356–78.

MEISSNER, G., CONMER, G. E. & FLEISHER, S. (1973). Isolation of sarcoplasmic reticulum by zonal centrifugation and purification of Ca^{2+}-pump and Ca^{2+}-binding proteins. *Biochim. Biophys. Acta*, **298**, 246–69.

MITCHELL, R. H. & HAWTHORNE, J. N. (1965). The site of diphosphoinositide synthesis in rat liver. *Biochem. Biophys. Res. Commun.*, **21**, 333–8.

MUSCATELLO, U., ANDERSSON-CEDERGREN, E. & AZZONE, G. F. (1962). The mechanism of muscle-fiber relaxation, adenosine triphosphatase and relaxing activity of the sarcotubular system. *Biochim. Biophys. Acta*, **63**, 55–74.

PEACHEY, L. D. (1970). Form of the sarcoplasmic reticulum and T system of striated muscle. In: *The Physiology and Biochemistry of Muscle as a Food*, Vol. 2, pp. 273–310. E. J. Briskey, R. G. Cassens and B. B. Marsh, eds., The University of Wisconsin Press, Madison, Wisc.

PEARSON, A. M., CARSE, W. A., DAVEY, C. L., LOCKER, R. H. & HAGYARD, C. J. (1973). Influence of epinephrine and calcium upon glycolysis, tenderness and shortening of sheep muscle. *J. Food Sci.*, **38**, 1124–7.

SRETER, F. A. (1969). Temperature, pH and seasonal dependence of Ca-uptake and ATPase activity of white and red muscle microsomes. *Arch. Biochem. Biophys.*, **134**, 25–33.

TISDALE, H. D. (1967). Preparation and properties of succinic-cytochrome c reductase (complex II–III). In *Methods in Enzymology, Vol. X*, pp. 213–15. P. W. Estabrook and M. E. Pullman, eds., Academic Press, New York.

WEINER, P. D. & PEARSON, A. M. (1966). Inhibition of rigor mortis by ethylenediamine tetraacetic acid. *Proc. Soc. Expt. Biol. Med.*, **123**, 185–7.

CHEMICAL COMPOSITION OF SOME LESS COMMONLY USED LEGUMES IN GHANA

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ABSTRACT

Three legumes, viz. mung bean, winged bean (seeds, pods and roots) and the yam bean have been analysed with respect to their moisture, protein, fat, soluble carbohydrate, fibre, mineral and energy content. The seeds provide a valuable source of protein, the winged bean having 29 %, *the mung bean* 23 % *and the yam bean* 19 %. *They are also high in energy content, the winged bean having* 400 *kcals/*100 *g, the mung bean* 310 *kcals/*100 *g and the yam bean* 327 *kcals/*100 *g. In addition to having the highest protein content, the winged bean seeds provide fair amounts of fat* (17·7 %) *and calcium* (204 *mg/*100 *g). The winged bean is significant in that most parts of the plant are edible such as the young leaves, pods and the roots. The roots contain significant amounts of protein and carbohydrate. The production and increased consumption of these legumes has been stressed.*

INTRODUCTION

The increased production and consumption of legumes offers a partial solution to increasing the available protein supplies to any given population. There are however some setbacks in the utilisation of legumes as sources of food and consequently of protein. There is the problem of storage because many legumes are easily infested with various kinds of pests which attack them. With regard to their use as foods there are factors such as palatability, flatulence effect, cooking time, etc., which militate against their increased consumption.

Although wide varieties of legumes are grown in many parts of the world only a few are commonly consumed by man. In recent years attempts have been made to bring about a better utilisation of legumes in the diet and in particular to try and introduce some of the less-used varieties. In Ghana the commonly used legumes

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include the cowpea, bambara bean, groundnut and lima bean. However, there are several other types of legumes which are already found or could be cultivated. This study was undertaken to provide data on the chemical composition of three different legumes found in Ghana but which are not consumed to any appreciable extent. The legumes studied were the mung bean (two varieties), the winged bean and the yam bean.

MATERIALS AND METHODS

Materials

The samples of mung bean and winged bean were obtained from the University research stations and that of the yam bean from the Volta Region of Ghana where it is commonly found. In addition to the seeds of the winged bean, the pods and the roots also were analysed since they are edible and can serve as sources of food.

Methods

The procedures carried out for the determination of moisture, protein, fat, crude fibre and ash were based on the standard techniques adopted by the Association of Official Agricultural Chemists (1970). The protein value was derived from the nitrogen content by multiplying by the factor 6·25. Of the minerals, calcium was precipitated as calcium oxalate and subsequently determined by titration against permanganate; both phosphorus and iron were determined colorimetrically, the former by the quinol/sodium sulphite method and the latter by the dipyridyl method. The carbohydrate values were computed by difference by subtracting from 100 the sum of the moisture, protein, fat, fibre and ash contents. The energy value was determined with a ballistic bomb calorimeter described by Miller & Payne (1959). The gross energy values thus determined are compared with the physical heats of combustion as computed from the carbohydrate, protein and fat contents using the following values: for carbohydrate 4·15 kcals/g; protein 5·65 kcals/g and fat 9·4 kcals/g. In the computation of the physical heats of combustion of carbohydrate, both the soluble carbohydrate and the fibre content of the foodstuff were taken into account. The metabolisable energy values were derived using the classical Atwater factors 4, 9 and 4 for protein, fat and carbohydrate respectively. However, in the case of contribution of carbohydrate to metabolisable energy, only the soluble carbohydrate content was used in the computation.

RESULTS AND DISCUSSION

The results giving the mean and range values for the various constituents are presented in Table 1.

TABLE 1

CHEMICAL COMPOSITION OF LEGUMES (VALUES IN g/100 g EDIBLE PORTION. EXCEPT MINERALS WHICH ARE EXPRESSED IN mg/100 g)

Foodstuff and description	*Moisture*	*Energy (kcals)*			*Protein*	*Fat*	*Carbo-hydrate*	*Fibre*	*Ash*	*Minerals*		
		Bomb	*Calculated*							*Ca*	*P*	*Fe*
			Physical	*Metab-olisable*								
Mung bean green (4)[a] *Phaseolus aureus*	15·2 (14·6–15·8)	374 (366–382)	379	306	23·0 (22·8–23·1)	1·3 (1·3–1·4)	53·3	3·8 (3·5–4·1)	3·4 (3·3–3·4)	108 (82–130)	430 (430–430)	8·0 (6·0–10·0)
Mung bean black (4) *Phaseolus mungo*	12·0 (11·7–12·2)	415 (385–440)	386	320	22·6 (22·1–22·9)	0·4 (0·3–0·5)	56·5	4·7 (4·4–4·9)	3·8 (3·6–4·0)	135 (127–140)	360 (320–395)	13·5 (11·0–16·5)
Winged bean pods (4) *Psophocarpus tetragonolobus*	86·8 (84·0–90·0)	64 (49–78)	62	43	2·6 (2·0–3·1)	0·6 (0·4–0·7)	6·8	3·2 (2·5–3·9)	0·6 (0·4–0·7)	45 (34–54)	36 (27–43)	15·0 (11·6–18·6)
Winged bean seeds (4)	11·2 (11·0–11·5)	450 (445–455)	490	402	29·1 (29·0–29·2)	17·7 (17·7–17·8)	31·6	6·8 (6·8–6·9)	3·6 (3·6–3·6)	204 (203–205)	376 (375–377)	9·6 (9·5–9·6)
Winged bean roots (4)	65·2 (63·2–67·6)	148 (139–158)	147	129	4·7 (4·4–5·0)	0·1 (0·1–0·15)	27·2	1·5 (1·4–1·6)	0·9 (0·8–0·9)	40 (37–42)	64 (59–67)	3·0 (2·7–3·1)
Yam bean (4) *Sphenostylis stenocarpa*	11·2 (10·8–11·8)	428 (417–446)	390	327	19·1 (18·3–19·9)	0·5 (0·4–0·6)	61·6	5·2 (4·5–6·4)	2·4 (2·3–2·5)	45 (44–46)	Not determined	11·5 (11·0–12·0)

[a] Figure in parentheses denotes number of samples analysed.

Of the legumes examined, the winged bean seeds have the highest protein content, 29 %, followed by the mung bean, 22–23 % and the yam bean, 19 %. The winged bean pods and roots have 2·6 % and 4·7 % protein respectively. All the legumes are low in fat with the exception of the winged bean seeds which contain 17·7 %. The winged bean seeds are also a moderate source of calcium, containing 204 mg/100 g. The results for the mung bean and the yam bean compare favourably with other published data such as those of Platt (1962) and FAO (1968). However, in the case of the winged bean seeds, the protein values obtained in this study are slightly lower (29 %) compared with those reported by other workers (Nicholls *et al.*, 1961, and Cerny *et al.*, 1971) who had protein values of 33 %.

The mung bean has been used extensively in parts of Asia where it is boiled and eaten whole or ground into a flour after the removal of the seed coat, the flour being used in the preparation of various dishes. In recent years it has been grown in Ghana where it has mainly been used as a poultry feed although there is no reason why it cannot be used for human consumption. One of the difficulties of introducing it is its unfamiliarity in this part of the world so that there is a need for education on how it may be used for incorporation into the local dishes.

The yam bean is consumed to a limited extent in Ghana and this is confined mainly to one particular region of the country, namely the Volta Region.

The winged bean is an interesting legume as most parts of the plant are edible. The young pods when tender are sliced and cooked like French beans; the tuberous roots are eaten like potatoes. The unripe seeds are used like peas in soups while the ripe seeds are roasted and eaten like peanuts. The seeds have a pleasant sweet taste even in the raw state although the skin is tough and more difficult to remove than that of the soya bean (Pospisil *et al.*, 1971).

Cerny *et al.* (1971) have shown that the amino acid composition of the protein in winged bean is very similar to that of the soya bean with methionine as the first limiting amino acid. The content of tocopherols, unsaturated fatty acids and that of polyunsaturated essential fatty acids was satisfactory. They showed that an active trypsin inhibitor found in the raw seeds could be destroyed by moist heat and that there was no detectable urease activity. They further demonstrated that the protein efficiency ratio (PER) and net protein utilisation (NPU) of the bean determined with rats were superior to those of groundnuts.

Cerny & Addy (1973) investigated the use of the winged bean as part of a mixed diet (two parts winged bean and three parts maize flour enriched with small amounts of skim milk) in the treatment of children suffering from kwashiorkor. They found that the diets were well accepted and tolerated and the children under study made good clinical progress such that the mixture gave results similar to those of a control diet in which 90 % of the protein content was supplied by skim milk.

The three legumes considered could serve as valuable sources of protein in the Ghanaian diet if their cultivation and consumption can be actively promoted.

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REFERENCES

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS (1970). *Methods of Analysis*, 11th Edition.

CERNY, K., KORDYLAS, J. M., POSPISIL, F., SVABENSKY, O. & ZAZIC, B. (1971). The nutritive value of the winged bean, *Brit. J. Nutr.*, **26**, 293–9.

CERNY, K. & ADDY, H. A. (1973). The winged bean in the treatment of kwashiorkor, *Brit. J. Nutr.*, **29**, 105–12.

FOOD AND AGRICULTURE ORGANISATION (1968). *Food composition tables for use in Africa.*

MILLER, D. S. & PAYNE, P. R. (1959). A ballistic bomb calorimeter, *Brit. J. Nutr.*, **13**, 501–8.

NICHOLLS, L., SINCLAIR, H. M. & JELLIFE, D. B. (1961). *Tropical nutrition and dietetics*, 4th Edition, Bailliëre, Tindall and Cox, London.

PLATT, B. S. (1962). Tables of representative values of foods commonly used in tropical countries, *Med. Res. Coun. Spec. Rep. Ser.*, No. 302.

POSPISIL, F., KARIKARI, S. K. & BOAMAH-MENSAH, E. (1971). Investigations on the winged bean in Ghana, *World Crops*, **23**, 250–3.

CHEMICAL STUDIES ON SOME NIGERIAN CARBONATED AND ALCOHOLIC BEVERAGES

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ABSTRACT

Carbonated beverages and different brands of lager beer bottled in Nigeria were analysed for carbohydrates, proteins, minerals, vitamins and alcohol, in order to provide basic data for the appraisal of their nutritive value. Seven carbonated beverages and five brands of lager beer were so analysed. The carbohydrate content of the carbonated drinks ranged from 9·52–13·93 g/100 g. They all contained sucrose, glucose and fructose. Calcium ranged from 0·97–1·71 mg/100 g; potassium, 0·21–0·27 mEq/100 g; sodium, 1·58–5·20 mEq/100 g. They contained no detectable amount of iron, traces of vitamin C, and negligible amounts of thiamin and riboflavin. The beers contained between 2·56 and 4·17 g carbohydrates/100 g. The main sugar was maltose. Alcohol (by weight) ranged from 3·77 % to 5·20 %. They were poor sources of minerals and vitamins. Some nutritional implications of these data are discussed.

INTRODUCTION

The demand of many Nigerians for carbonated beverages, simply referred to as 'soft drinks', and lager beers, has so greatly increased in recent times that many breweries and bottling companies have had to expand their factories to produce more of these beverages. The Federal Government of Nigeria, too, has been sympathetic enough as to allow the controlled importation of these beverages to discourage hoarding of the locally produced ones and for effective price control. Whilst the literature is replete with data on the nutrient content of such beverages in other countries (Jacobs, 1959; Davidson, 1961; McCance & Widdowson, 1967) no such published data are available on Nigerian beverages. This work was, therefore, embarked upon to estimate the nutrient content of these beverages for the purpose of providing data for the future compilation of Nigeria's Food Composition Tables, for the use of Food Consumption Survey teams, for the possible use of the Nigerian Standards

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Organisation and in view of the role of excessive and prolonged intake of alcohol in the aetiology of such ailments as cardiomyopathy (Asokan *et al.*, 1972; Godwin & Oakley, 1972), liver cirrhosis and some nutritional disorders (Davidson *et al.*, 1975).

MATERIALS AND METHODS

Materials

All the beverages analysed were bought from retailers. They included seven brands of carbonated beverages and five types of lager beer.

Methods

Alcohol was estimated using the AOAC methods of analysis (Association of Official Agricultural Chemists, 1970).

Total carbohydrate was determined using the phenol-sulphuric acid reagent of Dubois *et al.* (1951) and a glucose standard curve.

The beverages were qualitatively analysed for individual sugars by paper-chromatography using *n*-butanol, acetic acid and water (4:1:1 volumes) as irrigant. The sugars were identified using silver nitrate in acetone and ethanolic sodium hydroxide (Trevelyan *et al.*, 1950) and naphthoresorcinol and trichloroacetic acid (Partridge, 1948). Sucrose, fructose and glucose were found to be present in the carbonated beverages, with the latter two in greater proportions than sucrose. The beers contained maltose, and two other unidentified spots with Rf values lower than maltose and present in traces.

Protein was estimated by determining nitrogen using the Kjeldahl method and multiplying by 6·25.

Minerals were determined using atomic absorption spectrophotometry. Phosphorus was estimated by the method of Kitson & Mellon (1944).

Vitamins were estimated by the methods of vitamin assay of the Association of Vitamin Chemists (1966).

RESULTS AND DISCUSSION

Tables 1 and 2 below show the chemical composition of the beverages.

Carbonated beverages (Table 1)

The sugar content of the carbonated beverages ranged from 9·52 to 13·93%. Two cola beverages amongst them (C_2 and C_4) contained 12·68% and 11·31% respectively. These figures are slightly higher than those reported for similar drinks (10%–10·5%) by Toulousse (1933) and Jacobs (1959). The figure for a ginger ale (C_6) fell within the conventional range of 9·5–10% (Jacobs, 1959). These carbonated

TABLE 1
CHEMICAL COMPOSITION OF CARBONATED BEVERAGES

Carbonated beverages	*Carbohydrates (as glucose) (g/100 g)*	*Na*	*K*	*Ca*	*Mg*	*Fe*	*P*	*Thiamine*	*Riboflavin*	*Vit. C*
		(mEq/100 g)		*(mg/100 g)*				*(mg/100 g)*		
C_1	10·00	1·80	0·25	1·71	0·39	Nil	3·17	0·004	0·10	0·27
C_2	12·68	1·74	0·21	0·97	0·32	Nil	17·50	0·005	0·09	0·28
C_3	13·93	1·58	0·24	1·31	0·31	Nil	Nil	0·01	0·11	0·18
C_4	11·31	2·32	0·21	1·02	0·33	Nil	1·55	0·01	0·14	0·16
C_5	13·42	4·16	0·27	1·45	0·33	Nil	0·61	0·004	0·07	0·47
C_6	9·52	3·25	0·23	0·98	0·35	Nil	1·20	0·003	0·12	0·22
C_7	11·04	5·20	0·23	1·36	0·31	Nil	Nil	0·005	0·13	0·25

TABLE 2
THE CHEMICAL COMPOSITION OF LAGER BEERS

Beer types	*% Alcohol (by volume)*	*% Alcohol (by weight)*	*Carbohydrates (as glucose) (g/100 g)*	*Na*	*K*	*Ca*	*Mg*	*Fe*	*P*	*Thiamine*	*Riboflavin*	*Vit. C*
				(mEq/100 g)		*(mg/100 g)*				*(mg/100 g)*		
B_1	5·73	4·57	2·90	0·77	7·86	0·30	13·00	Nil	26·46	0·01	0·02	—
B_2	4·73	3·77	2·56	2·36	6·44	1·06	10·67	Nil	21·89	0·01	0·02	—
B_3	6·52	5·20	2·79	3·18	7·74	1·00	12·82	Nil	27·95	0·02	0·02	—
B_4	6·01	4·80	3·93	1·70	9·29	0·61	13·82	Nil	24·96	0·01	0·02	0·01
B_5	6·23	4·97	4·17	1·15	11·48	0·30	16·65	Nil	37·32	0·02	0·02	0·01

beverages are usually sold in bottles of minimum content 300 ml. By drinking 300 ml of these carbonated beverages, between 462 and 672 kJ of energy may be obtained. For school children, who are the major consumers of these beverages as refreshments in schools or at parties, a daily consumption of 300 ml may provide up to 9% of their daily energy requirement of 7·9 MJ (Idusogie, 1971). The vitamins were present in negligible amounts. However, the ascorbic acid content of the lemon-flavoured beverage (C_5) was outstanding, and up to 1·5 mg might be obtained per bottle of beverage. They were all low in minerals and contained no detectable iron. Sodium was, however, more abundant than other elements. Even at these levels (1·58–5·2 mEq/100 g), it is still possible for patients with congestive heart failure, on a daily sodium allowance of 40 mEq (Davidson *et al.*, 1975) per day, to drink any of these beverages.

Lager beers (Table 2)

They were all found to be low in vitamins. There is, therefore, a need for drinkers to eat a wide range of foodstuffs that are rich in vitamins to avoid avitaminosis. Thiamine was present in traces, the riboflavin figures of 15–19 μg/100 g fell below the range of 30–120 μg obtained by Davidson (1961) for some American beers. Though beers are generally regarded as poor sources of potassium (Davidson *et al.*, 1975), consumption of even a bottle of Nigerian beer (600 ml) may substantially contribute to potassium intake (between 3·86–6·89 mEq). They were all low in sodium and might be included in a low salt diet as previously suggested by Olmstead *et al.* (1954, 1955). Nigerian lager beers were found to be relatively high in alcohol (3·77% –5·20% by weight). Their alcohol content exceeded those cited by Watt & Merrill (1963)—3·6% by weight—and Platt (1962)—3% by weight for European beer. The nutritional implications of the relatively high alcohol content of Nigerian beers are that drinkers will derive more energy per unit weight of beer drunk, i.e. between 672 kJ and 916 kJ of energy per bottle; the period of alcohol clearance from the blood will be increased, and the rate of development of certain diseases precipitated by prolonged and excessive intake of alcohol will be faster in such drinkers than in those consuming beers of lower alcohol content. It must be noted that most Nigerian breweries prefer to sell their beer in bottles of 600 ml minimum content rather than in 333 ml cans or bottles.

The carbohydrate content ranged from 2·6% to 4·2%, and these carbohydrates may supply 246–398 kJ of energy per 600 ml bottle of beer.

The crude protein figures obtained were in close agreement with those earlier reported by Watt & Merrill (1963) and Davidson (1961).

CONCLUSION

In the light of the data above, it may be necessary for some Nigerians to modify

accordingly the quantities consumed of these beverages in consonance with their physiological integrity. In particular, the authority responsible for establishing standards in the country should formulate a policy on the level of inclusion of alcohol and carbohydrates in beverages to be consumed in Nigeria, taking into account Nigerians' great flair for parties.

REFERENCES

ASOKAN, S. K., FRANK, M. J. & WITHAM, A. C. (1972). Cardiomyopathy without cardiomegaly in alcoholics, *American Heart Journal*, **84**(1), 13–18.

Association of Official Agricultural Chemists (1970). *Official Methods of Analysis*, 11th Ed. pp. 157–9. Washington DC.

Association of Vitamin Chemists (1966). *Methods of Vitamin Assay*, 3rd Ed., pp. 120–64 and pp. 281–320. Interscience Publishers, London.

DAVIDSON, C. S. (1961). Nutrient content of beers and ales, *New Engl. J. Med.*, **264**, 185–6.

DAVIDSON, S., PASSMORE, R., BROCK, J. F. & TRUSWELL, A. S. (1975). *Human nutrition and dietetics*, 6th Ed. pp. 90–3 and p. 695. Churchill Livingstone, London.

DUBOIS, M., GILLES, K., HAMILTON, J. K., REBERS, P. A. & SMITH, F. (1951). A colorimetric method for the determination of sugars, *Nature*, **168**, 167.

GODWIN, J. F. & OAKLEY, D. M. (1972). The Cardiomyopathies, *British Heart Journal*, **34**, 545–52.

IDUSOGIE, E. O. (1971). The nutritional requirements of the Nigerian population, *African Journal of Medical Sciences*, **3**(1), 53–6.

JACOBS, M. B. (1959). *Manufacture and Analysis of Carbonated Beverages*, p. 47. Chemical Publishing Co. Inc., New York.

KITSON, R. E. & MELLON, M. G. (1944). The determination of phosphorus in plant material using phospho-vanadomolybdate complex, *Ind. Eng. Chem.* (Anal Ad.), **16**, 397.

MCCANCE, R. A. & WIDDOWSON, E. M. (1967). Alcohol and energy value of common alcoholic beverages, *Med. Res. Coun. Spec. Rep. Ser.*, No. 297, HMSO, London.

OLMSTEAD, E. G., CASSIDY, J. E. & MURPHY, F. D. (1954). Nutritional value of beer with reference to the low salt diet, *Amer. J. Clin. Nutrit.*, **2**, 392–5.

OLMSTEAD, E. G., CASSIDY, J. E. & MURPHY, F. D. (1955). The use of beer in the low salt diet with special reference to renal disease, *Amer. J. Med. Sci.*, **3**, 49–53.

PARTRIDGE, S. M. (1948). Filter-paper chromatography of sugars, *Biochem. J.*, **42**, 238.

PLATT, B. S. (1962). Tables of representative values of foods commonly used in tropical countries, *Med. Res. Counc. Spec. Rep. Ser.*, No. 302, HMSO, London.

TOULOUSSE, J. H. (1933). *Natl Bottlers' Gaz.*, **52**(618), 45 (cited by Jacobs (1959) above).

TREVELYAN, W. C., PROCTOR, D. O. & HARRISON, J. S. (1950). Detection of sugars on paper chromatograms, *Nature*, **166**, 444.

WATT, B. K. & MERRILL, A. L. (1963). Composition of foods—raw, processed, prepared. *US Department of Agriculture Handbook*, No. 8.

SOME FACTORS INFLUENCING CAECAL ENLARGEMENT INDUCED BY RAW POTATO STARCH IN THE RAT

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ABSTRACT

The phenomenon of caecal enlargement in rats fed raw potato starch as their sole dietary carbohydrate source is examined. The caeca of experimental animals were enlarged 800–1700% *. They contained undigested starch, an increased number of micro-organisms and a lower concentration of ions and were of lower pH* (5·5) *than those of control animals* (*pH* 7·0).

Duodenal contents had a lower specific activity of amylase expressed either as whole duodenal contents or on the basis of duodenal protein which was also depressed. This suggests that the potato starch either: (*a*) *contains an inhibitor of pancreatic amylase,* (*b*) *lacks an amylase activating or stabilising factor or* (*c*) *causes changes in the duodenal secretions.*

The resistance of potato starch to pancreatic amylase, together with the lower level of amylase in the duodenum, results in undigested starch reaching the caecum and there probably acting as a substrate for the caecal microflora. The mechanism whereby these events result in caecal enlargement is still unclear.

INTRODUCTION

Diets containing various types of glucose syrups and dextrins have been found to cause caecal enlargement in the rat (Birch & Etheridge, 1973; Birch *et al.*, 1973; Chalvardian & Stephens, 1970; Etheridge, 1974) and recently gross caecal

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enlargement has been reported in rats fed raw potato starch (El-Harith *et al.*, 1975, 1976*a* and *b*). The effect may be controlled by the osmotic value of the caecal contents (Leegwater *et al.*, 1974). Caecal enlargement was so gross as to be lethal in at least 21 % of rats fed raw potato starch at dietary levels of 30 % or more while no deaths occurred in control animals fed raw maize starch or in animals receiving gelatinised potato starch. Death of animals with grossly enlarged caeca appeared to result from respiratory insufficiency due to compression of the diaphragm (El-Harith *et al.*, 1976*a*). The effect is reversible since experimental animals which have maize starch substituted for potato starch in their diet revert to normal after a period of time (El-Harith *et al.*, 1975, 1976*a* and *b*).

Since caecal enlargement is quite commonly observed after feeding particular types of dietary carbohydrate such as lactose (Reussner *et al.*, 1963), analogous polyols such as sorbitol (Morgan & Yudkin, 1957), maltitol (Hosoya, 1972) and polyethylene glycol (Loeschke *et al.*, 1973) the effect deserves more detailed study.

MATERIALS AND METHODS

Animals and protocols

Weanling male Wistar albino rats of an inbred SPF-derived colony, of 40–50 g initial body weight, were used for all experiments. Animals were housed singly in cages with raised screen floors. Environmental conditions were controlled at a temperature of 20 °C ± 2°, a relative humidity of 45 % ± 5 %, and with a 12 h alternate light/dark cycle. Food and water were provided *ad libitum* and food intake and body weight were recorded weekly or more frequently as indicated below.

Materials and methods

Raw maize, potato and rice starches were obtained from BDH Chemicals Ltd (Poole, Dorset, Great Britain), cassava starch from Laing National Ltd (Manchester, Great Britain) and sorghum starch from the Food Processing Centre (Khartoum North, Sudan). Extracted maize and potato starches were obtained by refluxing with dioxan in a Soxhlet apparatus for 4 h. Autoclaved potato starch was prepared by moistening the raw starch with water and heating it in an autoclave at 120 °C for 2 h then drying at 40 °C for 24 h. Glucose syrups (DE 17 or DE 43) were obtained by a reverse osmosis fractionation procedure and freeze-dried (Birch & Kearsley, 1974).

Caecal fluid was obtained by homogenising caecal contents with deionised water (1:1 w/w). Electrolyte concentrations were determined in caecal fluid and blood serum as follows: sodium and potassium were determined in an EEL (Evans Electrosolenium Ltd) flame photometer and chloride by the method of Schales & Schales (1941). Osmolalities were determined with the Halbmicro-Knauer automatic osmometer. Pancreatin (Hopkin and Williams Ltd) was used for the

enzymatic hydrolysis of starches. The enzyme solution was prepared by shaking 250 mg of pancreatin with 50 ml of distilled water. The suspension was centrifuged and the supernatant liquid was used as the source of α-amylase. The starch (250 mg) was suspended in 40 ml of distilled water, phosphate buffer (Sorensen's, M/15, pH 7·0, 2 ml) and enzyme solution (30 iu amylase, 5 ml) were added and the volume made up to 50 ml with distilled water. The mixture was incubated at 37 °C with continuous shaking. Aliquots were withdrawn at intervals (2, 4, 8 and 12 h), centrifuged and the total soluble sugars determined in the supernatant by the method of Dubois *et al.* (1956).

For determination of amylase activity in the duodenum, an appropriate sample of the fluid from the duodenal lumen was collected immediately post-mortem after cervical dislocation and diluted with phosphate buffer (M/15, pH 7·0). Soluble maize or potato starch (BDH Chemicals, Poole, Dorset, Great Britain) made as 2% aqueous solution were then mixed with an equal volume of the diluted duodenal juice and the mixture incubated at 37 °C for 1 h. The reducing sugars (as maltose) were then determined by the 3,5-dinitrosalicylic acid method of Dahlqvist & Borgström (1961). For the determination of protein in the duodenal fluid the method of Lowry *et al.* (1951) was employed.

Experiment 1: Four groups of ten animals were maintained for three weeks on a diet with the following basic composition: carbohydrate 71 %, protein (casein) 16 %, corn oil 5 % (Craigmillar Ltd), vitamin mix 4 % (Cooper's Nutritional Products) and mineral mix 4 % (Cox Ltd). The carbohydrate source was varied between groups as follows: group 1A—raw maize starch; group 2A—extracted maize starch; group 1B—raw potato starch and group 2B—extracted raw potato starch.

At termination the animals were anaesthetised with Nembutal (20–35 mg/kg body weight) and bled from heart punctures and sacrificed by cervical dislocation. The caeca and intestinal sections were examined and weighed as necessary.

For the water balance study, three animals from each of groups 1A and 1B were singly housed in metabowls, on day 15 of the experiment. After a three-day period of adaptation to the metabowls, food intake, water intake, volume of urine and faecal weight were recorded daily for a period of three days (days 18–20, inclusive).

Experiment 2: To investigate whether a low molecular weight carbohydrate is needed for 'priming action' of the amylase activity (and hence to help reduce the phenomenon of caecal enlargement), animals were fed on small amounts of freeze-dried glucose syrup fractions.

Twelve groups of five animals were fed for three weeks on diets with a basic composition similar to the one described above. Six groups of these received maize starch as the main source of carbohydrate (groups $1C^1$, $2C^1$, $3C^1$ and $1D^1$, $2D^1$, $3D^1$). The six test groups ($1C^2$, $2C^2$, $3C^2$ and $1D^2$, $2D^2$, $3D^2$) received potato starch as the main source of carbohydrate.

Freeze-dried glucose syrups (DE 17 and DE 43) were included in the diets and partially replaced the 71 % carbohydrate part of the diet at levels of 5, 10 and 15 %.

The food intake and body weight were recorded weekly and at termination the animals were autopsied and the caeca excised and weighed.

RESULTS AND DISCUSSION

The inclusion of raw potato starch (whether extracted with dioxan or not) in the diet of rats resulted in significantly lower food efficiencies compared with similar diets containing maize starch (Table 1).

TABLE 1
MEAN FOOD EFFICIENCY VALUES OF RATS FED DIETS CONTAINING 71 % STARCH AS SOLE DIETARY CARBOHYDRATE

	1*A* (m)	2*A* (ext. m)	1*B* (p)	2*B* (ext. p)
First week	0·40	0·40	0·33	0·19
Second week	0·41	0·33	0·27	0·28
Third week	0·39	0·20	0·22	0·19
Third week (corrected)	0·37	0·19	0·14	0·09

m = raw maize starch; *p* = raw potato starch, *ext.* = dioxan extracted.
Food efficiency = increase in body weight (g)/food intake (g).
Corrected food efficiency = increase in body weight-weight of caecum (g)/food intake (g).
1A; 2A maize starch fed groups.
1B; 2B potato starch fed groups.

Dioxan treatment of the starches also resulted in a lowering of the food efficiency despite the fact that this treatment *increased* the rate of hydrolysis of maize starch by pancreatic amylase (Fig. 1) and had little effect on the rate of hydrolysis of raw potato starch. This effect of dioxan treatment may reflect a toxic effect of residues of dioxan in the extracted starches since this compound has been reported to be neurotoxic in the rat and to cause morphological changes in the hepatorenal system with an LC_{50} of 37 mg/litre by inhalation (Kulagina, 1960). Goldstein *et al.* (1970) reported that the maximum concentration of dioxan tolerated by rats and mice was 0·2 mg/litre.

Extraction of starches with dioxan removes certain minerals such as phosphorus compounds and polar lipids and, in the case of maize starch, this increased the rate of hydrolysis by amylase (Fig. 1) which is in accordance with the postulate that helical phospholipid/amylose complexes (Priestley, 1974) or phosphates (Bhotiyakornkiat & Birch, 1972; Turvey & Hughes, 1958) stabilise starches to hydrolytic attack by amylases. Potato starch retains more phosphate after dioxan

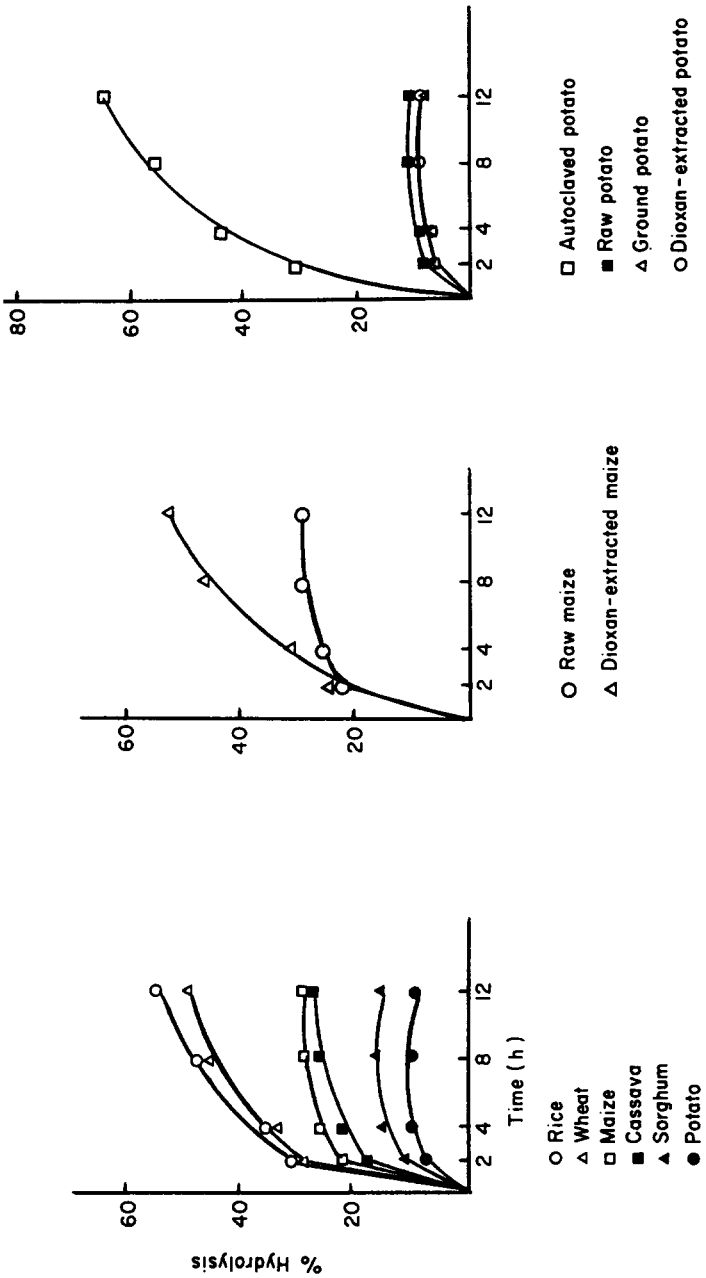

Fig. 1. The rate of hydrolysis of different starches (*in vitro*) by pancreatic α-amylase.

extraction than does maize starch (Bhotiyakornkiat & Birch, 1972) and this is probably involved in cross-linking between amylose molecules conferring a paracrystalline structure to the raw potato starch granule (Banks & Greenwood, 1975). Such a structure is probably responsible for the peculiar resistance of potato starch to amylase which results in the rate of hydrolysis being lower than any of the other starches examined, while destruction of this crystallinity by autoclaving greatly increases the rate of hydrolysis (Fig. 1).

As previously reported (El-Harith *et al.*, 1976), raw potato starch caused a gross enlargement of the caecum (Table 2) relative to maize starch; in addition, dioxan treatment itself caused further significant increases in caecal size. The animals receiving potato starch, raw or extracted, exhibited undigested starch granules in caecal contents and faeces while those receiving maize starch or autoclaved potato starch did not and this favours the view that the paracrystalline nature of the potato starch granule and its peculiar resistance to amylase are responsible for the caecal enlarging propensity. In this context potato starch granules are noted for their high swelling power (perhaps related to their esterified phosphorus content) and ability to absorb minerals, particularly Ca^{2+} and Mg^{2+}.

TABLE 2
ANALYSIS OF CAECAL CONTENTS AND SERUM ELECTROLYTES IN RATS FED RAW AND DIOXAN-EXTRACTED STARCHES

Parameter		1*A* (71% m)	2*A* (71% ext. m)	1*B* (71% p)	2*B* (71% ext. p)
Filled caecum wt g/100 g bd wt		1·23 ± 0·13	2·21^{a} ± 0·36	9·59^{b} ± 0·45	13·18^{b}† ± 1·95
Osmotic value m osml/Kg		228 ± 10	231 ± 14	202 ± 12	188^{a} ± 13
Caecal contents electrolytes					
μeqv./g wet wt	Na^+	107 ± 8	106 ± 8	67^{b} ± 5	83^{a}† ± 4
	K^+	31 ± 2	32 ± 2	24^{a} ± 3	29 ± 1
	Cl^-	20 ± 1	19 ± 3	16 ± 2	15 ± 3
Serum electrolytes m eqv./l	Na^+	125 ± 4	124 ± 4	127 ± 5	121 ± 6
	K^+	4·8 ± 0·2	4·7 ± 0·1	4·6 ± 0·1	4·2 ± 0·3
	Cl^-	109 ± 2	112 ± 3	115 ± 3	112 ± 2

m = raw maize starch, *p* = raw potato starch, *ext.* = dioxane extracted.
Mean values significantly different from those of group 1A are marked $^{a} = P < 0{\cdot}05$, $^{b} = P < 0{\cdot}001$.
The values of group 2B which are significantly different from group 1B values are marked † = $P < 0{\cdot}05$.
1A; 2A maize starch fed groups.
1B; 2B potato starch fed groups.

The ionic concentrations and osmolality of the caecal contents indicated a tendency towards lower values in the animals receiving potato starch (Table 2) and the Na^+/K^+ ratio (2·8–2·9) was lower in the potato starch than the maize starch fed groups (3·3–3·5), but this was without significant effect on serum electrolytes.

The greater caecal enlargement and lowered food efficiency consequent on dioxan extraction may reflect effects of residual dioxan on caecal microflora since some antibiotics also cause caecal enlargement (Savage & Dubos, 1968).

Partial replacement of dietary starch by dried glucose syrups (DE 17 or DE 43) had little effect on food efficiencies (Table 3) and in the case of DE 17 syrup the effect on caecal size was no more than expected on partial replacement of a refractory starch with a readily utilisable carbohydrate. Incorporation of DE 43 syrup in the potato starch-containing diet was without effect on caecal weight. Again, this is not altogether unexpected because DE 43 glucose syrup itself causes some caecal enlargement (Birch *et al.*, 1973).

TABLE 3
EFFECT OF FEEDING GLUCOSE SYRUPS (OF DE17 AND DE43) TO WEANLING RATS. ON THEIR FOOD EFFICIENCY AND CAECAL WEIGHT

Group	*Percent and source of dietary carbohydrate*	*Caecal weight g/100 g bd wt*	*Food efficiency First week*	*Second week*	*Third week*
$1C^1$	5*gs* + 66*m*	1·50 ± 0·05	—	0·34	0·57
$2C^1$	10*gs* + 61*m*	1·61 ± 0·06	—	0·31	0·62
$3C^1$	15*gs* + 56*m*	1·57 ± 0·13	—	0·41	0·66
$1C^2$	5*gs* + 66*p*	9·62 ± 1·38	—	0·39	0·34
$2C^2$	10*gs* + 61*p*	8·77 ± 0·37	—	0·35	0·39
$3C^2$	15*gs* + 56*p*	7·87 ± 0·99	—	0·34	0·35
$1D^1$	5*gs* + 66*m*	1·43 ± 0·07	0·49	0·37	0·32
$2D^1$	10*gs* + 61*m*	1·22 ± 0·06	0·51	0·37	0·32
$3D^1$	15*gs* + 56*m*	1·44 ± 0·10	0·51	0·31	0·37
$1D^2$	5*gs* + 66*p*	7·44 ± 0·78	0·35	0·26	0·24
$2D^2$	10*gs* + 61*p*	7·60 ± 1·05	0·43	0·28	0·21
$3D^2$	15*gs* + 56*p*	8·09 ± 1·03	0·43	0·19	0·27

gs = glucose syrup, *m* = raw maize starch and *p* = raw potato starch.
All the C groups received glucose syrup of DE = 17 while all the D group received glucose syrup with DE = 43.
Values listed are means of five animals/group.

When the amylase activity (against potato and maize starches) and protein content of the duodenal fluid of the animals used in the first experiment were determined *in vitro*, both were found to be lower in the group receiving raw potato starch (1B) than in the maize starch fed group (1A) (Fig. 2). The enzyme activities were, however, lowered more than the proteins. The fluid retention which is characteristic of all alimentary tissue below the stomach (in the raw potato starch fed groups) is underlined by examining the water balance in the two groups 1A and 1B (Table 4). This could account, in some measure, for the lower protein content in the duodenal fluid, but the marked lowering of the enzyme activity may possibly be ascribed to the presence of an inhibitor or the absence of an activator for amylase. Which of these two explanations best fits the facts must await more detailed enzyme study, but the marked caecal enlarging effect of the raw potato starch and the even more marked effect of the dioxan extracted material favours the notion that an activator is being lost. It is possible, for example, that a low molecular weight carbohydrate is needed for 'priming action' (Whelan, 1971) if the amylase is to successfully hydrolyse potato starch, and certain minerals such as Ca^{2+} and Mg^{2+}

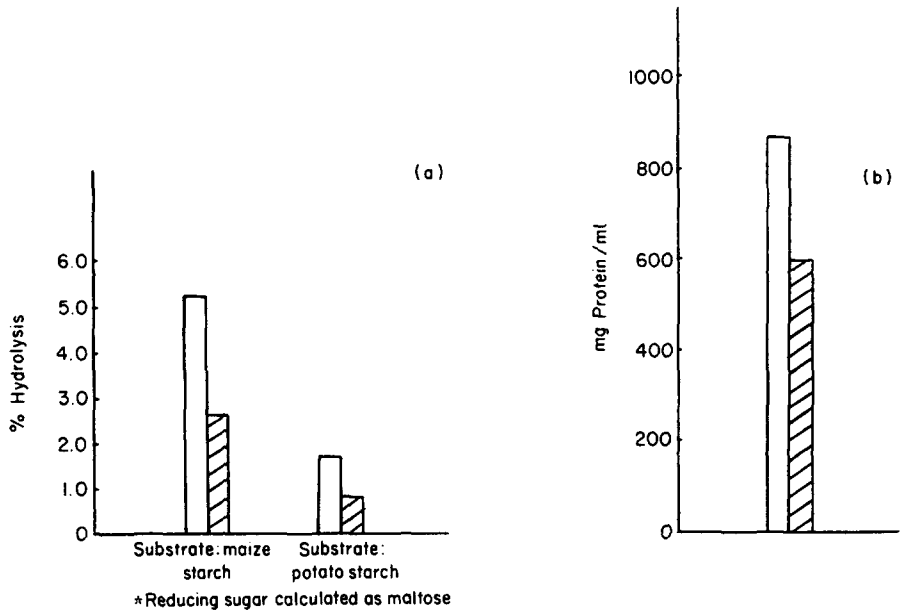

Fig. 2. (a) Amylase activity (reducing sugar calculated as maltose) of duodenal fluid in rats fed maize starch (white columns) or potato starch (hatched columns) diets. (b) Protein content of duodenal fluid in rats fed maize starch (white column) or potato starch (hatched column) diets.

are known stabilisers for amylase (Vallee *et al.*, 1959). The first of these possibilities was ruled out after the feeding experiment (Experiment 2) in which decreasing proportions of the dietary raw potato starch were replaced by DE 17 or DE 43 dried glucose syrup, a substance containing a complete range of possible priming sugars (Birch *et al.*, 1970). The results indicated that the glucose syrup addition conferred no protection against caecal enlargement, except for the proportional differences

TABLE 4

WATER BALANCE† IN YOUNG RATS FED ON RAW MAIZE OR RAW POTATO STARCHES

Parameter (per day per animal)	*1A (71% maize starch)*	*1B (71% potato starch)*
Water intake (g)	17·7 ± 0·5	16·7 ± 0·8
Volume of urine (ml)	8·8 ± 0·7	$5·2^a$ ± 0·6
Wet faecal weight (g)	0·7 ± 0·2	$7·4^b$ ± 1·0
Dry faecal weight (g)	0·6 ± 0·1	$4·5^b$ ± 0·5
Per cent moisture in faeces	23·9 ± 2·9	$36·7^a$ ± 2·5
Water balance	48·7 ± 4·3	51·4 ± 2·1

Values listed are mean daily determinations of three animals on three consecutive days.
Mean values which are significantly different from the values of the control group (1A) are marked $^a = P < 0·01$, $^b = P < 0·001$.

† Water balance calculated as $= \frac{(\text{water intake} - \text{water lost in urine and faeces}) \times 100}{\text{water intake}}$

expected by partial replacement of the potato starch (Table 3). The second possibility, that the divalent ions are absent, might not be anticipated in view of the mineral additions included in our experimental diets. However, pure polysaccharides may chelate metal ions (Angyal & Pickles, 1972*a* and *b*) and indeed the metal complexing properties of carbohydrates are already well known (Rendleman, 1966). Thus the mineral additions may not have been sufficient to compensate for the well known mineral absorbing property of the raw potato starch (Hollo *et al.*, 1962) which in particular absorbs Ca^{2+} and Mg^{2+} ions about twice as much as other starches. The rate of absorption of these ions by potato starch is again much higher than the rate of absorption by other starches.

The caecal microflora (especially lactic anaerobes) count is elevated in group 1B (receiving raw potato starch) and this will be the subject of a further paper from these laboratories. The pH of caecal contents is low (5·5) in group 1B compared with the control group 1A (7·0); this could be due to the apparent changes in the gut microfloral population. It is possible that maltodextrins, produced by limited amylase activity, are poorly absorbed and utilised, and that their humectant properties (Kearsley & Birch, 1975*a* and *b*) result in fluid retention.

The poor utilisation of raw potato starch has been recognised for some time (Booher *et al.*, 1951; Yoshida & Morimoto, 1955); Ketiku & Oyenuga (1973) have illustrated some similarity between yam and potato in this respect. More recently, Whittemore *et al.* (1975*a* and *b*) have studied the poor metabolisable energy associated with whole raw potato diets in pigs and have observed enlarged caeca engorged with particles of raw potato. These authors point out that there is a loss of measurable metabolisable energy when gut microflora convert undigested carbohydrate into volatile acids and intestinal gas, and such considerations may well undermine the significance of food efficiency calculations. Clearly, the phenomenon of caecal enlargement is not confined to rats fed on potato starch diets and it may possibly be a general manifestation of certain types of dietary carbohydrate interacting with the alimentary canal.

The mechanism by which caecal enlargement is effected remains unclear but the postulate that osmolality of the gut contents may play a part is worthy of consideration (Leegwater *et al.*, 1974). Although the osmolality tended to be lower in the enlarged caeca (Table 2) it is remarkable how similar these values are and this suggests that a homeostatic mechanism may be operative, controlling water absorption and osmolality in the caecum. In that case, caecal enlargement may be considered an adaptive rather than a toxic response which only produces pathologic sequelae in extreme cases.

REFERENCES

ANGYAL, S. J. & PICKLES, V. A. (1972*a*). Equilibria between pyranoses and furanoses I, *Aust. J. Chem.*, **25**, 1695.

ANGYAL, S. J. & PICKLES, V. A. (1972*b*). Equilibria between pyranoses and furanoses II. *Aust. J. Chem.*, **25**, 1711.

BANKS, W. & GREENWOOD, C. T. (1975). In: *Starch and its components*, Edinburgh University Press, 242.

BHOTIYAKORNKIAT, V. & BIRCH, G. G. (1972). Phosphate content in glucose syrup conversion, *Process Biochem.*, **7**, 25.

BIRCH, G. G. & ETHERIDGE, I. J. (1973). Chemical and physiological properties of glucose syrup components, *Starke*, **25**, 235.

BIRCH, G. G., ETHERIDGE, I. J. & GREEN, L. F. (1973). Short-term effects of feeding rats with glucose syrup fractions and dextrose, *B. J. Nutr.*, **29**, 87.

BIRCH, G. G., GREEN, L. F. & COULSON, C. B. (1970). *Glucose syrups and related carbohydrates*, Amsterdam. London and New York, Elsevier, 118.

BIRCH, G. G. & KEARSLEY, M. W. (1974). The fractionation of glucose syrups by reverse osmosis, *Starke*, **26**, 220.

BOOHER, L. E., BEHAN, I. & MCMEANS, E. (1951). Biological utilization of unmodified starches, *J. Nutrition*, **45**, 75.

CHALVARDIAN, A. & STEPHENS, S. (1970). Lipolitic effect of dextrin versus sucrose in choline deficient rats, *J. Nutr.*, **100**, 397.

DAHLQVIST, A. & BORGSTROM, B. (1961). Digestion and absorption of disaccharides in man, *Biochem. J.*, **81**, 411.

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**(3), 350.

EL-HARITH, E. A., WALKER, R. & DICKERSON, J. W. T. (1975). Some gastrointestinal effects of raw and processed potato starch, *J. Sci. Fd. Agric.*, **26**, 538.

EL-HARITH, E. A., DICKERSON, J. W. T. & WALKER, R. (1976*a*). Potato starch and caecal hypertrophy in the rat, *Fd. Cosmet. Toxicol.*, **14**, 115.

EL-HARITH, E. A., DICKERSON, J. W. T. & WALKER, R. (1976*b*). On the nutritive value of various starches for the albino rat, *J. Fd. Sci. Agric.*, **27**, 521.

ETHERIDGE, I. J. (1974). Ph.D. Thesis (Reading Univ.) Nutritional and technological studies on glucose syrups.

GOLDSTEIN, I., DUMITRU, E., DAVID, V. & MELINTE, L. (1970). Toxicity of glycol derivatives, *Igiena*, **19**(4), 209. *Chem. Abst.*, **75**, 33269.

HOLLO, J., HUSZAR, J., SZEJTH, J. & PETLO, M. (1962). Calcium and magnesium absorption in starch, *Starke*, **14**, 343.

HOSAYA, N. (1972). Effect of sugar alcohol on the intestine, *IX Int. Congr. Nutr.*, Mexico City.

KEARSLEY, M. W. & BIRCH, G. G. (1975*a*). Selected physical properties of glucose syrup fractions obtained by reverse osmosis: I—Specific rotation, average molecular weight, solubility rate, *J. Fd. Technol.*, **10**, 625.

KEARSLEY, M. W. & BIRCH, G. G. (1975*b*). Selected physical properties of glucose syrup fractions: II—Hygroscopicity, *J. Fd. Technol.*, **10**, 613.

KETIKU, A. O. & OYENUGA, V. A. (1973). Dehydrated yam and cassava as sources of energy to the laboratory rat. *W.A.J. Biol. Appl. Chem.*, **16**(2), 9.

KULAGINA, N. K. (1960). On the toxic action of dioxan, *Akad. Med. Nauk SSSR*, 142. *Chem. Abst.*, **58**, 869.

LEEGWATER, D. C., DE GROOT, A. P. & VAN KALMTHOUT-KUYPER, M. (1974). The aetiology of caecal enlargement in the rat, *Fd. Cosmet. Toxicol.*, **12**, 687.

LOESCHKE, K., UHLICH, E. & HALBACH, R. (1973). Caecal enlargement combined with sodium transport stimulation in rats fed polyethylene glycol, *Proc. Soc. Exptl. Biol. Med.*, **142**, 96.

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.

MORGAN, T. B. & YUDKIN, J. (1957). The vitamin-sparing action of sorbitol, *Nature (London)*, **180**, 543.

PRIESTLEY, R. J. (1974). Ph.D. Thesis (Reading Univ.)—Physiochemical studies of rice starch.

RENDLEMAN, J. A. (1966). Complexes of alkali metals and alkaline earth metals with carbohydrates. In: *Advances in carbohydrate chemistry*, Vol. 21 (ed. M. L. Wolfrom), London, Academic Press.

REUSSNER, G. (Jr.), ANDROS, J. & THISSEN, R. (Jr.) (1963). Studies on the utilization of various starches and sugars in the rat, *J. Nutr.*, **80**, 291.

SAVAGE, D. C. & DUBOS, R. (1968). Alterations in the mouse caecum and its flora produced by antibacterial drugs, *J. Exp. Med.*, **128**(1), 97.

SCHALES, O. & SCHALES, S. S. (1941). A simple and accurate method for the determination of chloride in biological fluids, *J. Biol. Chem.*, **140**, 879.

TURVEY, G. R. & HUGHES, R. C. (1958). The enzymic degradation of raw starch granules, *Proceedings of the Biochemical Society*, **69:1**, 4.

WHELAN, W. J. (1971). Enzymatic explorations of the structures of starch and glycogen, *Biochem. J.*, **122**, 609.

WHITTEMORE, C. T., TAYLOR, A. G., MOFFAT, I. W. & SCOTT, A. (1975*a*). Nutritive value of raw potato for pigs, *J. Sci. Fd. Agric.*, **26**, 255.

WHITTEMORE, C. T., MOFFAT, I. W. & TAYLOR, A. G. (1975*b*). Influence of cooking upon the nutritive value of potato and maize diets for growing pigs, *J. Sci. Fd. Agric.*, **26**, 1567.

VALLEE, B. L., STEIN, E. A., SUMMERWELL, W. N. & FISCHER, E. H. (1959). Metal content of amylases of various origins, *J. Biol. Chem.*, **234**, 2901.

YOSHIDA, M. & MORIMOTO, H. (1955). Utilization of sweet potato starch by rats and its effect on the digestion of dietary protein, *J. Nutr.*, **57**, 565.

ACCELERATED SHELF-LIFE TESTING FOR OXIDATIVE RANCIDITY IN FOODS—A REVIEW

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ABSTRACT

Accelerated shelf-life test (ASLT) methods for processed foods are receiving greater attention. In this paper, current ASLT methodology for fatty foods is reviewed with particular emphasis on the testing of antioxidant effectiveness.

In all the classical ASLT methods temperature is the dominant acceleration factor used. Its effect on the rate of lipid oxidation is best analysed in terms of the overall activation energy, E_A for lipid oxidation. It is an inherent assumption in these tests that the E_A is the same in both the absence and the presence of antioxidants. An analysis of the rate equations for the uninhibited versus the inhibited oxidation indicates, however, that the E_A may be considerably higher in the latter case. ASLT data collected at 60–65 °C bear this out and show that such tests lead to sizeable, but predictable, underestimation of the shelf-life extension by antioxidants for room temperature. In comparison, data collected at 98–100 °C are much less predictable. At this higher temperature E_A-variations are generally smaller and both under- and overestimation of shelf-life is found. In addition, the use of such high temperatures for complex foods is ruled out because of secondary reactions of other food components.

Other acceleration parameters for shelf-life used are the oxygen pressure, reactant contact and the addition of catalysts. The effect of these factors, although much less important than that of temperature, is discussed.

INTRODUCTION

Rancidity of edible oils and fatty foods due to lipid oxidation is a serious problem in some sectors of the food industry. Factors which have contributed to this problem in recent years are the increased emphasis on polyunsaturated dietary lipids and the fortification of certain foods with iron. Because of the unfortunate consequences of

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lipid oxidation in foods it is critical that information about the oxidative stability of susceptible food items be obtained before they are marketed.

The food manufacturer would like to employ methods which can give a reasonably accurate indication of the product shelf-life in a relatively short period of time. Many accelerated shelf-life tests (ASLT) are available. Since the rate of a reaction increases exponentially with the absolute temperature, this parameter is usually singled out to speed up the oxidation in such tests (for explanation of symbols and abbreviations used see Table 1).

The first step in a typical ASLT study is to select a suitable method for testing the food product under consideration. Next, a sample is placed under the conditions of the test and the induction period θ_s is measured, i.e. the time, usually in hours, required to reach a specified end-point. The last and the most difficult step is to translate the value for the induction period obtained into actual product shelf-life in months of storage. Usually this is done with some arbitrary factor based on prior experience. As most highly oxidisable foods contain added antioxidants these methods are often used to evaluate the effectiveness of antioxidants.

There are several reasons why a review of the ASLT methodology is presented here. Originally these methods were designed to be used for homogeneous lipids such as animal fats and vegetable oils. Unfortunately, only a few studies have been carried out to evaluate these methods critically. At least two such studies (Pohle *et al.*, 1964; Paul & Roylance, 1962) raise serious doubts about the usefulness of these tests even when applied to fats and oils. Pohle *et al.* (1964) concluded from their data that in order to obtain any useful information from the current ASLT methods, each test must be calibrated for each individual fat formulation.

Another serious drawback of the currently-used ASLT methods is the fact that, with the exception of the Active Oxygen Method, they are not standard methods. Different workers use different versions and modifications, which greatly complicates matters.

Finally, there is a growing interest in applying ASLT methods to formulated foods. The feasibility of using existing methods for this purpose should be evaluated.

Basically, four parameters are manipulated in ASLT procedures to speed up the oxidation and development of rancidity in foods or oils. These are listed in Table 2. Increased temperature is the most common and effective means of accelerating the oxidation. The rate of the reaction is exponentially related to the temperature; thus shelf-life should decrease logarithmically with increasing T. In *single-component* lipid systems oxidation can be represented by the following equation:

$$RH + O_2 \xrightarrow{K} ROOH \rightarrow \text{Secondary Products} = \text{rancidity or end of shelf-life}$$

where: RH = polyunsaturated lipid substrate; O_2 = oxygen; ROOH = lipid hydroperoxide (primary product) and K = overall rate constant for ROOH production.

TABLE 1
ABBREVIATIONS AND SYMBOLS USED

Symbol	*Meaning*
a	Inhibition, reaction (10)
A	Pre-exponential factor
A·	Antioxidant radical
AH	Primary antioxidant
AOM	Active Oxygen Method
ASLT	Accelerated Shelf-Life Test
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
c	Chain transfer, reactions (6) and (−10)
d	Dimerisation, reaction (9)
D	Bond dissociation energy, kcal/mole
E_a, E_i, E_p, E_t	Activation energies for elementary steps
E_A	Overall activation energy
EDTA	Ethylene diaminetetraacetic acid
f	Reaction (8)
HVO	Hydrogenated vegetable oil
i	Initiation, reaction (2)
k	Rate constant for elementary step
K	Overall reaction rate constant
K_A	Overall inhibited rate constant
K_M	Overall uninhibited rate constant
M	Metal ion
M^{n+}	Metal ion, lower oxidation state
$M^{(n+1)+}$	Metal ion, upper oxidation state
o	Propagation, reaction (4)
OAM	Oxygen Absorption Method
OBM	Oxygen Bomb Method
p	Propagation, reaction (5)
PG	Propyl gallate
psia	lb/in^2 absolute pressure
PUFA	Polyunsaturated fatty acid
PV	Peroxide value, meq ROOH/kg lipid
Q_{10}	Temperature coefficient, k_{T+10}/k_T
R	Universal gas constant, 1·987 cal/K-mole
R·	Lipid radical
RH	Lipid substrate
RO·	Oxyl radical
ROO·	Peroxyl radical
ROOH	Hydroperoxide
SOT	Schaal Oven Test
t	Termination, reaction (7)
T	Temperature in °C or K
TBHQ	Tertiary butylhydroquinone
θ	Time
θ_s	Induction period (elevated T) or shelf-life (room temperature)

On a kinetic basis $K = A\,e^{-E_A/RT}$ where A, the pre-exponential factor, and E_A, the activation energy, stay approximately constant as long as the mechanism does not change. Some workers, in analysing storage studies, use Q_{10}-values to determine the

TABLE 2
COMMON ACCELERATION PARAMETERS

Parameter	*Normal range*
Temperature (°C)	60–140
Oxygen pressure (psia)	3–165
Added metals (ppm in lipid)	25–100
Reactant contact	variable

accelerating effect instead of activation energies. These quantities are related through the following equation:

$$\log Q_{10} = (2{\cdot}189E_A)/(T + 10)T = \log\left[\frac{\text{shelf-life at } T + 10}{\text{shelf-life at } T}\right]$$

where: Q_{10} = increase in rate or decrease in shelf-life for a 10 °C increase in T; T = temperature in K and E_A = energy of activation in cal/mole.
Unlike the activation energy, the Q_{10}-value is a strong function of temperature and thus a poor criterion of the temperature sensitivity of the rate constants. As can be seen, the higher the E_A, the larger will be the increase in rate resulting from an increase in temperature. If the Q_{10} for the control is known it is usually applied to the test system containing the antioxidant in question. Obviously if E_A changes this Q_{10} factor is meaningless, as will be discussed.

Lipid oxidation can be broken down into a number of elementary step reactions where each step has its own activation energy. The controlling overall activation energy E_A can thus change as a result of a temperature elevation alone. Also, the addition of antioxidants, change in the oxygen pressure and other factors can alter the mechanism and hence the activation energy. These E_A changes, therefore, could cause erroneous prediction of the shelf-life at room temperature on the basis of data collected at a higher temperature, when the shelf-life of a control system is compared with that of one with added antioxidants.

In multi-component formulated food systems the effect of temperature can be very complex. A number of changes, both physical and chemical, may occur as the temperature is increased as listed in Table 3. Some of these changes could drastically affect the chemical reactivity through their effect on the distribution of reactants, metal binding properties, viscosity, metal prooxidant effectiveness and other variables. The water activity will increase as the temperature is increased and water can evaporate from the food. The fat—if solid at room temperature—can undergo crystal structure changes and eventually melt, contact new catalytic surfaces or drip out of the food. Once melted, oxidation will no longer be limited to the surface of the fat. Some end-products of the browning reaction between carbohydrates and proteins have antioxidative properties (Kirigaya *et al.*, 1968). Various proteins and

TABLE 3
EFFECTS OF 'HIGH' TEMPERATURES ON FOODS

Component	*Process*	*Critical T-Zone*
Water	Transfer	Above ambient
	Evaporation	Above 60 °C
Fat	Crystal changes	Close to melting point
	Melting	Above melting point
	Transfer	Above melting point
Starch	Gelatinisation	Above 60 °C
Carbohydrates	Non-enzymatic browning	Above 50 °C
	Caramelisation	Above 100 °C
	Charring	Above 100 °C
Proteins	Denaturation	Above 40 °C

enzymes will be denatured including lipoxigenases, peroxidases and other heme-proteins, with subsequent alteration in their pro-oxidative potential (Ericksson *et al.*, 1971). Heating can expose SH-groups which can act as antioxidants. Heme-bound metals may adopt a low-spin configuration, resulting in a loss of catalytic activity (Love & Pearson, 1974). Thus, the probability of a change in the mechanism occurring as a result of an increase in temperature is much greater in complex foods than in simple lipid systems. Some of the reactions listed in Table 3 can become significant at temperatures as low as 40 to 50 °C. This temperature range should therefore not be exceeded in ASLT studies for foods. The question remains as to whether such a moderate temperature elevation alone provides a sufficient acceleration in the oxidation rates for evaluation of shelf-life.

Other parameters besides the temperature which may accelerate the oxidation are listed in Table 2. At *oxygen pressures* close to atmospheric and higher, little effect of the oxygen pressure on the rate is obtained. However, at higher temperatures the effect of oxygen can become considerable (Bateman, 1954), especially for fats which are high in polyunsaturated fatty acids. Similarly, stirring, air bubbling and the use of 'inert' carriers or other means of promoting the *reactant contact* should have little effect provided the lipid is completely melted, the temperature is relatively low and no trace contaminants are introduced in the process. At the high temperatures of most ASLT methods, however, increasing the contact between oxygen and substrate can have an effect on the rate. Finally, by *adding pro-oxidants* such as transition metals one can accelerate the oxidation. This parameter is seldom used as it may cause changes in the mechanism of lipid oxidation. In conclusion, the temperature is generally by far the most important acceleration parameter used in ASLT methods.

THE EFFECT OF TEMPERATURE ON LIPID OXIDATION RATES

Lipid oxidation is caused by a free-radical chain reaction occurring through a series of steps. Each one has associated with it a rate constant. The most important steps in

the mechanism of the oxidation of polyunsaturated fatty acid esters are shown in Table 4. As indicated, the reaction can be divided into four phases: Initiation, propagation, termination and inhibition.

TABLE 4
MECHANISM OF LIPID OXIDATION

	$k_{30°C}$	E_a
Initiation		
(1) $RH + M^{(n+1)+} \longrightarrow R\cdot + H^+ + M^{n+}$	f (metal)	f (metal)
(2) $ROOH + M^{(n+1)+} \xrightarrow{k_2} ROO\cdot + H^+ + M^{n+}$	f (metal)	f (metal)
(3) $ROOH + M^{n+} \longrightarrow RO\cdot + OH^- + M^{(n+1)+}$	f (metal)	f (metal)
Propagation		
(4) $R\cdot + O_2 \xrightarrow{k_p} ROO\cdot$	10^7–10^9	0
(5) $ROO\cdot + RH \xrightarrow{k_p} ROOH + R\cdot$	10^2	5–7
(6) $A\cdot + RH \xrightarrow{k_c} AH + R\cdot$	slow	5–10
Termination		
(7) $ROO\cdot + ROO\cdot \xrightarrow{k_t} ROOOOR$	10^7	0–3
(8) $ROO\cdot + A\cdot \xrightarrow{k_f} ROOA$	fast	0–3
(9) $A\cdot + A\cdot \xrightarrow{k_d} A_2$	slow	0–3
Inhibition		
(10) $ROO\cdot + AH \underset{k_a}{\overset{k_a}{\rightleftharpoons}} ROOH + A\cdot$	10^4	0·8

RH = lipid substrate = methyl linoleate or linolenate
R· = substrate radical (di- or tri-allyl)
RO· = lipid oxyl radical
ROO· = lipid peroxyl radical
ROOH = lipid hydroperoxide
AH = primary antioxidant = BHT
A· = antioxidant radical (phenoxy)
$k_{30°C}$ = rate constant at 30 °C, litres/mole-sec
E_a = energy of activation, kcal/mole

Uri (1961*a*); Ingold (1973); Howard (1973); Howard & Furimsky (1973); Bateman (1954); Korcek *et al.* (1972); Ingold (1968).

The initiation can be either metal-, light- or thermally-catalysed. Singlet oxygen initiation has been implicated in the early initiation process (Rawls & van Stanten, 1970). When the lipid contains suitable sensitisers such as chlorophyll or polycyclic hydrocarbons, singlet oxygen initiation may dominate throughout in the presence of ultraviolet or visible light (Labuza, 1971; Grosch, 1975). Normally, however, as soon as hydroperoxides have formed, reactions (2) and (3) predominate. Waters (1971) does not believe that reaction (2) is important in metal-catalysed lipid oxidation. Kochi (1973), in contrast, has suggested that reaction (2) does occur and is rate-limiting in non-polar and poorly co-ordinating solvents such as lipids, especially in the presence of transition metals which are powerful oxidising agents such as Co and Mn.

The propagation occurs through steps (4) and (5). At ambient conditions the concentrations of oxygen and methyl linoleate in purified methyl linoleate are of the order of 10^{-3} molar and 1 molar respectively; thus reaction (5) is rate-limiting and the overall rate is approximately independent of the oxygen pressure. As the energy of activation for reaction (5), E_p is much higher than E_0 (Koreck *et al.*, 1972)—the same is not true at 100 °C. If weak, non-hindered antioxidants are present the A· radical can also act as a chain carrier through reactions (6) and (− 10), especially at high temperatures and at high antioxidant concentrations.

The termination is dominated by reaction (7) at atmospheric pressure. In the presence of primary antioxidants termination steps (8) and (9) also occur. The former is normally 'fast' whereas reaction (9) is very slow for 2,4,6-tri-substituted phenols (Ingold, 1973).

The inhibition takes place via reaction (10). Howard & Furimsky (1973) measured the E_A for a number of amines and phenolic antioxidants. For highly hindered phenols and amines the E_A was approximately 0–1 kcal/mole whereas the corresponding value for phenol and aniline was about 5 kcal/mole (Chenier *et al.*, 1974).

Mahoney (1969) has pointed out that for hydroquinones and sterically hindered phenols (hereafter referred to as ideal inhibitors) reactions (8) and (9) are much faster than reactions (6) and (− 10), whereas, for non-hindered phenolic antioxidants (i.e. non-ideal antioxidants), the four reactions can proceed at comparable rates. Commercial primary food antioxidants generally belong to the former category.

The low value of E_A obtained for hindered phenols and aromatic amines has been explained in terms of the formation of a hydrogen-bonded free-radical complex prior to the transfer of hydrogen (Howard & Furimsky, 1973). The activation energy for the back-reaction, E_{-A} may be roughly estimated by subtracting the bond dissociation energy of AH, D_{AH} from that for ROOH, D_{ROOH}. Generally the latter is about 88 (Howard, 1973) while the former is about 80 for hindered phenols, e.g. 81 kcal/mole for 2,6-di-tert-butylphenol (Chenier *et al.*, 1974). Therefore E_{-A} in this case is close to 7 kcal/mole. Hence, at high temperatures and once significant hydroperoxide has built up, the back-reaction can become important, giving the antioxidant some pro-oxidant properties. Reaction (6) also has a relatively high activation energy and would be expected to dominate the chain transfer process at low hydroperoxide concentration.

In the absence of primary antioxidants the limiting equation at 'high' oxygen pressures has been shown to be (Labuza, 1971):

$$[\mathrm{ROOH}]^{1\,2} = (k_i[\mathrm{M}]/k_t)^{1\,2} k_p[\mathrm{RH}]\theta/2 = K_M\theta/2 \tag{11}$$

thus

$$E_A = E_p + 0{\cdot}5(E_i - E_t) \approx 6 + 0{\cdot}5(E_i - 0) \tag{12}$$

where: θ = time and K_M = overall uninhibited reaction rate constant.

It should be noted that the rate does not depend on the oxygen pressure. At 'low' oxygen pressures, however, an oxygen-dependent rate-expression with a lower E_A is found.

Because of the change in termination with the presence of ideal primary inhibitors the following first order rate-expression is obtained (Labuza, 1971):

$$\ln [ROOH] = \ln [ROOH]_0 + k_i[M]k_p[RH]\theta/K_A[AH] = \ln [ROOH]_0 + K_A\theta/[AH] \quad (13)$$

thus:

$$E_A = E_i + E_p - E_a \approx E_i - E_a + 6 \quad (14)$$

where: $[ROOH]_0$ = initial hydroperoxide concentration and K_A = overall inhibited reaction rate constant.

In this case the rate is also oxygen-independent, even at quite low oxygen pressures. As shown, the rate of lipid oxidation is inversely proportional to the antioxidant concentration.

A comparison of eqns. (12) and (14) shows that the E_A will be considerably higher in the presence of a primary antioxidant than for the corresponding control if E_i is the same. This is, of course, quite logical and indicates that these inhibitors lower the rate of the oxidation at least partly by increasing the overall energy of activation. It must be realised, however, that eqn. (13)—and hence eqn. (14)—applies only for ideal primary inhibitors. Especially for relatively non-hindered phenolic antioxidants at high antioxidant concentrations, much more complex rate expressions with lower E_A-values can be expected. The same is probably true for the ideal inhibitors. At high temperatures chain transfer reactions become more important and eqns. (13) and (14) no longer apply.

To illustrate the significance of eqns. (12) and (14) one can consider a reaction, in this case lipid oxidation ($RH + O_2 \xrightarrow{K} ROOH$), which can occur via two different routes, path 1 and path 2, where $K_1 = A_1 e^{-E_1/RT}$ and $K_2 = A_2 e^{-E_2/RT}$. If path 1 represents lipid oxidation in the absence of primary antioxidants and path 2 in the presence of primary antioxidants then $E_2 > E_1$. The Arrhenius plots corresponding to $A_1 = A_2$ are shown in Fig. 1(a). As shown, the degree of protection by the primary antioxidant increases as the temperature is decreased.

In order to apply these principles to ASLT methodology one must first assume that the overall rate constant (K_1 or K_2) is proportional to the reciprocal of the 'induction period', θ_s, where the induction period (or the shelf-life at room temperature) is defined as the time to reach a constant percent oxidation of the substrate or end of shelf-life. Hence one can plot 'either' ln K or ln θ_s versus $1/T$ K (or T°C, if the temperature interval is small) and obtain linear plots as shown in Figs. 1(a) and (b). These plots both show that the effectiveness increases as T decreases. Thus the overall protection predicted at high temperatures for an antioxidant will usually be less than that found at lower temperatures. On the other hand, if the E_A

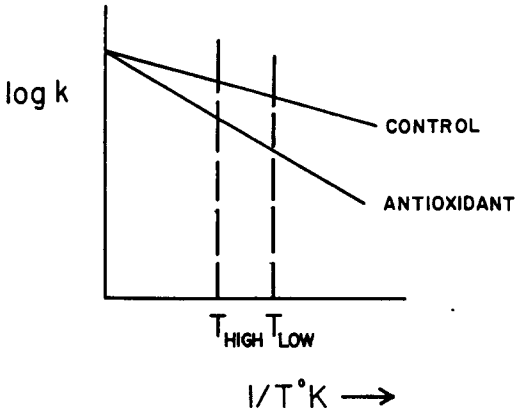

Fig. 1(a). Arrhenius plots for two parallel reactions.

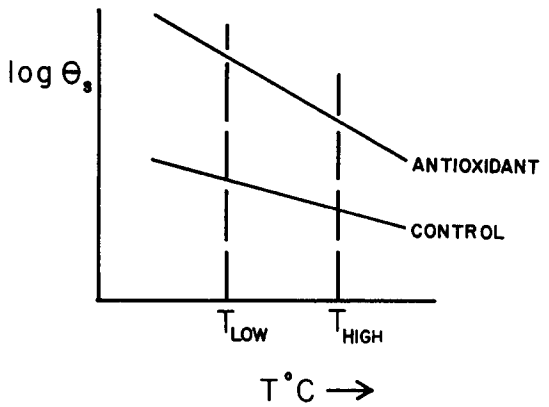

Fig. 1(b). ASLT plots for two parallel reactions.

decreases when the antioxidant is added, the degree of protection projected from high temperatures would overestimate the true shelf-life.

Bolland (1949) assumed that $E_i = 30$ kcal/mole for all purified olefins. In foods the E_i is much lower. Labuza (1972) found that in the range 37–52 °C for lipid oxidation in a chicken/cellulose/glycerol food system, E_A was about 10 in the absence of added antioxidants, indicating that E_i may have been as low as 8 kcal/mole. In the presence of added EDTA the E_A rose to about 17, presumably because the metal catalysts were inactivated, allowing an increase in E_i (to about 22 kcal/mole).

These considerations have important implications for high temperature testing of antioxidants at a single temperature. When fats such as animal fats, which contain

very low levels of primary antioxidants (*cf.* eqn. (12)), are tested against the same fat containing ideal antioxidants (*cf.* eqn. (14)) the temperature coefficients are quite different. Thus, any quantitative prediction for lower temperatures is impossible unless several temperatures are used. Besides, the order of effectiveness (ranking) at the higher temperature may not be the same at a lower temperature, since E_A may vary, depending on the structure of the antioxidant. The same is true for the evaluation of chelating agents since the E_i will change as compared with the control. However, if the only objective is to qualitatively rank antioxidants of similar structure (e.g. hindered phenols) at a single temperature, the test is probably useful.

Some other important considerations include: (1) Especially for weak, non-hindered antioxidants, very complex rate equations have been obtained, indicating that different mechanisms can occur (Scott, 1965, Ingold, 1970). (2) Volatility of antioxidants can become important at high temperatures, e.g. for BHT (Klaui, 1971). (3) If two phases, such as water and fat, are present the solubility in each phase and pH may become important, especially for the low-molecular weight gallates (Cornell *et al.*, 1970).

Lea (1960) prepared purified tocopherol-free cottonseed, linseed and cod liver oil esters which respectively were high in linoleate, linolenate and more unsaturated fatty acid esters. From the induction period (time to reach PV = 100 meq/kg), the E_A-values for the control oils respectively were about, 20, 11 and 13 kcal/mole. For samples containing various hindered phenols the corresponding E_A ranges were 35–40, 27–35 and 13–20. Agreement with eqns. (12) and (14) thus seems to have been excellent for cottonseed oil if E_i is taken as 30 kcal/mole. In this case the gallates and hydroquinones behaved very similarly to the phenols. Agreement could be considered fair for the other substrates if one assumes lower values for the E_i. However, in the linseed oil the gallates and hydroquinones did not show the same behaviour as did the phenolic antioxidants and thus the E_A-value was somewhat different.

ASLT METHODS FOR PREDICTING OXIDATIVE STABILITY

(a) *The Schaal Oven Test (SOT)*

This method was developed in the baking industry in the 1920s. No published reference by its originator exists (Dugan, 1955). Joyner & McIntyre (1938) recommended that 50 g samples be held in 250 ml beakers with watch-glass on the top and maintained at about 63 °C. The samples were smelled daily until the rancid point was reached. Lea (1962) advocated the use of peroxide values to monitor the oxidation and the use of much smaller samples (0·2 ml) which were kept in small glass cups, the oil forming about a 2 mm layer on the bottom.

The temperature called for in this method is much lower than in most other ASLT procedures. This method can therefore be recommended as the one having the fewest possible problems. Hartman *et al.* (1975) feel that this method gives a better

correlation with an actual shelf-life test than does the Active Oxygen Method. However, especially for complex foods, a temperature of 60 °C is too high. The end-points used—either a rancid odour or a peroxide value of 70–120—are appropriate for correlating with the shelf-life at lower temperature.

(b) *Oxygen Absorption Methods (OAM)*

Many versions of the Oxygen Absorption Method are available. The most commonly used procedures are those of Sylvester–Martin and Eckey. In the former method (Sylvester *et al.* (1942) as modified by Martin (1961)), 100 to 1000 mg samples of lipid are kept in 30 ml flasks connected to mercury manometers. These are connected to a pressure recorder. The sample is kept at atmospheric pressure in oxygen at 100 °C. The end-point was taken as the time when a marked drop in pressure occurred. If the sample absorbed oxygen only gradually throughout, the end-point was taken at the organoleptic rancid point. In order to get a sharp end-point with vegetable oil, Sylvester found it necessary to replace the air with oxygen.

Eckey (1946) proposed a somewhat similar design in which 1 g of lipid was suspended in 12·5 g of 'pure silica sand' in a 50 ml flask. The temperature was maintained at 80 °C. The end-point was taken as the time for the sample to absorb 3 ml of oxygen as calculated at 0 °C, 760 mm. The author used air as the surrounding atmosphere, but suggested a modified design that could be used with pure oxygen.

The temperature used in these methods is considerably higher than that used in the Schaal Oven Test. This is a serious disadvantage even for simple lipids. Of the two methods considered the Eckey method involves a somewhat lower temperature, but this method has two additional disadvantages. Firstly, the sand is bound to introduce trace pro-oxidants. Generally the use of the sand increased the rate 1·4 to 6 times. The least acceleration occurred with a sample containing phosphoric acid. This acid can act as a metal-chelating agent, indicating that the acceleration was probably due to metal contaminants. The other problem is the end-point chosen. The significance of the induction period obtained depends on the degree of unsaturation of the oil. If the lipid to be tested contains 10 % polyunsaturated fatty acids (PUFA), 1 g of lipid contains about 0·3 mmoles PUFA. Three millilitres of O_2 at 1 atm and 0 °C correspond to about 0·13 mmoles O_2. Thus, at the end-point, the sample would be about 40 % oxidised. In contrast, rancidity in foods usually occurs when the lipids are 0·1 to 3 % oxidised (Labuza, 1971).

A considerable disadvantage of both methods is that at the relatively low oxygen pressures used (3 and 15 psia in the Eckey and Sylvester–Martin methods, respectively) the rate can easily become dependent on the oxygen pressure and the rate of oxygen dissolution. There are three reasons why this can occur. At the high temperatures used: (a) a shift in the mechanism occurs so that now higher oxygen pressures are needed to remove the oxygen dependence of the reaction rate (Bateman, 1954); (b) the solubility of oxygen in the lipid decreases (Bateman *et al.*, 1951) and (c) the rate of oxidation increases dramatically, decreasing the oxygen

concentration in the lipid more and more as the reaction proceeds (Bateman & Gee, 1951). The dependence on oxygen will be expected to increase with the degree of unsaturation of the lipid.

Sylvester *et al.* (1942) found that for palm kernel oil the induction period was almost cut in half when the air was replaced with pure oxygen. Pohle *et al.* (1962), using a considerably higher oxygen pressure, found less than a 10% increase in rate as the oxygen pressure was increased from 65 to 115 psia. At higher temperatures the dependence was found to be even greater, as expected (Bennett, 1964).

(c) *The Active Oxygen Method (AOM)*

The Active Oxygen Method, or the Swift Stability Test, as it is sometimes called, was originally proposed by King *et al.* (1933) and later modified slightly by Riemenschneider *et al.* (1943). Twenty millilitre samples of lipid are kept in 1 in × 8 in glass tubes and clean dry air at 2·33 cm^3/sec is bubbled through. The temperature is maintained at 97·8 °C. Periodically about 0·2 ml samples are withdrawn and the peroxide value (PV) is determined until it reaches 120 meq/kg. Unlike most other ASLT methods, this one has been rigorously standardised.

The main problem with this method is the high temperature used. Generally, an arbitrary multiplying factor is used, based on previous experience, to give an estimate of the shelf-life at room temperature. The method obviously cannot be used with formulated foods.

Another problem is the arbitrary air bubbling rate. Since one is trying to compare the data with room temperature conditions where the rate is indeed oxygen-independent, ideally the rate in the AOM test should be oxygen-independent also. The use of bubbling does, of course, speed up the rate of dissolution. Air at atmospheric pressure, however, will not suffice to achieve oxygen independence even if one assumes that the oxygen concentration in the lipid equals the oxygen solubility under the conditions used. As oxygen solubility depends almost linearly on the oxygen concentration in the head space, the only way to maintain oxygen independence is to use higher oxygen pressures. The final problem is that antioxidants such as BHT which are relatively volatile can evaporate from the sample (Klaui, 1971).

Luckman *et al.* (1953) used specially prepared and cleaned iron tubes instead of glass tubes to shorten the induction period. A two- to six-fold increase in the rate was obtained with hydrogenated vegetable oil. In contrast, only a 20% increase was obtained when the fat contained 0·08% added isopropyl citrate. This modification has not been generally accepted. The easiest and most accurate way of increasing the level of pro-oxidants is to add the metal directly in the form of a salt.

(d) *The ASTM Oxygen Bomb Method (OBM)*

This method has long been used (ASTM Committee D-2, ASTM Standards on

Petroleum Products and Lubricants, pp. 254–7, 1955) to determine the resistance of petrols to gum formation (Scott, 1965). Gearhart *et al.* (1957) were the first to use this method for food lipids. The authors added 15–30 g of lipid to a glass container which was fitted into the bomb. The oxygen pressure used was either 65 or 115 psia and the temperature 99 °C. The induction period was taken as the time to reach the mid-point of the first hour during which a pressure drop of at least 2 psia/h was obtained. The reproducibility has been shown to be excellent (Pohle *et al.*, 1962). Stuckey *et al.* (1958) modified the method by using a smaller sample which was spread on tissue paper in order to increase the contact between the lipid and the oxygen. For lard a five- to eight-fold increase in the rate was obtained in this way.

Three problems occur with this method. Firstly, the temperature is too high. Secondly, as in the Eckey method, the degree of oxidation of the sample at the end-point depends on the % PUFA in the lipid. When a 6 g sample of 10 % PUFA-lipid is to be tested in a 200 ml bomb the sample will be about 40 % oxidised at the end of the induction period, if one assumes a total pressure drop of 2 psia. Finally, unwanted contaminants can be introduced from the tissue paper.

Pohle *et al.* (1963) proposed a modification whereby a fat soluble copper-salt was added to the oil at a level of 25 or 100 ppm. Approximately a ten-fold increase in the rate was obtained in this manner. This method is not generally accepted.

(e) *Other methods*

A number of other methods have been suggested for specific applications. The Weight-Gain Technique (Sherwin, 1968) is based on the increase in weight of the lipid as it continues to absorb oxygen. This method is not recommended. Unless the weight-gain is calibrated against oxygen uptake data the method is meaningless.

A variety of methods have been proposed specifically to evaluate antioxidant effectiveness. Cort *et al.* (1975) used a modification of the Schaal Oven Test (Lea's version) which is carried out at 45 °C and which they term the Thin Layer Test. This method is strongly recommended, but it will be slow when potent antioxidants are tested.

Because of the problems experienced with the high-temperature ASLT methods, alternative means of acceleration have received more interest recently. Most of these tests use some form of metal-containing pro-oxidants. According to Betts & Uri (1968) metal ions, when they act as pro-oxidants, usually increase the rate in proportion to their concentration to the one-half or first power. Hence the addition of such pro-oxidants can be used to give a powerful catalytic effect.

Uri (1961*b*) has pointed out that since the temperature coefficients of antioxidant efficiency vary with the nature of the antioxidant, high-temperature ASLT studies are open to criticism. Furthermore, since lipid oxidation is generally trace-metal catalysed, adding metallic pro-oxidants may be a more meaningful method of acceleration. The author used ferrous phthalocyanine as a catalyst for linoleic acid

oxidation in an ethyl benzoate solvent at 25 °C. He compared the effectiveness of five antioxidants (propyl gallate and four flavonoids) at a concentration close to 50 ppm. The order of effectiveness was found to be similar to that obtained using a control containing no added pro-oxidant. It should be pointed out that the advantage of using complexed iron is that it is equally effective in the presence of metal chelating agents.

Berner *et al.* (1974) used hemin catalyst which they added to a lipid emulsion at 45 °C, pH 7·2 and measured the oxygen uptake using an oxygen analyser. The method was used for lard containing 0·02 % of one of several antioxidants. The AOM method was used for comparison. As expected, the two methods ranked the antioxidants quite differently. In particular the more water-soluble antioxidants (PG and TBHQ) were much less effective in the former method, probably in part because of their higher partition into the water phase.

The use of metallic pro-oxidants in ASLT studies, perhaps in conjunction with a moderate temperature elevation, should be given careful consideration. One of the problems involved is that some foods contain higher levels of endogenous metals than others. Therefore, different amounts must be added to different foods to give the same percent acceleration. If metal salts are used, problems will be encountered when metal-chelating compounds are present. By using metal complexes such as phthalocyanins or hematin-catalysts, this may be avoided. However, the possibility of a change in the mechanism can become much greater, e.g. when hematins are used (Kendrick & Watts, 1969). In selecting a suitable catalyst, the emphasis should be on using one which is already present in the product and which is likely to be the dominating catalyst for lipid oxidation in that particular food. One would question, therefore, the use of heme-catalysts for accelerating the oxidation of vegetable oils.

ASLT STUDY RESULT ANALYSIS

Unfortunately, ASLT studies where the overall rate constants K_A and K_M are calculated are almost non-existent. In calculating E_A-values for such studies one must rely on data for the induction period obtained. The use of different end-points and the concurrent presence of other acceleration parameters makes the calculated values inaccurate at best. An added obstacle, when it comes to interpreting the data, is the loose characterisation of the substrates given. Neither trace metal content nor the fatty acid distribution is generally known. Obscure terms such as 'stabilised lard,' 'shortening A' and 'hydrogenated vegetable oil' are often the only information given.

Because of the different temperatures used for different methods it is convenient to divide this survey into two parts depending on the T-zone under consideration.

(a) *Ambient to* 65 °C

In this range the Shelf-Life Study carried out at or close to room temperature and the Schaal Oven Test are the most commonly used methods. The most interesting data available are those of Pohle *et al.* (1964) who compared various different ASLT methods. When comparing Shelf-Life Test data collected at 29·5 °C with data obtained using the Schaal Oven Test at 60 °C an E_A-value of about 14 kcal/mole can be calculated for both lard and tallow. When 0·01 % BHA was added, the value increased to 18–20 kcal/mole. In contrast, the E_A for hydrogenated vegetable oil (HVO) was about 18–20 and did not change when BHA was added. These results are in line with what would be predicted on the basis of eqns. (12) and (14) and the fact that most vegetable oils already contain close to an optimum concentration of primary antioxidants (tocopherols).

(b) *Ambient to* 100 °C

The most important ASLT methods carried out in the vicinity of 100 °C are the AOM, OBM and the two oxygen absorption methods. Of these methods only the OBM, especially if used with a dispersant, will be close to giving oxygen-independent kinetics. The dispersant, however, is bound to be a source of pro-oxidative contaminants. Of the other methods the AOM is intermediate and the absorption methods are the most severely oxygen-dependent.

Pohle *et al.* (1962) found the Eckey OAM at 100 °C to be twice as fast as the AOM. Stuckey *et al.* (1958) found the OBM *without* a dispersant to be 1·4 times faster than the AOM when lard was the substrate. *With* a dispersant the OBM was three to six times faster still. Based on these considerations, the OBM seems to be the best of the high-temperature ASLT methods.

Pohle *et al.* (1964) made extensive comparisons between different high-temperature ASLT methods and concluded that the OBM was the most precise and gave the best correlation with product stability. When comparing their shelf-life test data at 29·5 °C with AOM data, the calculated E_A for lard is 20 and for tallow 14 kcal/mole. In the presence of 0·01 % BHA these values dropped to 16 and 11 kcal/mole, respectively instead of increasing as expected. In this case, predictions about antioxidant effectiveness, based on the control, would cause the degree of protection afforded by the antioxidant under normal storage conditions to be overestimated. Why the E_A dropped is unexplainable.

When the OBM-data for these same fats are compared to the Shelf-Life Test data the calculated E_A becomes about 19 kcal/mole for lard and 10 kcal for tallow. The corresponding values in the presence of 0·01 % BHA are 19 and 13 kcal/mole. Similarly, the value of HVO is 16 kcal/mole both with and without added BHA.

Thompson & Sherwin (1966) collected data at 43·3 °C (storage studies) and 98·9 °C (AOM) for safflower oil. Their data show that E_A can vary from 13–24 kcal/mole, depending on the antioxidant used. Generally, however, the value

increased from 17 for the control to 19–20 when antioxidants were added. In this case predictions would lead to underestimation of protection at room temperature.

Paul & Roylance (1962) collected data at 10–20 °C (Shelf-Life Test) and 98·4 °C (AOM) for two batches of peanut oil: (1) factory oil, refined in iron vessels and (2) further purified oil, using batch 1 as starting material. The E_A for the batch 1 control was about 9 and dropped to 7·5 kcal/mole in the presence of some primary antioxidants (0·01 % level). This difference may seem slight, but in this case indicated a 60–70 % increase in the AOM-induction period whereas no corresponding increase in shelf-life occurred. For batch 2 the E_A for the control was about 17 kcal/mole, indicating that the purification procedure (Crossley *et al.*, 1962) removed active pro-oxidants, possibly trace metals. In the presence of primary antioxidants (0·01 or 0·02 % level) the E_A appears to have been considerably lower.

The data of Paul & Roylance (1962) show that the AOM is unreliable, not only for testing antioxidants but also for testing batches of oil processed in different ways. The shelf-life test data collected indicated that the purification procedure increased the shelf-life of the control—in some cases by over 100 %—while the AOM data showed a dramatic drop in the induction period and thus a shorter shelf-life.

These results illustrate that temperature-accelerated test procedures for rancidity are generally more accurate for vegetable oils and stabilised animal fats. However, even for such lipids, high temperature test procedures can give very misleading results. In some cases the protection at 100 °C is greater than at room temperature, whereas in other cases the reverse is true. The Schaal Oven Test seems to yield much more predictable results although more data are needed to prove this. It seems quite possible that in this test one may be able to correct for E_A-differences between samples with and without antioxidants using eqns. (12) and (14).

The erratic behaviour at high temperatures is undoubtedly caused at least in part by the increased importance of pro-oxidative side-reactions of the antioxidants, when the antioxidant radical, A·, becomes an effective chain carrier as discussed above, causing a change in mechanism of antioxidation and possibly a drop in the activation energy. It seems that in the studies reviewed here, these reactions are not sufficiently important at 60 °C to greatly affect the results. Shelf-life studies for both model systems and foods are currently being carried out in our laboratories using oxygen absorption in the range 25–45 °C. Results will be presented in the future.

ACKNOWLEDGEMENT

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REFERENCES

BATEMAN, L., BOLLAND, J. L. & GEE, G. (1951). Determination of absolute rate constants for olefinic oxidations by measurement of photochemical pre- and after-effects. Part II—At 'low' oxygen pressures. *Trans. Faraday Soc.*, **47**, 274–85.

BATEMAN, L. & GEE, G. (1951). Determination of absolute rate constants for olefinic oxidations by measurement of photochemical pre- and after-effects. Part I—At 'high' oxygen pressures. *Trans. Faraday Soc.*, **47**, 155–64.

BATEMAN, L. (1954). Olefin oxidation. *Quart. Reviews*, **8**, 147–67.

BENNETT, J. E. (1964). Studies of the oxygen bomb method for determining shortening stabilities, *J.A.O.C.S.*, **41**, 505–7.

BERNER, D. L., CONTE, J. A. & JACOBSON, G. A. (1974). Rapid method for determining antioxidant activity and fat stability, *J.A.O.C.S.*, **51**, 292–6.

BETTS, A. T. & URI, N. (1968). Catalyst-inhibitor conversion in autoxidation reactions, *Adv. Chem. Series*, (75) (Americ. Chem. Soc.), 160–81.

CHENIER, J. H. B., FURIMSKY, E. & HOWARD, J. A. (1974). Arrhenius parameters for reaction of tertbutylperoxy and 2-ethyl-2-propylperoxy radicals with some nonhindered phenols, aromatic amines and thiophenols, *Can. J. Chem.*, **52**, 3682.

CORNELL, D. G., DEVILBISS, E. D. & PALLANSCH, M. J. (1970). Partition coefficients of some antioxidants in butteroil-water model systems, *J. Dairy Sci.*, **53**, 529.

CORT, W. M., SCOTT, J. W. & HARLEY, J. H. (1975). Proposed antioxidant exhibits useful properties, *Food Technol.*, **29**, 46.

CROSSLEY, A., DAVIES, A. C. & PIERCE, J. H. (1962). Keeping properties of edible oils. Part III. Refining by treatment with albumin, *J.A.O.C.S.*, **39**, 150–65.

DUGAN, L. (1955). Stability and rancidity, *J.A.O.C.S.*, **32**, 605–9.

ECKEY, E. W. (1946). An oxygen absorption method for examination of fat, *Oil and Soap*, **23**, 38–45.

ERICKSSON, C. E., OLSSON, P. A. & SVENSSON, S. G. (1971). Denatured hemoproteins as catalysts in lipid oxidation, *J.A.O.C.S.*, **48**, 442.

GEARHART, W. M., STUCKEY, B. N. & AUSTIN, J. J. (1957). Comparison of methods for testing the stability of fats and oils, and of foods containing them, *J.A.O.C.S.*, **34**, 427–30.

GROSCH, W. (1975). Auflauf und Analytik des oxydativen Fettverderbs, *Z. Lebensm. Unters.-Forsch.*, **157**, 70–83.

HARTMAN, L., ANTUNES, A. J., GARRUTI, R. S. & CHAIB, M. A. (1975). The effect of free fatty acids on the taste, induction periods and smoke points of edible oils and fats, *Lebensm.-Wiss. u.-Technol.*, **8**, 114–18.

HOWARD, J. A. (1973). Homogeneous liquid-phase autoxidation. In: *Free radicals*, ed. J. K. Kochi. New York, Interscience, Vol. II, 4–61.

HOWARD, J. A. & FURIMSKY, E. (1973). Arrhenius parameters for reaction of tert-butylperoxy radicals with some hindered phenols and aromatic amines, *Can. J. Chem.*, **51**, 3738–45.

INGOLD, K. U. (1968). Inhibition, *Adv. Chem. Series* (75) (Amer. Chem. Soc.), 296–305.

INGOLD, K. U. (1970). The inhibition of autoxidation by phenols. In: *Essays in free-radical chemistry*, Spec. Publ. 24 (Chem. Soc.), 285–93.

INGOLD, K. U. (1973). Rate constants for free radical reactions in solution. In: *Free radicals*, ed. J. K. Kochi, New York, Interscience, Vol. I, 36–113.

JOYNER, N. T. & MCINTYRE, J. E. (1938). The oven test as an index of keeping quality, *Oil and Soap*, **15**, 184–6.

KENDRICK, J. & WATTS, B. M. (1969). Acceleration and inhibition of lipid oxidation by heme compounds, *Lipids*, **4**, 454–8.

KING, A. E., ROSCHEN, H. L. & IRWIN, W. H. (1933). An accelerated stability test using the peroxide value as an index. *Oil and Soap*, **10**, 105–9.

KIRIGAYA, N., KATO, H. & FUJIMAKI, M. (1968). Studies on antioxidant activity of nonenzymic browning reaction products. Part I. Relations of color intensity and reductones with antioxidant activity of browning reaction products, *Agr. Biol. Chem.*, **32**, 287–90.

KLAUI, H. (1971). The functional (technical) uses of vitamins. In: *Vitamins*, University of Nottingham Seminar, ed. M. Stein, London, Churchill Livingstone, 110–41.

KOCHI, J. K. (1973). Oxidation-reduction reactions of free radicals and metal complexes. In: *Free radicals*, ed. J. K. Kochi, New York, Interscience, Vol. I, 591–683.

KORCEK, S., CHENIER, J. H. B. & INGOLD, K. U. (1972). Absolute rate constants for autoxidation. XXI. Activation energies for propagation and correlation of propagation rate constants with carbon-hydrogen bond strengths, *Can. J. Chem.*, **50**, 2285–97.

LABUZA, T. P. (1971). Kinetics of lipid oxidation in foods, *CRC Crit. Rev. Food Technol.*, **2**, 355–404.

LABUZA, T. P. (1972). Mechanisms of deterioration of intermediate moisture foods, NASA, *Contractor Report*, NASA CR-114861.

LEA, C. H. (1960). Antioxidants in dry fat systems: Influence of the fatty acid composition of the substrate. *J. Sci. Food Agr.*, **11**, 143–50.

LEA, C. H. (1962). The oxidative deterioration of food lipids. In: *Lipids and their oxidation*, ed. H. W. Schultz, the AVI Publishing Co., Inc., 3–28.

LOVE, J. D. & PEARSON, A. M. (1974). Metmyoglobin and nonheme iron as pro-oxidants in cooked meat, *Agr. Food Chem.*, **22**, 1032–4.

LUCKMANN, F. H., MELNICK, D. & MILLER, J. D. (1953). A new accelerated holding test involving aeration of oils in iron tubes, *J.A.O.C.S.*, **30**, 602–6.

MAHONEY, L. R. (1969). Antioxidants, *Angew. Chem.* Internat. Edition. **8**, 547–55.

MARTIN, H. F. (1961). A modified recording oxygen absorption apparatus for the determination of oxidative stability of lipid material, *Chem. and Ind.*, (10) 364–7.

PAUL, S. & ROYLANCE, A. (1962). Keeping properties of edible oils.—Part I. The use of accelerated tests for assessment of keeping properties of oils and the value of antioxidants, *J.A.O.C.S.*, **39**, 163–5.

PIERCE, J. H., CROSSLEY, A. & DAVIES, A. C. (1962). Keeping properties of edible oils. Part II Refining by treatment with alumina, *J.A.O.C.S.*, **39**, 165–8.

POHLE, W. D., GREGORY, R. L. & TAYLOR, J. R. (1962). A comparison of several analytical techniques for prediction of relative stability of fats and oils to oxidation, *J.A.O.C.S.*, **39**, 226–9.

POHLE, W. D., GREGORY, R. L. & VAN GIESSEN, B. (1963). A rapid oxygen bomb method for evaluating the stability of fats and shortenings, *J.A.O.C.S.*, **40**, 603–5.

POHLE, W. D., GREGORY, R. L., WEISS, T. J., VAN GIESSEN, B., TAYLOR, J. R. & AHERN, J. J. (1964). A study of methods for evaluation of the stability of fats and shortenings, *J.A.O.C.S.*, **41**, 795–8.

RAWLS, H. R. & VAN SANTEN, P. J. (1970). Singlet oxygen: A possible source of the original hydroperoxides in fatty acids, *Ann. N.Y. Acad. Sci.*, **171**, 135–7.

RIEMENSCHNEIDER, R. W., TURER, J. & SPECK, R. M. (1943). Modifications of the Swift Stability Test, *Oil and Soap*, **20**, 169.

SCOTT, G. (1965). Atmospheric oxidation and antioxidants. Amsterdam, Elsevier Publishing Co.

SHERWIN, E. R. (1968). Methods for stability and antioxidant measurement. *J.A.O.C.S.*, **45**, 632A–49A.

STUCKEY, B. N., SHERWIN, E. R. & HANNAH, F. D. (1958). Improved techniques for testing fats and oils by the oxygen bomb method, *J.A.O.C.S.*, **35**, 581–4.

SYLVESTER, N. D., LAMPITT, L. H. & AINSWORTH, A. N. (1942). Determination of the stability of oils and fats, *J. Soc. Chem. Ind.*, **61**, 165–9.

THOMPSON, W. & SHERWIN, E. R. (1966). Investigation of antioxidants for polyunsaturated edible oils, *J.A.O.C.S.*, **43**, 683–6.

URI, N. (1961*a*). Physico-chemical aspects of autoxidation. In: *Autoxidation and antioxidants*, ed. W. O. Lundberg, Vol. X, New York, Interscience, 55–106.

URI, N. (1961*b*). Mechanism of antioxidation. In: *Autoxidation and antioxidants*, ed. W. O. Lundberg, Vol. I, New York, Interscience, 133–69.

WATERS, W. A. (1971). The kinetics and mechanism of metal-catalyzed autoxidation, *J.A.O.C.S.*, **48**, 427–33.

EFFECTS OF HYPOCHLORITE TREATMENTS ON A METHIONYL PEPTIDE

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ABSTRACT

Treatment of glycyl-L-methionyl-glycine with up to 0·4% (w/v) sodium hypochlorite causes oxidation of methionine residues into methionine sulphoxide and methionine sulphone, and probable deamination of free α amino groups.

INTRODUCTION

Hypochlorite can be used to destroy contaminating aflatoxins of protein meals and concentrates (Natarajan *et al.*, 1975*a*). However, the detoxifying treatment of peanut protein isolates with 0·3% sodium hypochlorite at pH 8 to 9 was found to bring a 50% reduction in tryptophan and tyrosine contents, probably through oxidation (Natarajan *et al.*, 1975*b*).

A more systematic study of the effects of hypochlorite on specific amino acid residues of proteins is needed.

In the present study, using the tripeptide glycyl-L-methionyl-glycine as a substrate, it is shown that 0·01 to 0·1% sodium hypochlorite causes methionine oxidation into methionine sulphoxide. Higher hypochlorite concentrations, longer reaction times, an acid pH and a temperature of 80°C appear to provoke the deamination of free α amino groups and the formation of methionine sulphone.

The molarity (5 mM) of glycyl-L-methionyl-glycine selected for hypochlorite treatments approximately corresponds to that of methionine residues in the experiments with peanut protein isolates (Natarajan *et al.*, 1975*b*).

MATERIALS AND METHODS

5 mM glycyl-L-methionyl-glycine (purchased from Interchim, Montluçon, France) in a 0·1 M, pH 8·0 phosphate buffer is made 0·1% (w/v) in sodium

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hypochlorite and held at 60 °C for 30 min. Similar incubations are also performed in other conditions of hypochlorite concentration, pH, temperature and duration, as indicated. In all cases, the incubation medium also contains free norleucine (at a 4·5 mM concentration).

The incubation medium is then frozen in liquid nitrogen and immediately freeze-dried (no remaining hypochlorite can be detected at this stage by iodometric assays). The dried residue is dissolved in distilled water. A part of this solution is analysed by ion-exchange chromatography (see below). The remaining part is made 3N in NaOH (1·31 mg of tripeptide per 2 ml final volume) and hydrolysed for 16 h at 110 °C under nitrogen (a technique known to permit the direct analysis of methionine sulphoxide and methionine sulphone, Neumann, 1967; Cuq *et al.*, 1973). The hydrolysate is brought to pH 2 with 2N HCl; a sample of less than 100 μl is then assayed on a Technicon NC1 analyser. A rapid amino acid separation and analysis (Fig. 1) is obtained as follows: the column (25 cm × 0·6 cm) of Chromobeads C_2 resin is maintained at 60 °C. It is first cleaned with 0·2N NaOH for 5 min, then equilibrated with pH 3·10 citrate buffer for 15 min. The amino acid sample is next introduced into the top of the column and then eluted with pH 3·8 citrate buffer at a rate of

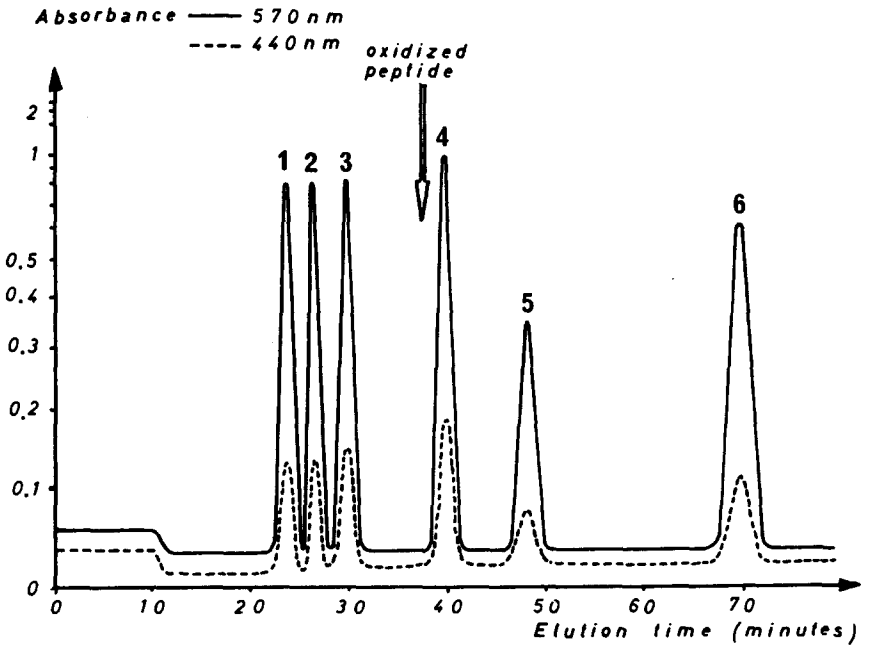

Fig. 1. Chromatogram of glycyl-L-methionyl-glycine and of various amino acids. 125 nmoles methionine sulphoxide (1), 125 nmoles methionine sulphone (2), 100 nmoles glycine (3), 250 nmoles methionine (4), 125 nmoles norleucine (5) and 250 nmoles glycyl-L-methionyl-glycine (6). Chromobeads C_2 resin. 25 cm × 0·6 cm column. 60 °C. NC1 Technicon amino acid analyser.

0·8 ml/min. Both buffers contain per litre, 14·71 g sodium citrate, $2H_2O$; 25 ml of 2N NaOH; 5 ml of thiodiglycol; 10 ml of a saturated solution of Brij 35, and enough 6N HCl for pH adjustment.

Amino acid contents are given as moles of individual amino acid per mole of glycyl-L-methionyl-glycine.

RESULTS AND DISCUSSION

Chromatography on Chromobeads C_2 resin of glycyl-L-methionyl-glycine submitted to 0 to 0·1% (w/v) hypochlorite treatments indicates a decrease in the tripeptide level and the corresponding formation of a derivative (elution time 37 min, Fig. 1) which may well be glycyl-L-methionyl-sulphoxide-glycine. Treatment with 0·2% hypochlorite causes the complete disappearance of glycyl-L-methionyl-glycine and a decrease in free norleucine (forty per cent of the initial concentration), while the level of oxidised peptide remains constant (Fig. 2). Treatment with 0·4% hypochlorite causes the total disappearance of the peptide, of the oxidised peptide and of free norleucine. This is probably due to deamination of N terminal amino groups.

Alkaline hydrolysis of non hypochlorite-treated glycyl-L-methionyl-glycine is

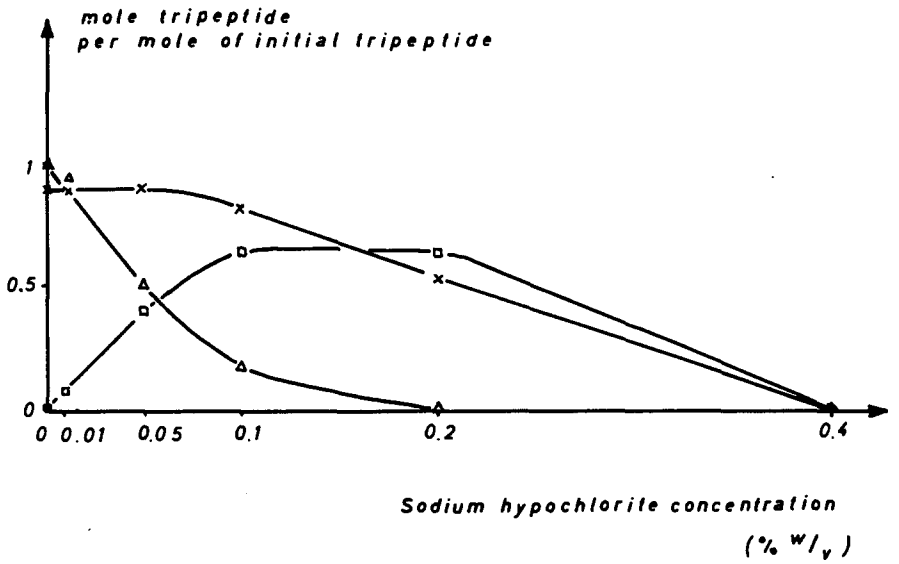

Fig. 2. Effects of sodium hypochlorite treatments on glycyl-L-methionyl-glycine. Influence of sodium hypochlorite concentration (60 °C, 30 min, pH 8·0). △—△ peptide, □—□ oxidised peptide, (× — × norleucine).

complete: no residual peptide can be detected, and the release of glycine is close to the theoretical maximum of 2 moles (Fig. 3). There is, however, a 10% loss in the recovery of methionine, a loss probably due to partial destruction during alkaline hydrolysis.

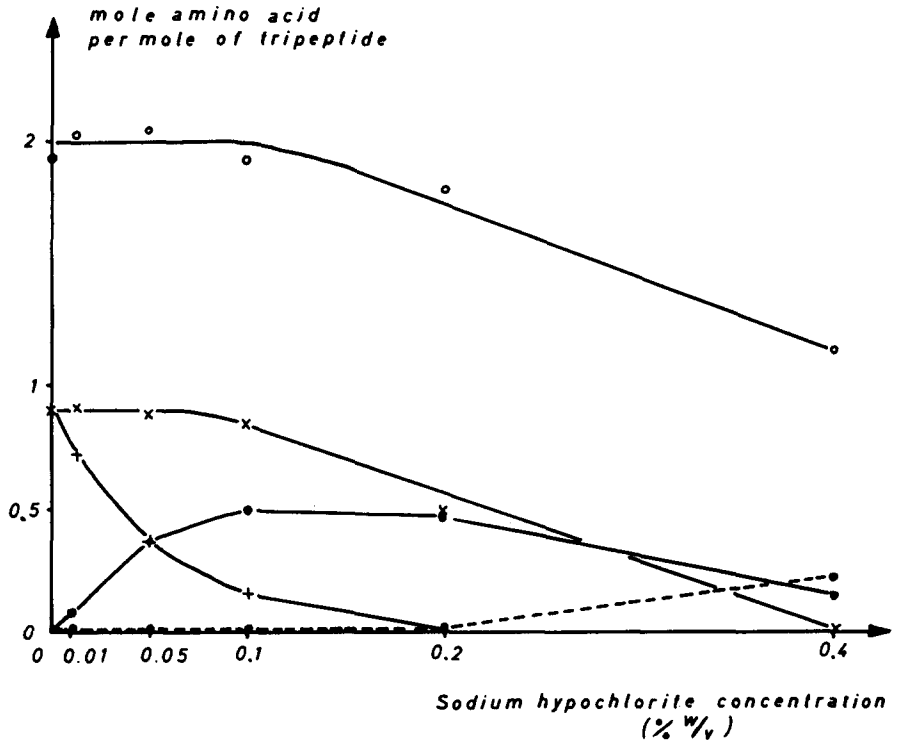

Fig. 3. Effects of sodium hypochlorite treatments on the amino acid composition of glycyl-L-methionyl-glycine. Influence of sodium hypochlorite concentration (60 °C, 30 min, pH 8·0). Amino acid analysis after alkaline hydrolysis. O—O glycine, + — + methionine, ●—● methionine sulphoxide, ●---● methionine sulphone (× — × norleucine).

Treatments with 0·01 to 0·1% hypochlorite cause significant decreases in the methionine content and the formation of methionine sulphoxide (Fig. 3). The latter does not account totally for the decrease in methionine. This effect of hypochlorite differs from that of 0·1 M hydrogen peroxide, which can quantitatively oxidise the methionyl residues of a protein (Cuq *et al.*, 1973). It can be mentioned that Natarajan *et al.* (1975*b*) did not investigate the formation of methionine sulphoxide in hypochlorite-treated peanut proteins.

Treatment with 0·2% hypochlorite provokes an almost complete destruction of methionine, while the content of methionine sulphoxide remains constant (fifty per cent of initial methionyl residues). Of interest is the concomitant decrease in the

glycine and norleucine contents, probably due to oxidative deamination reactions (Fig. 3). No new ninhydrin-positive peak can be detected on the amino acid chromatogram.

Treatment with 0·4% hypochlorite brings about the total disappearance of norleucine and the destruction of one mole of glycine out of the initial two (Fig. 3). It is likely that deamination affects the N terminal glycine residue. It would be of interest to check if hypochlorite can provoke the deamination of free α and ε-amino groups of proteins.

The 0·4% hypochlorite treatment also causes a decrease in the content of methionine sulphoxide (fifteen per cent of initial methionine residues) and the marked formation of methionine sulphone (twenty-five per cent of the initial methionine residues: Fig. 3). No methionine can be detected on the chromatogram, and therefore 60% of the initial methionine residues cannot be accounted for. The partial N-chlorination of peptide bonds is not ruled out; it could modify the stability of methionine residues or of their oxidation derivatives during alkaline hydrolysis.

It is usually assumed that interaction of hypochlorite with proteins results in oxidative reactions at neutral and alkaline pH while acid pH favours chlorination

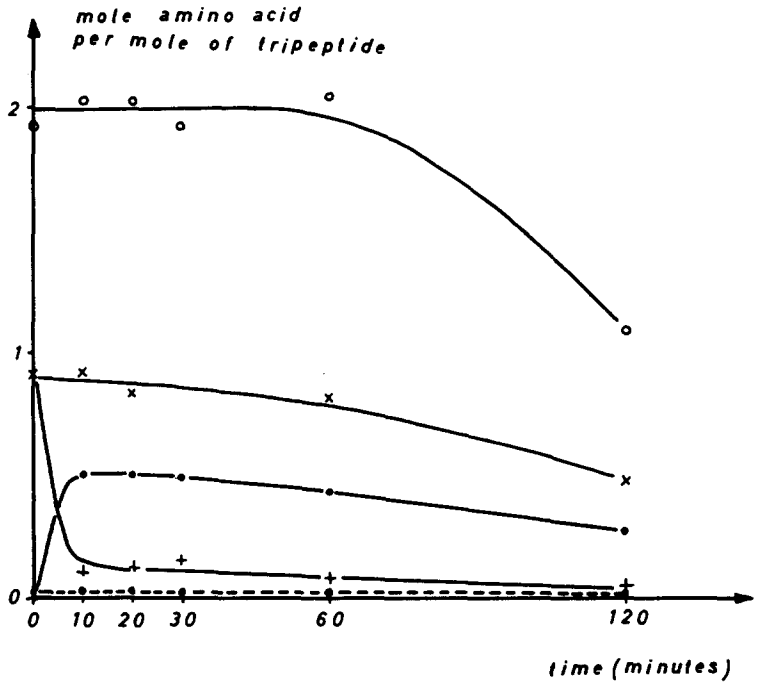

Fig. 4. Effects of sodium hypochlorite treatments on the amino acid composition of glycyl-L-methionyl-glycine. Influence of incubation time (60 °C, pH 8·0, 0·1% w/v sodium hypochlorite). Amino acid analysis after alkaline hydrolysis, O—O glycine, + — + methionine, ●—● methionine sulphoxide, ●---● methionine sulphone (× — × norleucine).

reactions (Natarajan *et al.*, 1975*b*). Such pH effects have not been confirmed here, when glycyl-L-methionyl-glycine was treated at 60 °C for 30 min with 0·1 % (w/v) sodium hypochlorite at pH 3·8, 6·5, 8·2, 10·5 and 11·9. Results obtained in the range of pH 6·5 to 11·9 are similar to those of Fig. 3 (for the same sodium hypochlorite concentration). However, at pH 3·8, the content of methionine sulphoxide decreases by half, while methionine sulphone is formed (corresponding to 13 % of the initial residues). At pH 3·8, also, the content of norleucine and glycine is slightly reduced. It therefore appears that an acid pH favours the oxidation of methionine sulphoxide, and possibly also the deamination of free α-amino groups.

Changes in the temperature of incubation from 0 to 80 °C (for a 30 min, 0·1 % (w/v) sodium hypochlorite treatment at pH 8·0) do not modify the amino acid contents except for a significant formation of methionine sulphone at 80 °C (seventeen per cent of the initial methionine residues).

Experiments have also been performed (with 0·1 % (w/v) sodium hypochlorite at pH 8·0 and 60 °C) for different incubation times (Fig. 4). The decrease in methionine and the formation of methionine sulphoxide are already maximal after 10 min. An incubation time of 2 h brings marked decreases in the contents of glycine, methionine sulphoxide (without significant formation of methionine sulphone) and, to a lesser extent, norleucine.

The effects of hypochlorite treatments on the amino acid composition of tryptophanyl and lysyl peptides are presently under investigation in this laboratory.

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REFERENCES

CUQ, J. L., PROVANSAL, M., GUILLEUX, F. & CHEFTEL, C. (1973). Oxidation of methionine residues of casein by hydrogen peroxide. Effects on *in vitro* digestibility, *J. Food Sci.*, **38**, 11–13.

NATARAJAN, K. R., RHEE, K. C., CATER, C. M. & MATTIL, K. F. (1975*a*). Destruction of aflatoxins in peanut protein isolates by sodium hypochlorite, *J. Amer. Oil Chem. Soc.*, **52**, 160–3.

NATARAJAN, K. R., RHEE, K. C., CATER, C. M. & MATTIL, K. F. (1975*b*). Effect of sodium hypochlorite on peanut protein isolates, *J. Food Sci.*, **40**, 1193–8.

NEUMANN, N. P. (1967). Analysis for methionine sulphoxides. In: *Methods in enzymology*, ed. C. H. W. Hirs, S. P. Colowick and N. O. Kaplan, Vol. XI. New York and London, Academic Press. 487–90.

LESSER KNOWN SOURCES OF PROTEIN IN SOME NIGERIAN PEASANT DIETS

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ABSTRACT

The proximate mineral composition and indispensable amino acid pattern were determined for samples of Snail (Vivapara quadrata), *periwinkle* (Littorina littorea), *crayfish* (Palamonetes varians) *and some locally smoked fresh water fish* (Pisces *spp.*).

The crude protein contents of smoked fish, crayfish, snail and periwinkle were 75·31, 74·84, 65·29 *and* 60·93 % *dry matter, respectively. These values were* 56·9, 54·9, 35·2 *and* 26·1 %, *respectively higher than that of whole hen's egg. All the samples, except snail, also had higher ash values. Whole hen's egg, however, contained more crude fat* (40·23 %) *and gross energy* (34·74 *Kj/g*) *than the test samples.*

The minerals calcium and phosphorus were higher in the test samples than in whole hen's egg. Samples analysed contained higher amounts of tryptophan than whole hen's egg and, with the exception of snail, the samples also contained more lysine. The test samples have high chemical scores (A/E ratios). Comparison has also been made of the indispensable amino acid patterns of these samples with those of cow's milk, human milk and the FAO (1957) *provisional pattern.*

INTRODUCTION

There has been, for a long time, an awareness of the persistent shortage of animal protein in the diet of the Nigerian peasant. Evidence from reviews of the literature has indicated clearly that among the factors limiting animal protein supply (particularly via livestock and its products) to these Nigerians have been the prohibitive prices and unavailability (Idusogie, 1971, 1973; Bassir, 1962; Ekpo, 1974). There is, therefore, a natural urge to bridge the wide gap existing between the physiological requirement for protein and the supply from the available

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conventional sources in the diets. While the peasants in the Northern States of Nigeria consume cereals, cereal products, fermented milk and milk products and some legumes, in the Southern States—and especially in the riverine areas—they resort to small terrestrial game, fish and other aquatic protein foodstuffs. In general, the Nigerian peasants from the Southern States derive their protein from such animal sources as tortoises, turtles, snails, lizards, snakes and other reptiles, caterpillars, locusts, grasshoppers, crickets, termites, beetles and similar insects, crayfish, fish, crabs, periwinkle, small game and low flying birds. In some areas of Nigeria our survey results (Bassir & Umoh, 1973) have shown that these foods are the only sources of protein in the peasant diets.

It is, however, now being appreciated that, more than anything else, lack of nutritional information on these protein sources is the major problem, rather than the actual shortage of the sources of protein, as was, until now, widely believed. There is a dearth of information on nutrient composition (particularly protein content), indispensable amino acid pattern and mineral composition of these foodstuffs consumed by the Nigerian peasants. The availability of such information would lead to more judicious use of these materials. This paper is a report of a series of analytical results aimed at elucidating the nutrient composition of some of these protein sources. The results are compared with freeze-dried hen's egg.

MATERIALS AND METHODS

Sampling and preparation of the material for analysis

Live samples of edible snails (*Vivapara quadrata*), periwinkle (*Littorina littorea*) and the locally preserved dry forms of both crayfish (*Palamonetes varians*) and smoked fish (*Pisces* spp.) were bought from the local market. Hen's eggs were also bought in the same market.

Snail

The hard shells were broken and the edible portion removed. This was then washed with lime juice to remove the slime, sliced to pieces and then freeze-dried. The dried material was then milled and stored until needed for analysis.

Periwinkle

These were first steeped in hot water for 20 min. The edible portion was removed from the shell with a sharp needle. A sufficient quantity of the material was washed once with water to remove any particles of the shell, then freeze-dried, milled and stored.

Smoked fish

Medium-sized smoked fish of moderate cost, as revealed by our previous survey

(Bassir & Umoh, 1973), was bought. Since the natives usually consume these whole (bones, head and flesh), there was no separation of the bones from the dried flesh. The sample was then milled and stored for analysis.

Crayfish

Samples of the dry crayfish were milled as bought from the market.

Whole hen's egg

Fresh eggs bought from the market were de-shelled. The egg white and the yolk were mixed and freeze-dried. The dried samples were then milled.

ANALYTICAL PROCEDURES

Proximate chemical composition

Analyses of the samples for moisture, crude protein (sample—NX6.25), ether extract, and ash were carried out using the method of the AOAC (1970). The nitrogen-free extract was obtained by difference. Analyses were carried out in triplicate on three separate batches of the samples.

Mineral composition

Calcium and iron were determined by the dry ashing method of the AOAC (1970). Phosphorus was determined from aliquots of the mineral solution by the method of Gomori (1942). The other minerals were estimated from the wet-digested samples (AOAC, 1970), using the Perkin-Elmer Atomic Absorption Spectrophotometer 290. The calorific values of the specimens were determined using a ballistic bomb calorimeter. The results were expressed on a dry matter basis.

Amino acid analysis

The quantitative estimation of the amino acids was carried out according to a procedure based on the report of Spackman *et al.* (1956). Tryptophan was, however, estimated chemically (Miller, 1967) using *p*-dimethylaminobenzaldehyde (DMAB) and sodium nitrite solutions.

RESULTS AND DISCUSSION

Table 1 shows the proximate nutrient composition of snail (*Vivapara quadrata*), periwinkle (*Littorina littorea*), crayfish (*Palamonetes varians*), locally smoked fresh water fish (*Pisces* spp.) and whole hen's egg for comparison purposes. These lesser known foodstuffs were quite rich in crude protein. The ash contents of all the samples except snail were higher than that of whole hen's egg. Whole hen's egg, on

TABLE 1

PROXIMATE NUTRIENT COMPOSITION OF SOME PROTEIN SOURCES AND WHOLE HEN'S EGG (g/100 g DRY MATTER) (Mean value ± SD)

Nutrient	*Smoked fish*	*Periwinkle* (Littorina littorea)	*Crayfish* (Palamonetes varians)	*Snail* (Vivapara quadrata)	*Whole hen's egg*
Dry matter	98·55 ± 4·06	20·12 ± 3·71	97·98 ± 6·34	23·58 ± 4·34	25·34 ± 1·34
Crude protein	75·31 ± 2·82	60·93 ± 2·85	74·84 ± 5·01	65·29 ± 6·51	48·31 ± 0·93
Ash	13·34 ± 1·12	8·40 ± 1·11	8·80 ± 1·88	4·10 ± 0·95	4·63 ± 0·54
Ether extract	11·01 ± 4·33	2·34 ± 0·90	5·53 ± 1·75	3·10 ± 0·33	40·23 ± 0·54
Nitrogen-free extract	—	28·33 ± 3·31	10·83 ± 1·51	27·50 ± 3·18	6·83 ± 1·02
Energy value (Kj/g)	26·39 ± 2·01	19·52 ± 2·15	19·14 ± 2·00	20·13 ± 3·25	34·74 ± 1·98

TABLE 2

MINERAL COMPOSITION OF SOME PROTEIN SOURCES AND WHOLE HEN'S EGG (g/100 g DRY MATTER) (Mean value ± SD)

Mineral	*Smoked fish*	*Periwinkle* (Littorina littorea)	*Crayfish* (Palamonetes varians)	*Snail* (V. quadrata)	*Whole hen's egg*
Calcium	15·31 ± 3·51	5·13 ± 0·35	6·50 ± 0·98	2·31 ± 0·15	0·81 ± 0·02
Phosphorus	2·61 ± 0·55	0·39 ± 0·01	1·40 ± 0·03	0·53 ± 0·09	0·79 ± 0·01
Iron	0·03 ± 0·00	0·04 ± 0·00	0·16 ± 0·01	0·04 ± 0·01	0·02 ± 0·00
Magnesium	0·14 ± 0·01	2·77 ± 0·15	0·45 ± 0·03	0·24 ± 0·01	0·06 ± 0·01
Chloride	0·81 ± 0·04	0·57 ± 0·02	1·65 ± 0·10	0·82 ± 0·11	0·91 ± 0·13
Sodium	0·53 ± 0·01	0·28 ± 0·05	1·22 ± 0·19	0·64 ± 0·08	0·06 ± 0·02
Potassium	1·09 ± 0·11	0·50 ± 0·80	1·20 ± 0·20	0·51 ± 0·01	0·58 ± 0·11
Manganese (mg/100 g)	2·80 ± 0·06	3·22 ± 0·17	5·10 ± 0·18	1·61 ± 0·22	0·95 ± 0·04
Copper (mg/100 g)	1·20 ± 0·10	33·15 ± 2·11	4·10 ± 0·55	2·48 ± 0·18	0·98 ± 0·14
Calcium/Phosphorus ratio	5·90	13·20	4·64	4·36	1·03

the other hand, contained more ether extractable material which may be attributable to the presence of the yolk with its high content of carotene. The gross energy value of 34·74 Kj for whole hen's egg was also higher than for the test samples. Of all these lesser known protein sources, smoked fish contains higher crude protein, ash, ether extractable material and energy than the others.

The mineral composition of the samples and whole hen's egg is shown in Table 2. The calcium contents of the samples were quite high, being 15·31, 5·13, 6·50 and 2·31% dry matter, respectively. Considering these lesser known peasant foods individually, periwinkle seems to be the best source of the trace elements copper, manganese and magnesium. Crayfish is richer in iron, chloride, sodium and potassium than the other test samples and whole hen's egg. In all cases, these lesser known foodstuffs contain higher quantities of iron and copper than whole hen's egg. The importance of these trace elements in the nutrition of the peasant cannot be overemphasised, especially in the villages where there is abundant evidence of anaemia arising from gastroenteritis, hookworm and similar ailments which precipitate iron and copper deficiences.

In Table 3, the indispensable amino acid pattern of the protein sources and whole hen's egg is shown. While whole hen's egg contains more isoleucine (390 mg/gN) and valine (420 mg/gN), all the test samples are better sources of tryptophan. With the exception of *Vivapara quadrata*, the samples are also richer in lysine. Periwinkle (*Littorina littorea*) also contains more leucine, threonine and phenylalanine than whole hen's egg. The protein in these lesser known foodstuffs is also of considerably high quality with chemical scores of 70·89, 75·95, 61·96 and 62·50 for smoked fish, periwinkle, crayfish and snail, respectively. The most limiting amino acids were valine in locally smoked fresh water fish, isoleucine in *Littorina littorea*, threonine in *Palamonetes varians* and methionine in *Vivapara quadrata*.

In Table 4, the indispensable amino acid patterns of the lesser known protein sources are compared with cow's milk, human milk and the FAO (1957) provisional patterns reported by Lunven *et al.* (1973). Periwinkle looks quite outstanding in its indispensable amino acid pattern. Its isoleucine content is 122·6% of that in the FAO (1957) provisional pattern, 112·2% of that in cow's milk and 130·3% of that in human milk. It also contains 224·8% leucine, 220·0% lysine, 211·7% threonine, 153·3% tryptophan, 136·7% valine and 210·6% total aromatic amino acids when compared with the FAO (1957) provisional pattern. It is also richer than cow's milk and human milk in all the indispensable amino acids except the total sulphur amino acids which were not determined. On the other hand *Vivapara quadrata*, *Palamonetes varians* and *Pisces* spp. contained less isoleucine than the FAO (1957) provisional pattern, cow's milk and human milk.

These results certainly indicate that these foodstuffs could provide, on a dry weight basis, much more dietary protein than whole hen's egg. The protein is also of good quality judging from the high chemical scores. Such foodstuffs, like the periwinkle (*Littorina littorea*), are even richer in indispensable amino acids than

TABLE 3

INDISPENSABLE AMINO ACID PATTERN OF SOME PROTEIN SOURCES AND WHOLE HEN'S EGG (mg/gN)

Amino acid	*Smoked fish*	*Periwinkle* (Littorina littorea)	*Crayfish* (Palamonetes varians)	*Snail* (V. quadrata)	*Whole hen's egg*
Isoleucine	244	331	238	238	390
Leucine	439	688	476	469	550
Lysine	750	594	742	439	538
Threonine	231	381	181	249	320
Tryptophan	236	138	219	156	102
Valine	262	369	250	297	420
Methionine	119	129	119	65	133
Phenylalanine	193	384	237	196	244
Total indispensable amino acids	2374	3014	2462	2109	2697
Chemical score	70·89	75·95	61·96	62·50	—

TABLE 4

COMPARISON OF INDISPENSABLE AMINO ACID PATTERN OF SOME PROTEIN SOURCES WITH FAO (1957) PROVISIONAL PATTERN,† COW'S MILK† AND HUMAN MILK† (mg/gN)

Amino acid	*Smoked fish*	*Periwinkle* (Littorina littorea)	*Crayfish* (P. varians)	*Snail* (V. quadrata)	*FAO provisional pattern*† (*FAO*, 1957)	*Cow's milk*† (*FAO*, 1970)	*Human milk*† (*FAO*, 1970)
Isoleucine	244	331	238	238	270	295	254
Leucine	439	688	476	469	306	596	548
Lysine	750	594	742	439	270	487	428
Threonine	231	381	181	249	180	278	280
Tryptophan	136	138	219	156	90	88	105
Valine	262	369	250	297	270	362	284
Total S-amino acids	—	—	—	—	270	208	185
Total aromatic amino acids	331	758	446	340	360	633	421

† Lunven *et al.* (1973).
(—) Not determined.

cow's milk and human milk and contain more than the FAO (1957) recommended quantity of indispensable amino acids. Except in the case of snail, methionine was not limiting. With their high content of lysine, these foods could effectively supplement the cereals and legumes which are widely consumed in many villages in Nigeria. These foodstuffs can also provide mineral elements, especially the trace elements which act at enzyme levels during metabolism. It may be added that despite the high chemical scores of the protein and the high nutrient contents of these lesser known foods, their physiological availability to the consumer is still in doubt. Work is, however, in progress to evaluate their nutritional values by *in vivo* studies with rats.

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REFERENCES

AOAC (1970). *Methods of analysis of the Association of Official Analytical Chemists* (11th ed.), Washington, D.C., 123–37.

BASSIR, O. (1962). In: *Biochemical aspects of human malnutrition in the Tropics*, ed. A. Junk Den Hang, The Netherlands, 10–15.

BASSIR, O. & UMOH, I. B. (1973). The nutritive adequacy of some peasant Nigerian diets, *Ecology of Food and Nutr.*, **2**, 297–306.

EKPO, E. U. (1974). Field problems in nutrition among peasant communities and suggested remedial measures in relation to developmental programmes with special reference to Nigeria, *Proceedings of the 1st African Nutrition Congress*, University of Ibadan, Ibadan, Nigeria, 1975 (in press).

FAO (1957). The FAO Committee on Protein Requirements. FAO Nutritional Studies, No. 16. FAO. Rome.

GOMORI, G. (1942). A modification of the colorimetric phosphorus determination for use with the photoelectric colorimeter, *J. Lab. Clin. Med.*, **27**, 955.

IDUSOGIE, E. O. (1973). Centuries of changing food consumption patterns in African communities. Paper presented at the Conference on Factors Affecting Food Utilization in the Semi-Arid Tropics. Zaria, Nigeria, ABU.

IDUSOGIE, E. O. (1971). The relation of population and nutritional health problems in African communities. Paper presented at the African Population Conference. Accra, Ghana.

LUNVEN, P. & DE ST. MAREQ, E. LEC. (1973). Amino acid composition of hen's egg. *Brit. J. Nutr.*, **30**, 189–94.

MILLER, E. L. (1967). Determination of the tryptophan content of feedingstuffs with particular reference to cereals, *J. Sci. Food Agric.*, **18**, 381.

SPACKMANN, D. H., STEIN, W. H. & MOORE, S. (1956). Automatic recording apparatus for use in chromatography of amino acids, *Fed. Proc.*, **15**, 358.

UMOH, I. B. (1973). Changes in the nutritive values of some Nigerian diets after cooking by certain South Eastern Nigerian traditional methods. Ph.D. Thesis. University of Ibadan, Ibadan, Nigeria.

BOOK REVIEW

Amine und Nitrosamine. By Ahmed Asker. Vorkommen, Bedeutung, Stoffwechsel und Bestimmung.

Quite apart from their probable contributions to the flavour, aroma and acceptability of items of the diet, amines have become important trace components of foods and drinks as potential precursors to N-nitroso compounds and as possible toxic agents where medication with monoamine oxidase inhibitors is involved or in initiating migraine attacks.

A feature of this book is its list of amines which occur in foods and drinks, including milk and cheese, meat and sausages, fish, wine, beer, etc. The contents of tyramine, which has been found to precipitate attacks of migraine, in food sources are particularly well represented, along with the simpler primary, secondary and tertiary alkylamines. Further sections of this book are concerned with the importance of amines in relation to aroma, non-enzymic browning, quality control, etc., as well as the functions of amines *in vivo* and as diagnostic aids in clinical chemistry. The pathways of the biosynthesis and metabolism of many amines are dealt with in detail and presented conveniently in a tabulated form together with some of the physiological actions of amines.

Finally, so far as amines are concerned, the various methods available for their detection and determination are reviewed in a further section, including their separation and concentration and such methods of detection as paper, thin layer and gas chromatography, mass spectrometry, etc. In fact, this book provides a comprehensive but concise treatise of many of the various aspects of the chemistry and biochemistry of amines. Its treatment of nitrosamines, the other topic of its title, is very cursory in comparison, being restricted to abbreviated accounts of their synthesis, actions and occurrence, and that of their precursors in foods. For those researchers interested in amines *per se*, however, this book is well worth while, but it would have been an advantage to provide an adequate binding to prevent its early collapse, in spite of gentle treatment by the reader.

C. WALTERS

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