ISSN 0022-1163

Volume 16 Number 1 February 1981

Journal of Food Technology

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

JOURNAL OF FOOD TECHNOLOGY

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

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The Journal of Food Technology is published bimonthly, six issues form one volume. The annual subscription is £64.00 (U.K.), £77.50 (Overseas), $195.00 (N. America, including cost of airfreight). Back volumes are still available.

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D000153679

J. Fd Technol. (1981) **16**, 1–11

Recent applications of high pressure liquid chromatography* to food analysis

R. MACRAE

Summary

The range of applications of HPLC to food analysis has widened considerably in recent years. In this paper an attempt is made to review the latest applications, especially those in novel areas of food analysis.

Introduction

The number of papers being published on applications of HPLC, especially in the area of food analysis, is increasing very rapidly. In many respects this dramatic increase in popularity of the method is analogous to that of GC some 10–15 years ago. However, in many instances, as also happened with GC, very similar work has been published by a number of workers simultaneously. The technique of HPLC is now well proven in food analysis and thus the need for publications *solely* illustrating separations of standard mixtures will decline. Rather the emphasis will shift to the solution of real analytical problems in food studies.

Development of instrumentation for HPLC continues unabated and in particular a number of interesting improved detectors have appeared on the market, such as the electrochemical detector, the conductivity detector and the mass detector. The successful development of HPLC/MS has still not materialized, with only a very limited number of units in operation.

Column performance is still being improved although with the possible exception of the introduction of the first commercially available 3 μm packing

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*The terms 'high pressure liquid chromatography', 'high performance liquid chromatography' and 'high speed liquid chromatography' are synonymous. In this paper the abbreviation HPLC is used to cover these, and all other expressions, describing chromatography with microparticulate columns.

0022–1163/81/0200–0001 $02.00

material no major innovations in this area have arisen recently. There is now a marked trend to 'in-house' packing of columns to avoid the high costs of replacement columns. This will also result in more chromatographers producing their own packing materials at a fraction of the commercial cost.

This paper contains a review of those recent publications concerning applications of HPLC to food analysis, subsequent to those already cited in the review by Macrae (1980). Whilst the majority of such papers are mentioned, the coverage is not comprehensive and where several papers describe essentially similar methods, the least accessible references are omitted.

Carbohydrates

Carbohydrates in foods, particularly the simple mono- and disaccharides, are being increasingly analysed by HPLC. The majority of methods utilize partition columns with acetonitrile/water as the mobile phase followed by refractive index detection (e.g. Hurst, Martin & Zoumas, 1979; Dunmire & Otto, 1979). The poor sensitivity of refractive index detectors is not usually a problem in food analysis where the sugars are present at high levels. Methods using ion-exchange columns are still being published (e.g. Engel & Olinger, 1979) and indeed a combination of ion-exchange and partition columns (Woidich, Pfannhauser & Blaicher, 1978b). An alternative approach is to use a silica column with an amine modifier in the mobile phase, which it is claimed gives similar resolution to an amino-bonded phase but at greatly reduced cost (Aitzetmuller, 1978).

The main areas covered in recent publications include soft drinks (Anon., 1979), sugar refinery products (Clarke & Brannan, 1979a,b; Rydel, 1979), lactose in dairy products (Euber & Brunner, 1978, 1979; Warthesen & Kramer, 1979; Hemmati & Keeney, 1979), sugars in honey (White, 1979); gluconic acid in wines, as a result of contamination by *Botrytis cinerea* (Kupina & Gump, 1979), sugars in stored onions with an interesting comparison with enzymatic methods (Gorin, 1979), inversion of sucrose in grapes (Rapp, Ziegler & Steffan, 1977), and an excellent review containing a useful comparison with classical methods by Buckee & Hargitt, 1978.

Lipids

HPLC offers few advantages over GC in the analysis of lipids for fatty acid composition. However, there are three specific areas where HPLC is being used more, namely in the study of triglycerides, fatty acid oxidation products and the separation of *cis* and *trans* isomers. Triglycerides are difficult to analyse by GC but several workers (e.g. Herslof, Podlaha & Toeregard, 1979), have shown that molecules differing by only two methylene groups can be separated by HPLC. Karleskind *et al.* (1978) have employed gradient elution to obtain a

good separation of complex triglyceride mixtures. The triglyceride composition of beef fat has also been reported (Wada *et al.*, 1978).

HPLC is ideally suited to the analysis of those compounds that are unstable at elevated temperature (cf. GC) or to atmospheric oxygen (cf. TLC). A number of papers covering fatty acid autoxidation (e.g. Neff *et al.*, 1978; Gasparoli & Fedeli, 1979), and enzymatic oxidation (e.g. Pattee & Singleton, 1979; Hatanaka *et al.*, 1979) have been published.

An interesting alternative to a silver loaded stationary phase has been proposed by Chan & Levett (1978), in which silver nitrate is incorporated in the mobile phase. This provides increased resolution of *cis* and *trans* isomers on a reverse phase column.

An excellent review covering physical methods for analysis of polyunsaturated fatty acids has been written by Khan & Scheinmann (1978) in which a useful comparison with GC is made.

Other areas covered in recent papers include determination of diglycerol in partial glycerides (Aitzetmueller & Bohrs, 1979), vegetable oil unsaponifiable matter (Cortesi, Fedeli & Tiscornia, 1978), lecithins in egg (Porter, Wolf & Nixon, 1979) and the use of 4-bromomethy-7-methoxycoumarin to form fluorescent fatty acid derivatives, which greatly increases sensitivity (Zelenski & Huber, 1978).

Vitamins

The range of applications of HPLC to vitamin analysis continues to widen. In general there are few problems in obtaining an adequate chromatographic separation between the various vitamins, or indeed between the isomeric forms of a particular vitamin. However, many problems still exist in the development of rapid methods for obtaining clean extracts free from interfering substances. In addition for some vitamins such as those of the B group which are only present at very low levels, there still remains a problem of sensitivity, particularly for the non-fluorescent members of the group.

The majority of the water soluble vitamins, e.g. thiamin, riboflavin and niacin, have received considerable attention from workers eager to abandon lengthy microbiological methods (Richardson *et al.*, 1978) or wet chemical methods (Toma & Tabekhia, 1979). In general the correlation of data between the methods is good. The B_6 group of water soluble vitamins has also received extensive study (Gregory & Kirk, 1978; Wong, 1978; Vanderslice, Stewart & Yarmas, 1979).

Vitamin D_3 can be readily separated from vitamin D_2 by reverse phase chromatography and indeed in those cases where D_3 alone is found, as in fish products, D_2 can be used as an internal standard (Egass & Lambertsen, 1979). Other foods in which vitamin D has been determined include cod liver oil (Syed Laik Ali, 1978) and milk (Henderson & Wickroski, 1978).

Koshy & Vanderslik (1979a,b) have published methods for the determina-

tion of 25-hydroxycholecalciferol in egg yolk and cow's milk, illustrating the complex separation procedures often required to obtain sufficiently clean extracts for HPLC, namely a combination of extraction, and partition and adsorption chromatography.

Vitamin A and its precursors have been extensively studied in a variety of foods, including margarine (Aitzetmueller, Pilz & Tasche, 1979; Landen & Eitenmiller, 1979), milk products (Nilsson, 1978) and tomatoes (Zakaria *et al.*, 1979). The last paper shows clearly how HPLC can be used to separate the bio-active carotenoids from the inactive, e.g. lycopene.

Many separations of vitamin E isomers (tocopherols) have recently been published, mainly employing adsorption chromatography, applied to milk (Pickston, 1978), vegetable oils (Podlaha, Eriksson & Toeregard, 1978) and miscellaneous foods, (Tangney *et al.*, 1979). In his paper Podlaha also presents an interesting comparison with a polarographic method.

In addition to the above publications several papers have appeared showing how more than one of the fat-soluble vitamins can be determined simultaneously, (e.g., Macleod & Wiggins, 1978; Widicus & Kirk, 1979; Mankel, 1979). The K group vitamins have also been studied by Rittich *et al.*, 1978, who claimed a complete separation of the members of the group using a silicagel column.

Flavour compounds

GC is well established as the most important technique for the analysis of flavour compounds, particularly when coupled to a mass spectrometer. However, the range of applications of HPLC in this area is increasing with the realization that many compounds of comparatively low volatility can still have a significant effect on both taste and flavour.

The major area of study using HPLC has undoubtedly been in the brewing industry, with a large number of publications mainly concerned with the analysis of hop bitter principles (Davis & Palamand, 1976; Siebert, 1976; Verzele & Potter, 1978; Otter & Taylor, 1978; Slotema, 1979; Gill, 1979). A review in French, covering research on polyphenols in brewing also illustrates the use of HPLC (Jerumanis, 1979).

Other applications cover a very wide range of foods and include beverages (Bricout, 1978; Qureshi, Prentice & Burger, 1979; Jeuring, 1979), liquorice (Beasley, Ziegler and Bell, 1979), peppers (Verzele, Mussche & Qureshi, 1979), capsaicin (Iwai *et al.*, 1979; Sticker, Soldati & Joshi, 1978) and citrus and other volatile oils (Latz & Ernes, 1978; Ross, 1978; Rousseff & Fisher, 1978). The xanthine alkaloids, particularly caffeine, have provided the subject matter for a number of papers. These alkaloids can be readily determined in tea and soft drinks simultaneously with saccharin and benzoate (Tweedy, Heffelfinger & Waldrop, 1978; Woodward, Heffelfinger & Ruggles, 1979). An

interesting comparison between HPLC and a voltammetric determination for caffeine has also been reported (Sontag & Kral, 1979).

HPLC is also finding application in the study of the development of off-flavours in foods, for example the production of methyl ketones during spray drying of milk (Reineccius, Anderson & Felska, 1978) or the degradation of tryptophan in irradiated food samples (Yong & Lau, 1979).

Toxic compounds

The increasing interest in food safety has led to an increase in the use of HPLC for the analysis of minor food contaminants. Present and future legislation, largely from the EEC, will demand rapid and sensitive methods of analysis for a wide range of contaminants, including mycotoxins, nitroso-compounds, pesticides and hydrocarbons (particularly polynuclear aromatics).

Mycotoxins

Norpoth & Boesenberg (1977) published a paper reviewing the range of mycotoxins encountered in foods, together with analytical methods for their detection including HPLC methods. Since that date a vast literature has grown up in this area, mainly concerned with aflatoxins. Methods for aflatoxins employ adsorption chromatography, (e.g. Blanc, Midler & Karleskind, 1977) or reverse phase chromatography (e.g. Koch, Nothhelfer & Treiber, 1978; Colley & Neal, 1979) with fluorescence detection (e.g. Siegfried & Ruckemann, 1978; Hunt *et al.*, 1978b) or ultraviolet detection (e.g. Blanc *et al.*, 1977). The various methods have been applied to a wide range of foods including: peanuts, or their products (Beebe, 1978; Hurst & Toomey, 1978; Kmieciak, 1977; Pons & Franz (1978), rice (Manabe, Goto & Matsuura, 1978), corn (Luethy *et al.*, 1978; Pons, 1979; Diebold *et al.*, 1979b), milk (Winterlin, Hall & Hsieh, 1979) and breakfast cereals (Hunt *et al.*, 1978b).

Other mycotoxins which have received attention in recent years include zearalenone (Frischkorn, Frischkorn & Ohst, 1978; Hunt, Bourdon & Crosby, 1978a; Scott *et al.*, 1978; Ware & Thorpe, 1978; Moeller & Josefsson, 1978; Diebold, Karny & Zare, 1979a; Josefsson & Moeller, 1979), rubratoxin (Unger & Hayes, 1978), patulin (Hunt *et al.*, 1978a; Leuenberger, Gauch & Baumgartner, 1978; Stray, 1978; Woidich *et al.*, 1978a; Brackett & Marth, 1979), xanthomegnin (Stack, Brown & Eppley, 1978) and territrems, mycotoxins of *Aspergillus terreus* (Ling, Yang & Huancy, 1979).

Nitroso-compounds

The use of HPLC for the analysis of nitroso-compounds does not appear to have become as established as might have been anticipated from the large

number of earlier publications. However, the use of fluorometric labelling, for example with dansyl chloride, would appear to provide an elegant and sensitive method for the determination of nitrosamines (e.g. Heyns, Roeper & Stolzenburg, 1978). HPLC has also been applied to the determination of specific nitrosamines in meat, for example nitrosoproline (Baker & Ma, 1978).

Hydrocarbons (polynuclear aromatics)

The increasing demands for more stringent acceptable limits for polynuclear aromatics in foods has led to many analytical problems. These are discussed in two papers with reference to drinking water (Crathorne & Fielding, 1978) and to foods (Knowles, 1978). Two further specific food examples that have received attention are oysters (Hanus *et al.*, 1979) and alcoholic beverages (Toussaint & Walker, 1979).

Pesticides

The potential use of HPLC for the analysis of pesticides has been demonstrated by a number of workers (e.g. Lawrence & Turton. 1978; Hoodless *et al.*, 1978). However, in general, HPLC has not replaced GC as a routine analytical method, mainly because of the relatively greater sensitivity of the latter. Even in those cases where GC is not ideal, for example with carbamates, HPLC still requires a fluorometric labelling stage to increase sensitivity to provide a viable alternative (Krause, 1978; Lawrence & Leduc, 1978a). In spite of these limitations a number of methods continue to be published for specific pesticides, including diflubenzuron (Di Prima *et al.*, 1978), carbaryl in potato and corn (Lawrence & Leduc, 1978b), paraquat in sunflower (Paschal *et al.*, 1979), pentachlorophenol residues (Faas & Moore, 1979), ethoxyquin in apples (Ernst & Verveld-Roeer, 1979), 2-imidazoline in fruit (Newsome & Panopio, 1978), and naphthaleneacetic acid, a plant growth regulator, in fruit (Moye & Wheaton, 1978; Cochrane & Lanouetre, 1979).

Drugs

Another area where applications of HPLC are beginning to appear is in the analysis of drug residues in animal products. Here again the main problem is one of sensitivity as the drugs, or their metabolites, are only present at very low levels. Some examples include the analysis of nitrofurazone in milk (Vilum & Macintosh, 1979), furazolidone in turkey tissue (Hoener, Lee & Lundergan, 1979) and chloramphenicol in meat (Ruessel, 1978).

Colourants

The present trend away from synthetic colourants in foods has increased the interest in characterization of natural pigments, as these may be used in foods

without the same scrutiny as applied to synthetic dyes. Flavonoid glycosides may be successfully chromatographed with both adsorption and reverse phase columns (Henning, 1979). Flavanoid derivatives have been studied in a wide range of foods including oats (Strack, Fuisting & Popovici, 1979), orange juice (Rouseff & Ting, 1979; Ting *et al.*, 1979), plant tissue (Galensa & Herrmann, 1978), cranberry juice (Williams *et al.*, 1978), wines (Israelian, 1978), cider, procyanidins (Lea, 1979), redbeet, betacyanins (Vincent & Scholz, 1978) and plums (Henning, 1979).

The use of HPLC for the characterization of synthetic dyes in foods is well established (e.g. Chudy, Crosby & Patel, 1978; Martin *et al.*, 1978). The technique is also employed to study individual synthetic dyes in more detail such as the separation of the colourants E110 and E111 (Frede, 1978) or as a criterion of purity since chemically similar impurities may have toxicological effects (e.g. Jones, Hoar & Sellings, 1978).

Food additives

Judged by the relatively few recent publications in this area the potential of HPLC as a technique for studying food additives has not, in general, been fully realized. In many cases a chromatographic separation can be readily achieved but it is in the more arduous stage of obtaining a chromatographically 'clean' extract that problems arise. For certain food additives, for example saccharin and benzoate in soft drinks, the problems of interfering compounds are minimal (Leuenberger, Gauch & Baumgartner, 1979; Woodward *et al.*, 1979). However, in other cases such as emulsifiers and stabilisers, obtaining a clean extract with good recovery is very difficult. Recent publications covering food additives include the surfactant sodium 6-0-palmitoyl ascorbate in bread, (Mauro *et al.*, 1979), mono- and diacyl-glycerols of saturated fatty acids (Riisom & Hoffmeyer, 1978) and the antioxidants butylated hydroxyanisole and butylated hydroxytoluene (Hammond, 1978; Majors & Johnson, 1978; Ciraolo, Calabro & Clasadonte, 1978).

Miscellaneous

The vast range of applications of HPLC in food analysis clearly demonstrates the versatility of the technique. It is not possible to include all the recently developed methods but the following examples will illustrate the almost universal applicability of HPLC: the sterols testosterone in meat (Stan & Hohls, 1979) and coumestrol in soyabeans (Lookhart, Jones & Finney, 1978), Amadori compounds in model Maillard browning systems (Takeoka, 1979), amines in wines (Subden *et al.*, 1979), fish products (Schmidtlein, 1979) and chocolate (Ingles, Tindale & Gallimore, 1978), total and available lysine (Peterson & Warthesen, 1979), methionine in fortified foods (O'Keefe & Warthesen, 1978)

and cysteine in fruit (Saetre & Rabenstein, 1978), isothiocyanates in rapeseed (Maheshwari *et al.*, 1979) and in vegetables (Mullin, 1978), glycoalkaloids in potatoes (Walter, Purcell & McCollum, 1979), esters of hydroxy cinnamic and tartaric acids in grapes (Ong & Nagel, 1978), amides and phenols in coffee beans (Hunziker & Miserez, 1979; Folstar *et al.*, 1979) and organic acids in cranberries (Coppola, Conrad & Cotler, 1978), wines (Symonds, 1978) and apple juice (Jeuring *et al.*, 1979).

Conclusion

The importance of HPLC as an analytical tool in food studies is now well established. The speed of analysis, in most instances, is not governed by the final chromatographic stage but by the prior sample preparation and extraction. Consequently it is in this area that most progress needs to be made.

The development of more sensitive detectors, possibly coupled with more widespread use of fluorometric labelling, will allow trace contaminants, for example pesticides, to be more readily analysed.

HPLC has already become an essential technique for the well equipped food analysis laboratory and its range of applications will doubtless expand.

References

Aitzetmueller, K. (1978) *J. Chromat.* **156,** 354.
Aitzetmueller, K. & Boehrs, M. (1979) *Z. Lebensmittelunters. u. Forsch.* **169,** 155.
Aitzetmueller, K., Pilz, J. & Tasche, R. (1979) *Fette Seifen Anstrichmittel,* **81,** 40.
Anon (1979) *Soft Drinks Trade J.* **33,** 18.
Baker, J.K. & Ma., C.-Y. (1978) *J. agric. Fd Chem.* **26,** 1253.
Beasley, T.H., Ziegler, H.W. & Bell, A.D. (1979) *J. Chromat.* **175,** 350.
Beebe, R.M. (1978) *J. Ass. offic. analyt. Chem.* **61,** 1347.
Blanc, M., Midler, O. & Karleskind, A. (1977) *Arch. Inst. Pasteur Tunis,* **54,** 305.
Brackett, R.E. & Marth, E.H. (1979) *Z. Lebensmittelunters. u. Forsch.* **169,** 92.
Bricout, J. (1978) *Ann. Nutr. Alimentation,* **32,** 947.
Buckee, G.K. & Hargitt, R. (1978) *J. Inst. Brew.* **84,** 13.
Bushway, R.J., Barden, E.S., Bushway, A.W. & Bushway, A.A. (1979) *J. Chromat.* **178,** 533.
Camire, A.L. & Clydesdale, F.M. (1979) *J. Fd Sci.* **44,** 926.
Chan, H. W.-S. & Levett, G. (1978) *Chemy Ind.,* No. **15,** 578.
Chudy, J., Crosby, N.T. & Patel, I. (1978) *J. chromat.* **154,** 306.
Ciraolo, L., Calabro, G. & Clasadonte, M.T. (1978) *Rassegna Chimica,* **30,** 145.
Clarke, M.A. & Brannan, M.A. (1979a) *Proc. 1978 Technical Session on Cane Sugar Refining Research,* New Orleans, U.S.A., p. 136.
Clarke, M.A. & Brannan, M.A. (1979b) *Proc. 1978 Technical Session on Cane Sugar Refining Research,* New Orleans, U.S.A., p. 149.
Cochrane, W.P. & Lanouetle, M. (1979) *J. Ass. offic. analyt. Chem.* **62,** 100.
Colley, P.J. & Neal, G.E. (1979) *Analyt. Biochem.* **93,** 409.
Coppola, E.D., Conrad, E.C. & Cotler, R. (1978) *J. Ass. offic. analyt. Chem.* **61,** 1490.
Cortesi, N., Fedeli, E. & Tiscornia, E. (1978) *Riv. Ital. Sostanze Grasse,* **55,** 168.
Crathorne, B. & Fielding, M. (1978) *Proc. analyt. Div. Chem. Soc.* **15,** 155.

Davis, D.P. & Palamand, S.R. (1976) *J. Am. Soc. Brew. Chem.* **34,** 55.
Diebold, G.J., Karny, N. & Zare, R.N. (1979a) *Analyt. Chem.* **51,** 67.
Diebold, G.J., Karny, N., Zare, R.N. & Seitz, L.M. (1979b) *J. Ass. offic. analyt. Chem.* **62,** 564.
Di Prima, S.J., Cannizzaro, R.D., Roger, J.-C. & Ferrell, C.D. (1978) *J. agric. Fd Chem.* **26,** 968.
Dunmire, D.L. & Otto, S.E. (1979) *J. Ass. offic. analyt. Chem.* **62,** 176.
Egass, E. & Lambertsen, G. (1979) *Int. J. Vit. Nutr. Res.* **49,** 35.
Engel, C.E. & Olinger, D.M. (1979) *J. Ass. offic. analyt. Chem.* **62,** 527.
Ernst, G.F. & Verveld-Roeer, S.Y. (1979) *J. Chromat.* **174,** 269.
Euber, J.R. & Brunner, J.R. (1978) *J. Dairy Sci.* **61,** (Suppl. 1.), 103.
Euber, J.R. & Brunner, J.R. (1979) *J. Dairy Sci.* **62,** 685.
Faas, L.F. & Moore, J.C. (1979) *J. agric. Fd Chem.* **27,** 554.
Folstar, P., Van der Plas, H.C., Pilnik, W., Schols, H. & Melger, F. (1979) *J. agric. Fd Chem.* **27,** 12.
Frede, W. (1978) *Dt. Lebensmittel Rdsch.* **74,** 263.
Friberg, T.S. (1978) *Diss. Abstr. Int.* **B 39(2),** 693.
Frischkorn, C.G.B., Frischkorn, H.E. & Ohst, I.M. (1978) *Z. Lebensmittlunters. u. Forsch.* **167,** 7.
Galensa, R. & Herrmann, K. (1978) *Z. Lebensmittelunters. u. Forsch.* **166,** 355.
Gasparoli, A. & Fedeli, E. (1979) *Riv. Ital. Sostanze Grasse,* **56,** 2.
Gill, R. (1979) *J. Inst. Brew.* **85,** 15.
Gorin, N. (1979) *J. agric. Fd Chem.* **27,** 195.
Gregory, J.F. & Kirk, J.R. (1978) *J. Fd Sci.* **43,** 1801.
Hammond, K.J. (1978) *J. Ass. pub. Anal.* **16,** 17.
Hanus, J.P., Guerrero, H., Biehl, E.R. & Kenner, C.T. (1979) *J. Ass. offic. analyt. Chem.* **62,** 29.
Hatanaka, A., Kajiwara, T., Sekiya, J. & Fujimura (1979) *Agric. biol. Chem.* **43,** 175.
Hemmati, P.F. & Keeney, P.G. (1979) *J. Fd Sci.* **44,** 1357.
Henderson, S.K. & Wickroski, A.F. (1978) *J. Ass. offic. analyt. Chem.* **61,** 1130.
Henning, W. (1979) *Lebensmittelchemie u. Gerichtliche Chemie,* **33,** 80.
Hersloef, B., Podlaha, O. & Toeregard, B. (1979) *J. Am. Oil Chem. Soc.* **56,** 864.
Heyns, K., Roeper, H. & Stolzenburg, K. (1978) *Chemie Mikrobiologie Technologie Lebensmittel.* **5,** 155.
Hoener, B.A., Lee, G. & Lundergan, W. (1979) *J. Ass. offic. analyt. Chem.* **62,** 257.
Hoodless, R.A., Sidwell, J.A., Skinner, J.C. & Treble, R.D. (1978) *J. Chromat.* **166,** 279.
Hunt, D.C., Bourdon, A.T. & Crosby, N.T. (1978a) *J. Sci. Fd Agric.* **29,** 239.
Hunt, D.C., Bourdon, A.T., Wild, P.J. & Crosby, N.T. (1978b) *J. Sci. Fd Agric.* **29,** 234.
Hunziker, H.R. & Miserez, A. (1979) *Mitt. Geb. Lebensmittelunters. u. Hyg.* **70,** 142.
Hurst, W.J. & Toomey, P.B. (1978) *J. chromat. Sci.* **16,** 372.
Hurst, W.J., Martin, R.A. & Zoumas, B.L. (1979) *J. Fd Sci.,* **44,** 892.
Ingles, D.L., Tindale, C.R. & Gallimore, D. (1978) *Chemy. Ind.* No. **12,** 432.
Israelian, C. (1978) *Vignes et Vins,* No. 267, 50.
Iwai, K., Suzuki, T., Fujiwake, H. & Oka, S. (1979) *J. Chromat.* **172,** 303.
Jerumanis, J. (1979) *Cerevisia,* **4,** 9.
Jeuring, H.J., Brands, A. & Doorninck, P. Van, (1979) *Z. Lebensmittelunters. u. Forsch.* **168,** 185.
Jeuring, H.J., Hoevan, W. Van den, Doorninck, P. Van & Broeke, R. Ten (1979) *Z. Lebensmittelunters. u. Forsch.,* **169,** 281.
Jones, A.D., Hoar, D. & Sellings, S.G. (1978) *J. Chromat.* **166,** 619.
Josefsson, E. & Moeller, T. (1979) *J. Ass. offic. analyt. Chem.* **62,** 1165.
Karleskind, A., Valmalle, G., Midler, O. & Blanc, M. (1978) *Rev. Franc. Corps. Gras.* **25,** 551.
Khan, G.R. & Scheinmann, F. (1978) *Prog. Chem. Fats and Other Lipids,* **15,** 343.
Kmieciak, S. (1977) *Arch. Inst. Pasteur Tunis,* **54,** 389.
Knowles, M.E. (1978) *Proc. analyt. Div. Chem. Soc.* **15,** 153.
Koch, E., Nothhelfer, A. & Treiber, H. (1978) *Lebensmittelchemie u. Gerichliche Chemie,* **32,** 78.

Koshy, K.T. & Vanderslik, A.L. (1979a) *J. agric. Fd Chem.* **27,** 180.
Koshy, K.T. & Vanderslik, A.L. (1979b) *J. agric. Fd Chem.* **27,** 650.
Krause, R.T. (1978) *J. chromat. Sci.* **16,** 281.
Kupina, S.A. & Gump, B.H. (1979) *Abstr. Pap. Am. Chem. Soc.* **177,** AGFD 7.
Landen, W.O. & Eitenmiller, R.R. (1979) *J. Ass. offic. analyt. Chem.* **62,** 283.
Latz, H.W. & Ernes, D.A. (1978) *J. Chromat.* **166,** 189.
Lawrence, J.F. & Leduc, R. (1978a) *J. Chromat.* **152,** 507.
Lawrence, J.F. & Leduc, R. (1978b) *J. Ass. offic. analyt. Chem.* **61,** 782.
Lawrence, J.F. & Turton, D. (1978) *J. Chromat.* **159,** 207.
Lea, A.G.H. (1979) *J. Sci. Fd Agric.* **30,** 833.
Leuenberger, U., Gauch, R. & Baumgartner, E. (1978) *J. Chromat.* **161,** 303.
Leuenberger, U., Gauch, R. & Baumgartner, E. (1979) *J. Chromat.* **173,** 343.
Ling, K.H., Yang, C.K. & Huancy, H.C. (1979) *Appl. environment. Microbiol.* **37,** 358.
Lookhart, G.L. Jones, B.L. & Finney, K.F. (1978) *Cer. Chem.* **55,** 967.
Leuthy, J., Zweifel, U.. Balsiger, C. & Schlatter, C. (1978) *Mitt. Geb. Lebensmittelunters. u. Hyg.* **69,** 281.
Macleod, I.W. & Wiggins, R.A. (1978) *Proc. analyt. Div. Chem. Soc.* **15,** 329.
Macrae, R. (1980) *J. Fd Technol.* **15,** 93.
Maheshwari, P.N., Stanley, D.W., Gray, J.I. & Voort, F.R. (1979) *J. Am. Oil Chem. Soc.* **56,** 837.
Majors, R.E. & Johnson, E.L. (1978) *J. Chromat.* **167,** 17.
Manabe, M., Goto, T. & Matsuura, S. (1978) *Agric. biol. Chem.* **42,** 2003.
Mankel, A. (1979) *Dt. Lebensmittel Rdsch.* **75,** 77.
Martin, G.E., Tenebaum, M., Alfonso, F. & Dyer, R.H. (1978) *J. Ass. offic. analyt. Chem.* **61,** 908.
Mauro, D.J., Wetzel, D.L., Seib, P.A. & Hoseney, R.C. (1979) *Cer. Chem.* **56,** 152.
Moeller, T.E. & Josefsson, E. (1978) *J. Ass. offic. analyt. Chem.* **61,** 789.
Moye, H.A. & Wheaton, T.A. (1978) *J. agric. Fd Chem.* **27,** 291.
Mullin, W.J. (1978) *J. Chromat.* **155,** 198.
Neff, W.E., Frankel, E.N., Scholfield, C.R. & Weisleder, D. (1978) *Lipids,* **13,** 415.
Newsome, W.H. & Panopio, L.G. (1978) *J. agric. Fd Chem.* **26,** 638.
Nilsson, T. (1978) *XX Int. Dairy Congr.* **E,** 372.
Norpoth, K. & Boesenberg, H. (1977) Publ. 1978. *Berichte ueber Landwirtschaft,* **55,** 835.
O'Keefe, L.S. & Warthesen, J.J. (1978) *J. Fd Sci.* **43,** 1297.
Ong, B.Y. & Nagel, C.W. (1978) *J. Chromat.* **157,** 345.
Otter, G.E. & Taylor, L. (1978) *J. Inst. Brew.* **84,** 160.
Paschal, D.C., Needham, L.L., Rollen, Z.J. & Liddle, J.A. (1979) *J. Chromat.* **177,** 85.
Pattee, H.E. & Singleton, J.A. (1979) *J. agric. Fd Chem.* **27,** 216.
Peterson, W.R. & Warthesen, J.J. (1979) *J. Fd Sci.* **44,** 994.
Pickston, L. (1978) *New Zealand J. Sci.* **21,** 383.
Podlaha, O., Eriksson, A. & Toeregard, B. (1978) *J. Am. Oil Chem. Soc.* **55,** 530.
Pons, W.A. & Franz, A.O. (1978) *J. Ass. offic. analyt. Chem.* **61,** 793.
Pons, W.A. (1979) *J. Ass. offic. analyt. Chem.* **62,** 586.
Porter, N.A., Wolf, R.A. & Nixon, J.R. (1979) *Lipids,* **14,** 20.
Qureshi, A.A., Prentice, N. & Burger, W.C. (1979) *J. Chromat.* **170,** 343.
Rapp, A., Ziegler, A. & Steffan, H. (1977) *Vitis,* **16,** 112.
Reineccius, G.A., Anderson, H.C. & Felska (1978) *J. Fd Sci.* **43,** 1494.
Richardson, P.J., Favell, D.J., Gidley, G.C. & Jones, A.D. (1978) *Proc. analyt. Div. Chem. Soc.* **15,** 53.
Riisom, T. & Hoffmeyer, L. (1978) *J. Am. Oil Chem. Soc.* **55,** 649.
Rittich, B., Krska, M., Simek, M. & Coupek, J. (1978) *Roczn. Naukowe Zootech.* **5,** 33.
Ross, M.S.F. (1978) *J. Chromat.* **160,** 199.
Rousseff, R.L. & Fisher, J.F. (1978) *Abstr. Pap. Am. Chem. Soc.* **176,** AGFD 15.
Rousseff, R.L. & Ting, S.V. (1979) *J. Chromat.* **176,** 75.

Ruessel, H.A. (1978) *Chromatographia,* **11,** 341.
Rydel, S. (1979) *Gazeta Cukrownicza,* **87,** 79.
Saetre, R. & Rabenstein, D.L. (1978) *J. agric. Fd Chem.* **26,** 982.
Schmidtlein, H. (1979) *Lebensmittelchemie u. Gerichtliche Chemie,* **33,** 81.
Scott, P.M., Panalaks, T., Kanhere, S. & Miles, W.F. (1978) *J. Ass offic. analyt. Chem.* **61,** 593.
Siebert, K.J. (1976) *J. Am. Soc. Brew. Chem.* **34,** 79.
Siegfried, R. & Ruckemann, H. (1978) *Chemiker Z.* **102,** 403.
Slotema, F.P. (1979) *Voedingsmiddelentechnol.* **12,** 11.
Sontag, G. & Kral, K. (1979) *Mikrochimica Acta,* **1,** 229.
Stack, M.E., Brown, N.L. & Eppley, R.M. (1978) *J. Ass. offic. analyt. Chem.* **61,** 590.
Stan, H.J. & Hohls, F.W. (1979) *Z. Lebensmittelunters. u. Forsch.* **169,** 266.
Sticher, O., Soldati, F. & Joshi, R.K. (1978) *J. Chromat.* **166,** 221.
Strack, D., Fuisting, K. & Popovici, G. (1979) *J. Chromat.* **176,** 270.
Stray, H. (1978) *J. Ass. offic. analytic. Chem.* **61,** 1359.
Subden, R.E., Duitschaever, C., Kaiser, K. & Noble, A.C. (1979) *Am. J. Enol. Vitic.* **30,** 19.
Syed Laik Ali (1978) *Z. Anal. Chemie.* **293,** 131.
Symonds, P. (1978) *Ann. Nutr. Alimentation.* **32,** 957.
Takeoka, G.R., Coughlin, J.R. & Russell, G.F. (1979) *Abstr. Pap. Am. Chem. Soc.* **177,** AGFD 19.
Tangney, C.C., Driskell, J.A. & McNair, H.M. (1979) *J. Chromat.* **172,** 513.
Ting, S.V., Rouseff, R.L., Dogherty, M.H. & Attaway, J.A. (1979) *J. Fd Sci.* **44,** 69.
Toma, R.B. & Takekhia, M.M. (1979) *J. Fd Sci.* **44,** 263.
Toussaint, G. & Walker, E.A. (1979) *J. Chromat.* **171,** 448.
Tweedy, J.D., Heffelfinger, G.P. & Waldrop, A. (1978) *Fd Development,* **12,** 42.
Unger, P.D. & Hayes, A.W. (1978) *J. Chromat.* **153,** 115.
Vanderslice, J.T., Stewart, K.K. & Yarmas, M.M. (1979) *J. Chromat.* **176,** 280.
Verzele, M. & Potter, M. (1978) *J. Chromat.* **166,** 320.
Verzele, M., Mussche, P. & Qureshi, S.A. (1979) *J. Chromat.* **172,** 493.
Vilim, A.B. & Macintosh, A.I. (1979) *J. Ass. offic. analyt. Chem.* **62,** 19.
Vincent, H.R. & Scholz, R.G. (1978) *J. agric. Fd Chem.* **26,** 812.
Wada, S., Koizumi, C., Takiguchi, A. & Nonaka, J. (1978) *Int. Congr. Fd Sci. Technol.* Abstr. 199.
Walter, W.M., Purcell, A.E. & McCollum, G.K. (1979) *J. agric. Fd Chem.* **27,** 938.
Ware, G.M. & Thorpe, C.W. (1978) *J. Ass. offic. analyt. Chem.* **61,** 1058.
Warthesen, J.J. & Kramer, P.L. (1979) *J. Fd Sci.* **44,** 626.
White, J.W. (1979) *J. Ass. offic. analyt. Chem.* **62,** 515.
Widicus, W.A. & Kirk, J.R. (1979) *J. Ass. offic. analyt. Chem.* **62,** 637.
Williams, M., Hrazdina, G., Wilkinson, M.M., Sweeny, J.G. & Iacobucci, G.A. (1978) *J. Chromat.* **155,** 389.
Winterlin, W., Hall, G. & Hsieh, D.P.H. (1979) *Analyt. Chem.* **51,** 1873.
Woidich, H., Pfannhauser, W. & Blaicher, G. (1978a) *Lebensmittelchemie u. Gerichtliche Chemie,* **32,** 61.
Woidich, H., Pfannhauser, W. & Blaicher, G. (1978b) *Lebensmittelchem. u. Gerichtliche Chemie.* **32,** 74.
Wong, F.F. (1978) *J. agric. Fd Chem.* **26,** 1444.
Woodward, B.B., Heffelfinger, G.P. & Ruggles, D.I. (1979) *J. Ass. offic. analyt. Chem.* **62,** 1011.
Yong, S. & Lau, S. (1979) *J. Chromat.* **175,** 343.
Zakaria, M., Simpson, K., Brown, P.R. & Krstulovic, A. (1979) *J. Chromat.* **176,** 109.
Zelenski, S.G. & Huber, J.W. (1978) *Chromatographia.* **11,** 645.

(Received 12 June 1980)

J. Fd Technol. (1981) **16,** 13–19

Principles and practice of near infra-red (NIR) reflectance analysis

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Summary

Near infra-red (NIR) analysis is now widely used for the rapid analysis of many agricultural and food products for protein, moisture, oil, starch, sucrose, fibre, grain texture and lysine. Research has shown that the technique can also be used for the prediction of malting quality of barley, baking quality of wheat and measurement of the degree of starch damage in flour.

The technique of NIR analysis uses very small differences in absorption of NIR radiation at wavelengths corresponding to overtones and combinations of fundamental IR frequences of chemical functional groups that are characteristic of particular analytes. Complex regression mathematics is used to transform these absorption measurements into an analytical result.

Introduction

Near infra-red (NIR) reflectance analysis is becoming a widely accepted technique for the rapid analysis of a variety of food and agricultural products including cereals and cereal products (Hart, 1976; Hart, 1979; Hooton, 1978; Wingfield, 1979), animal feedingstuffs (Norris & Barnes, 1976), forages (Barton & Burdick, 1979; Norris *et al.*, 1976), milk (Ben-Gera & Norris, 1968a), meat (Ben-Gera & Norris, 1968b), seeds and pulses (Ben-Gera & Norris, 1968c; Williams *et al.*, 1978), confectionery (Roberts, 1980) and tobacco (McClure, Norris & Weeks, 1977). It is commended by its speed, low running costs, safety and simplicity combined with accuracy. It is necessary, however, as with any instrumental technique, to appreciate the principles behind its operation in order to understand the scope and the limitations of NIR analysis.

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0022–1163/81/0200–0013 $02.00

Infra-red (IR) absorption

The part of the electromagnetic spectrum visible to the human eye extends from about 400 to 700 nm while the infra-red (IR) extends from 2500–15000 nm. The intermediate region between the IR and the visible is termed the near infra-red (NIR). The IR spectrum was discovered about 1800 by Herschel when he set out to discover which colours of light carry the sun's warmth. He concluded that the warmth is carried by waves longer than those of visible light which are invisible to the human eye. He termed these rays 'infra-red' (beyond the red). Early this century Coblentz observed that compounds with similar chemical groupings have characteristic absorption bands in the IR. For instance, aldehydes, ketones, organic acids and their esters, anhydrides and salts, which all have a carbonyl ($>C = O$) functional group, have absorption bands near 6000 nm. Coblentz's findings have since been extended and refined to show that nature of the chemical structure bonded to the carbonyl group influences the exact wavelengths of the IR absorption. Thus, it is now possible to learn much about the chemical structure of compounds from their IR absorption spectra.

Chemical bonds in molecules are natural oscillators which are vibrating all the time. Thus, the molecule will absorb radiation when the vibrations occur at the frequency* of the radiation wave. For anything as small as a molecule, vibration can only occur at fixed frequencies so that radiation is absorbed in discrete packets (quanta). Thus, the molecule can only have characteristic absorption bands corresponding to these fixed frequences and the amount of radiation absorbed at each frequency is proportional to the number of similar chemical bonds which are vibrating.

Near infra-red (NIR) spectra

Surprisingly, despite the success of IR in other fields, it was not used for food analysis until 1964 when Norris developed a new method for measurement of moisture in grain using NIR (Norris, 1964). Norris and others soon developed way to measure fat in milk, fat and moisture in meat, and protein, oil and moisture in grains. Their measurement procedures were fast and uncomplicated although the underlying technology was sophisticated. The NIR region of the spectrum had been rather neglected because measurement was not possible until photoelectric detectors had been invented, whereas the IR had long been capable of measurement by thermal-sensitive devices. However, Norris discovered that the NIR offered important advantages for food analysis.

Like any simple harmonic motion, the vibrations which give rise to fundamental IR absorption bands have corresponding overtones at twice and three times the frequency which occurs in the NIR. However, the molecular vibra-

* Frequency $\nu = \dfrac{1}{\text{Wavelength } \lambda}$

tions do not obey the laws of simple harmonic motion exactly and the frequency of the overtones is governed by the rules of quantum theory. Overtones will only be easily detected when the band causing the fundamental vibration is between two very unlike atoms, e.g. carbon and oxygen. In addition to overtones, combination bands also occur in the NIR due to the simultaneous vibration of two or more bands when their frequencies ($\nu_1, \nu_2, \ldots$) are summed according to the formula

$$\nu_{comb} = n_1\nu_1 + n_2\nu_2 + \ldots$$

where n_1, n_2, . . . are positive integers.

Each of the many constituents of a food absorbs strongly at the wavelengths of the fundamental absorption bands in the IR. The IR spectra are therefore difficult to measure accurately enough for analysis but the NIR region of weaker overtone and combination bands is less complex, since not all constituents absorb, while the absorption bands due to constitutents such as protein, oil and moisture are still strong enough to be measured accurately.

Application of NIR to food analysis

Norris pioneered the technique of multiple wavelength readings on a sample, making possible the simultaneous determination of several constituents giving rise to overlapping absorption bands. One wavelength is chosen because the constituent to be measured absorbs strongly there but other constituents also absorb at the same wavelength (Law & Tkachuk, 1977a; Wetzel & Mark, 1978). For example, there is a prominent peak at 1930 nm in the spectrum of water and measurement of the magnitude of this peak can be related to the amount of water present in a sample. However, there is also a peak in this position in the spectrum of starch so that in a complex mixture containing starch, it is not possible to measure moisture from the absorption of the sample at one wavelength only. Similarly, protein is measured at 2180 and 2100 nm but a correction must also be made for the absorption due to starch at these wavelengths. By making absorption measurements on a number of calibration samples at six wavelengths and solving three multiple linear regression equations, the NIR instrument could be calibrated for the measurement of protein, moisture and oil in a food sample. However, it has since been discovered that it is not always necessary to use as many as six readings for a regression and often a sub-set of two or three can be selected by statistical analysis (Osborne, Douglas & Fearn, 1980).

Theory of diffuse reflectance

If light is directed onto a sample it may either be transmitted or reflected and since most foods are opaque, reflectance measurements are more convenient

for NIR analysis. Since some of the light is absorbed and the remainder is reflected, study of this diffused reflected light can be used to measure the amount absorbed. A reflectance spectrum cannot be obtained from a totally reflecting surface because by definition no light is absorbed and this reflectance is called specular. Since no surface is either perfectly matt or perfectly reflecting there will be both diffuse and specular reflectance from a real surface and since only the diffuse portion can be used for NIR analysis, the optics of NIR instruments are designed to measure only the diffuse reflectance from a sample surface.

The requirement by definition for reflectance to be diffuse is that the intensity of reflected light is isotropic but for a powdered sample both scattering and absorption occur, and, since the scattered radiation is angularly distributed, it is by no means isotropic. However, for a large number and sufficiently thick layer of closely packed particles an isotropic scattering distribution can still be anticipated and therefore the emerging light will still be diffuse (Kortum, 1969). As no rigorous theory exists to explain this phenomenon, several empirical theories based on the concept of two constants called the absorption coefficient (K) and the scattering coefficient (S) have been proposed. The most popular of such theories is that due to Kubelka & Munk who related the values of these constants to the total absolute reflectance of an infinitely thick layer R_∞ by the equation.

$$\frac{K}{S} = \frac{(1 - R_\infty)^2}{2 R_\infty}$$

where the reflectance term is called the Kubelka-Munk function F (R_∞). Since for dilute mixtures $K = 2.30 \varepsilon c$ where ε is the molar extinction coefficient and c is the concentration, F (R_∞) α c providing S is constant. This relationship has been shown to be valid for the absolute measurement of moisture in wheat without calibration against samples of known composition (Law & Tkachuk, 1977b). Unfortunately, the measurement of R_∞ is very tedious and therefore it is usual to measure apparent reflectance referred to a reflectance standard (Tkachuk & Law, 1978). Because the signal from the detector is amplified using a logarithmic response amplifier, data are recorded as $\log R/R'$, where R is the reflectance of the reference and R' is the reflectance of the sample. $\log R$ is constant because a reference is chosen such that its reflectance does not change with wavelength, and therefore $\log 1/R'$, carries all the information. The $\log 1/R'$, data on a number of samples must be calibrated against values obtained by an accepted method (such as Kjeldahl for protein) before an NIR instrument may be used for analysis.

Limitations of NIR analysis

Multiple regression techniques coupled with different data treatments give very

high correlations with a number of analytical parameters. However, the reflectance change caused by a significant change in composition is very small, so that extremely sensitive reflectance measurements are required to measure small changes in composition. For example, a change of 1% in protein content produced a change of only 0.002 in the log $1/R'$ value for wheat at the most sensitive wavelength. Present instruments have adequate signal to noise performance to be able to measure these small changes in reflectance and, in general, the measurement is not limited by instrument noise but by sample preparation.

Ideally, reflectance measurements should be made on a homogeneous sample of uniform small particles; therefore, for samples such as grains the method of grinding of the sample is extremely important (Hunt *et al.*, 1978; Williams & Thompson, 1978). The scattering coefficient for small particles depends on the ratio of mean particle size to wavelengths of the radiation used (d/λ) and on the ratio of the refractive index of the particles to that of the surrounding medium (n/n_0). Therefore, both the particle size of the powder and the moisture content of the sample (which will influence the refractive index of the medium) will influence S and thus determine the validity of the calibration between reflectance and concentration. Both of these variables can be taken into account by the use of multiple linear regression, but it is often necessary to produce a less universal calibration for better accuracy. For example, it is better to calibrate separately for hard and soft texture wheats (Hart, 1976; Watson *et al.*, 1977).

Scope of NIR analysis

With the cost of basic food commodities rising steadily, the need for the food processing industries to monitor their use is increasing and many of the classical analytical methods are far too time-consuming for effective quality control. NIR analysis is an ideal technique for this purpose as it combines speed with reliability and it does not need to be operated by highly skilled personnel. Instruments capable of measuring protein, moisture, oil, starch, sugar and fibre are commercially available and recent research has shown that many other applications are possible. Examples of such developments are the measurement of lysine content of wheat (Rubenthaler & Bruinisma, 1978) and barley (Gill, Starr & Smith, 1979), the determination of total reducing sugars in tobacco (McClure *et al.*, 1977), the prediction of malting quality of barley by measurement of β-glucan (Allison, Cowe & McHale, 1978) or malt hot water extract (Morgan and Gothard, 1979), and the measurement of starch damage in, and prediction of potential baking quality of flour (Osborne, Douglas & Fearn, 1980).

NIR analysis is widely used in grain trading for the analysis of protein and moisture and the determination of protein in wheat by NIR has been validated by collaborative study and shown to be capable of accuracy equal to that of the Kjeldahl method (Hunt *et al.*, 1977). In addition, hard and soft texture wheats

may be distinguished by NIR (Hart, 1976; Williams, 1979) and this distinction is of considerable value in assessing the milling quality of the wheat. The measurement of fibre in flour together with protein and moisture has facilitated the rapid control of the flour milling operation (Wingfield, 1979) and NIR has been developed into an on-line quality control technique for protein, moisture and oil in a soybean processing plant (Webster, 1979). A further application has been the control of sucrose content of chocolate crumb in confectionery manufacture (Roberts, 1980). It is apparent from these applications that NIR analysis would be a useful quality control technique for the food industry generally.

Conclusions

In summary, therefore, near infra-red reflectance analysis has possible application to a wide range of components because most components of food have absorption bands in the NIR region. Indeed, research now under way indicates that the NIR reflectance may be developed into a general purpose technique for the compositional analysis of many agricultural and food commodities.

References

Allison, M.J., Cowe, I.A. & McHale, R. (1978) *J. Inst. Brew.* **84,** 153.
Barton, F.E. & Burdick, D. (1979) *J. agric. Fd Chem.* **27,** 1248.
Ben-Gera, I. & Norris, K.H. (1968a) *Israel J. agric. Res.* **18,** 117.
Ben-Gera, I & Norris, K.H. (1968b) *J. Fd Sci.* **33,** 64.
Ben-Gera, I & Norris, K.H. (1968c) *Israel J. agric. Res.* **18,** 125.
Gill, A.A., Starr, C. & Smith, D.B. (1979) *J. agric. Sci. Camb.* **93,** 727.
Hart, H.V. (1976) *FMBRA Annual Report,* p. 24.
Hart, H.V. (1979) *FMBRA Annual Report,* p. 25.
Hooton, D. (1978) *Cereal Foods World* **23,** 176.
Hunt, W.H., Fulk, D.W., Elder, B. & Norris, K.H. (1977) *Cereal Fds Wld,* **22,** 534.
Hunt, W.H., Fulk, D.W., Thomas, T. & Nolan, T. (1978) *Cereal Fds Wld* **23,** 143.
Kortum, G. (1969) *Reflectance Spectroscopy Principles, Methods, Applications,* Springer-Verlag, Berlin.
Law, D.P. & Tkachuk, R. (1977a) *Cereal Chem.* **54,** 256.
Law, D.P. & Tkachuk, R. (1977b) *Cereal Chem.* **54,** 874.
Morgan, A.G. & Gothard, P.G. (1979) *J. Inst. Brew.* **85,** 339.
McClure, W.F., Norris, K.H. & Weeks, W.W. (1977) *Beitr. Tabakforsch.* **9,** 13.
Norris, K.H. (1964) *Agric. Engng, St. Joseph, Mich.* **45,** 370.
Norris, K.H. & Barnes, R.F. (1976) *Proceedings of the First International Symposium on Feed Composition,* Utah State University.
Norris, K.H., Barnes, R.F., Moore, J.E. & Shenk, J.S. (1976) *J. Anim. Sci.* **43,** 889.
Osborne, B.G., Douglas, S. & Fearn, T. (1980) *65th Annual Meeting of the American Association of Cereal Chemists,* Sept. 21–25, Chicago, Illinois.
Roberts, G. (1980) *Neotec International News Items* No. 12, p.2.
Rubenthaler, G.L. & Bruinisma, B.L. (1978) *Crop Sci.* **18,** 1039.
Tkachuk, R. & Law, D.P. (1978) *Cereal Chem.* **55,** 981.
Watson, C.A., Shuey, W.C., Banasick, O.J. & Dick, J.W. (1977) *Cereal Chem.* **54,** 1264.

Webster, D. (1979). *ASA Meeting*. Brussels, 12 Sept.
Wetzel, D.L. & Mark, H. (1978) *Sixth International Bread Congress*, paper no. S2–1, Winnipeg, Canada.
Wingfield, J. (1979) *Bulletin of the Association of Operative Millers*, 3769.
Williams, P.C. (1979) *Cereal Chem.* **56,** 169.
Williams, P.C., Stevenson, S.G., Starkey, P.M. & Hawtin, G.C. (1978) *J. Sci. Fd Agric.* **29,** 285.
Williams, P.C. & Thompson, B.N. (1978) *Cereal Chem.* **55,** 1014.

(Received 2 August 1980)

J. Fd Technol. (1981) **16,** 21–30

The evaluation of water activity in aqueous solutions from freezing point depression

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Summary

The water activity of various non-electrolyte and electrolyte solutions of interest for a_w inhibition of microorganisms, has been calculated from freezing point data and compared with a_w values measured at 25°C. It is shown that in the important range of a_w, 0.96–0.85, the error introduced by assuming that the a_w at the freezing point is the same as that at 'room' temperature, is relatively small. In the majority of cases the difference is not likely to be larger than about 0.01 a_w units.

Introduction

It is well known that water activity (a_w) largely influences microbial activity (and also various chemical reactions) in foods (Scott, 1957; Labuza *et al.*, 1970). For this reason there is a need for convenient and accurate methods of measuring a_w in bacteriological media as well as in food systems. Most methods for the measurement of water activity, either in solutions or solid food systems have been recently reviewed by Troller & Christian (1978). The a_w of aqueous solutions can be determined, among many other methods, by measuring the freezing point depression. Wodizinski & Frazier (1960) and Strong, Foster & Duncan (1970) utilized this technique to study the effect of a_w on bacterial growth. It has been stated, however, (Ayerst, 1965; Troller & Christian, 1978) that this method has the drawback that it provides the a_w at the freezing temperature and not at the 'normal' microbiological growth temperature (i.e. 25–30°C). A similar restriction applies to methods of a_w evaluation employing

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0022–1163/81/0200–0021 $02.00

boiling point determinations. Admittedly, the above objection is a reasonable one because the differences between room and freezing temperatures of solutions having a_w in order to 0.87–0.90 are about 35–40°C. This aspect, however, has not been quantitatively examined; i.e. how large is the error introduced by assuming that the a_w at 'normal' microbiological growth temperature is the same that at the corresponding freezing point.

This study compares the a_w of various non-electrolyte and some electrolyte solutions of interest in the food area, calculated from freezing point data, with a_w values measured at 25°C. The study is restricted to the microbiologically important range of a_w about 0.96–0.85, which is the one of most practical interest regarding intermediate moisture foods (Benmergui *et al.*, 1979). The boiling point elevation technique is also discussed to some extent.

Results and discussion

The depression of freezing point

The relationship between freezing point of an aqueous solution and its water activity is given by (Robinson & Stokes, 1965)

$$-\ln a_W = \frac{1}{R}\ (L_o - \bar{J}T_o)\left(\frac{1-1}{T_F\ \ T_o}\right) + \frac{\bar{J}}{R}\ln\frac{T_o}{T_F} \qquad (1)$$

where L_o is the latent heat of fusion at the freezing point of pure water (T_o), $\bar{J}$ is the difference of the molal heat capacities of liquid water and ice, and T_F is the freezing point of the solution. Equation (1) was obtained assuming that $\bar{J}$ is independent of temperature in the range $T_o - T_F$. It is easy to show that this assumption does not result in any significant loss of accuracy in the calculation of a_w; between 0°C and −15°C ($a_w = 0.864$) $\bar{J}$ varies 7.0% and this would change a_w by only 0.0004 a_w units.

Introducing the lowering of the freezing point, $\theta_F = T_o - T_F$, equation (1) may be written

$$-\ln a_w = \frac{L_o\,\theta_F}{R\,T_o^2} + \left[\frac{L_o}{R\,T_o} - \frac{\bar{J}}{2\,R}\right]\frac{\theta_F^2}{T_o^2} \qquad (2)$$

by a series development up to second order in θ_F. At 0°C the latent heat of fusion of ice is 1435.5 cal/mole while the difference of the molal heat capacities of liquid water and ice is 9.0990 cal/°C mole (Robinson & Stokes, 1965). Equation (2) may be numerically approximated to

$$-\ln a_w = 9.6934.\ 10^{-3}.\ \theta_F + 4.761\ .\ 10^{-6}\ .\ \theta_F^2 \qquad (3)$$

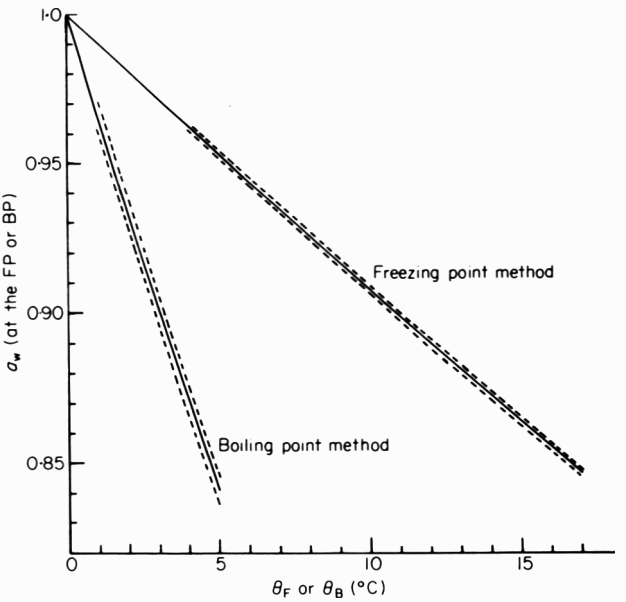

Figure 1. Graphical evaluation of a_w freezing point depression or boiling point elevation. FP,---, ± 0.15°C in θ_F; BP,---, ± 0.15°C in θ_B

which is accurate enough for our purposes. The a_w obtained from equation (3) is, of course, that at the temperature T_F. Another way of evaluating a_w is

$$(a_w)\ (T_F) = p_{ice}\ (T_F)/p_{water}\ (T_F) \tag{4}$$

that is, a_w is given by the ratio of vapour pressure of ice and supercooled liquid water at T_F. An alternative procedure to test the accuracy of simplified equation (3), which includes the assumption of constancy of $\bar{J}$ with temperature, consists in comparing a calculated value of a_w with that computed from relationship (4) using literature vapour pressure data of ice and supercooled liquid water at T_F (Troller & Christian, 1978). This calculation was performed for a $T_F = -15$°C and both a_w values were found to differ by only 0.0005 a_w units.

Equation (3) is given in graphical form in Fig. 1 and permits the direct evaluation of a_w from knowledge of the freezing point depression. It is also shown which is the accuracy in the calculated a_w value as a result of an error of ± 0.15°C in the determination of freezing points.

The elevation of the boiling point

The relationship between the boiling point of an aqueous solution containing non-volatile solute/s and its water activity is given by (Denbigh, 1964)

$$-\ln a_w = \frac{(L_B - \bar{G}\,T_B)}{R}\left(\frac{1}{T_{100}} - \frac{1}{T_B}\right) + \frac{\bar{G}}{R}\ln\frac{T_B}{T_{100}} \qquad (5)$$

where L_B is the latent heat at the boiling point of pure water (T_{100}), $\bar{G}$ is the difference of the molal heat capacities of water as a vapour and as a liquid, and T_B is the boiling point of the solution. Equation (5) may be also adequately approximated to

$$-\ln a_w = 35.127 \,.\, 10^{-3} \,.\, \theta_B - 1.1195 \,.\, 10^{-4} \,.\, \theta_B \qquad (6)$$

where $\theta_B = T_B - T_{100}$. The a_w obtained from equation (6) is that at the temperature T_B.

Equation (6) is also given in graphical form in Fig. 1 which shows the accuracy of computed a_w values when the error in boiling point determinations is ± 0.15°C. It can be seen that boiling points must be measured with much more accuracy than freezing points to obtain similar accuracy in a_w calculation.

Equation (6) on p. 24 should read
$-\ln a_w = 35.127.10^{+3}\,\theta_B - 1.1195.10^{-1}\,\theta_B^2$

Calculation of a_w

Freezing point depressions and boiling point elevations for a number of solutes of interest are reported in the International Critical Tables (1926) as well as in other laboratory handbooks (Perry, 1963; Hodgman, 1944). Equations (3) and (6) were used to evaluate a_w for a number of compounds including, sucrose, glycerol, citric acid, tartaric acid, ethyl alcohol, ethylene glycol, sodium chloride and calcium chloride. The results are shown in Figs 2–8, as compared with literature data of a_w measured at 25°C. Various a_w data at temperatures differing from 25°C or at freezing/boiling point, were also included. In most cases they were calculated from osmotic coefficients, taken from the literature

$$a_w = \exp(-0.018\, m\, \nu\, \psi)$$

where m is the molal concentration of the solute, ψ is the osmotic coefficient and ν is the number of ions generated by each molecule of solute (for non-electrolytes $\nu = 1$).

Before discussing the results, it is convenient to specify what accuracy is needed in a_w determination in the food area. Troller & Christian (1978) indicated that most methods for a_w estimation or range finding should be accurate to within 0.02 a_w units. For research applications in a critical range of a_w an accuracy of 0.005 a_w units is often required; accuracy greater than 0.005 a_w is probably unnecessary for most food-related applications. A collaborative study between different laboratories (Labuza *et al.*, 1976) showed that on the basis of most available a_w measurement units, a_w values should be rounded to 0.01 a_w. Examination of Figs 2–6 shows that in spite of the very large tempera-

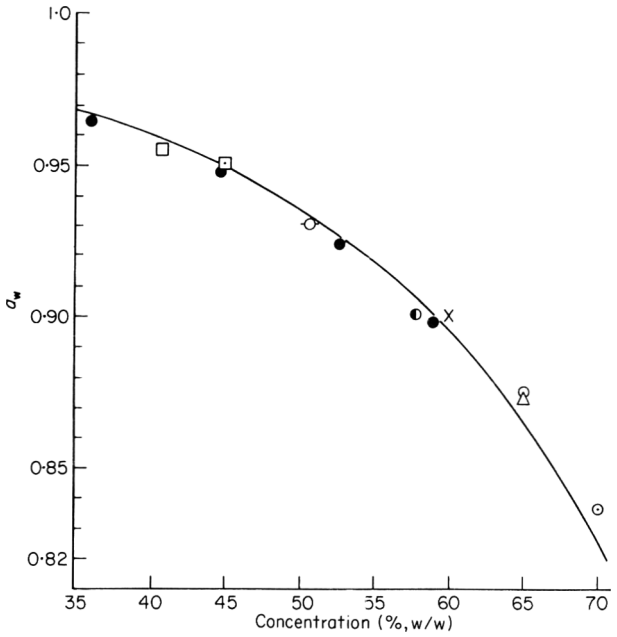

Figure 2. Comparison of a_w values calculated from freezing point depression with values measured at other temperatures, for sucrose solutions. ● (0°C), — (25°C), Robinson & Stokes (1965); ◐ (−10.8°C, FP), ⊖ (−7.5°C, FP), □ (−4.6°C, FP), *International Critical Tables* (1926) ⊙,○, × (60°C), ⊡ (75°C), △ (80°C), Dunning *et al.* (1951).

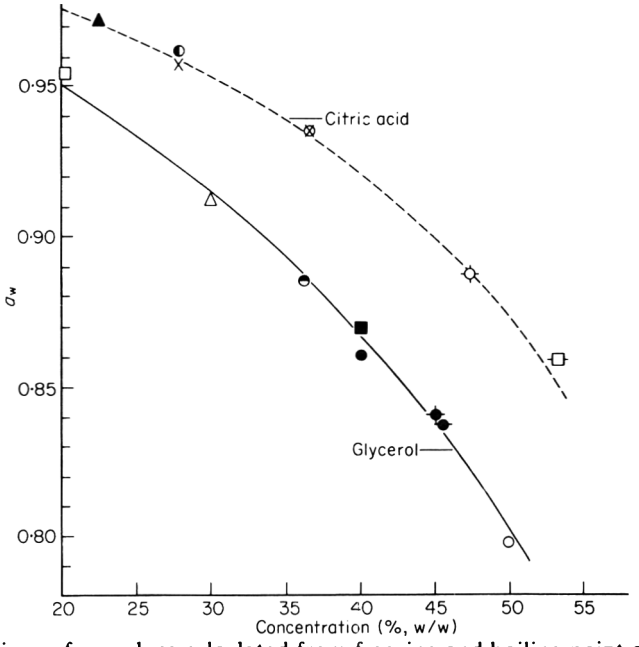

Figure 3. Comparison of a_w values calculated from freezing and boiling point data with values measured at other temperatures for glycerol and citric acid solutions. --- (25°C), Chirife & Ferro Fontán (1979); — (25°C), Teng & Lenzi (1974); ○ (−23°C FP), ▲ (−2.94°C FP), ◐ (−4°C FP), ○ (−23°C FP), ● (−15.4°C), △ (−9.5°C FP), □ (−4.8°C FP), ⊹ (−12.22°C FP), × (101.21°C BP), ⊗ (101.91°C BP), ⊡ (104.4°C BP), ◓ (103.5°C BP), ■ (104.03°C BP), ⊕ (105.12°C BP), *International Critial Tables* (1926); ✦ (70°C), Newman (1968).

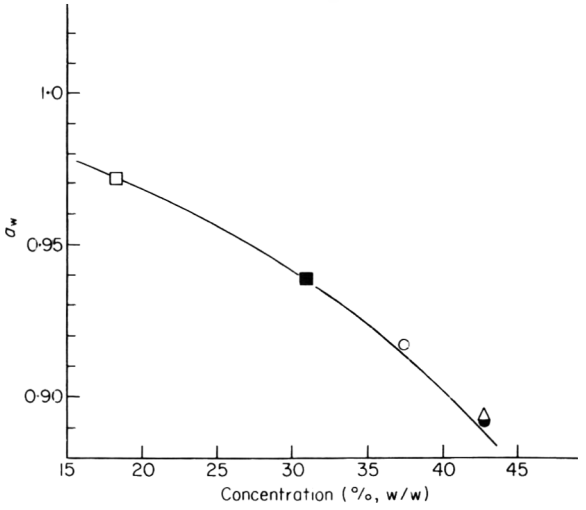

Figure 4. Comparison of q_w values calculated from freezing and boiling point data with values measured at 25°C for d-tartaric acid solutions. — (25°C), Teng & Lenzi (1974); ● (−11.75°C FP), ○ (−8.95°C FP), □ (−3.02°C FP), ■ (−6.42°C FP, 101.83°C BP), △ (103.26°C BP), *International Critical Tables* (1926).

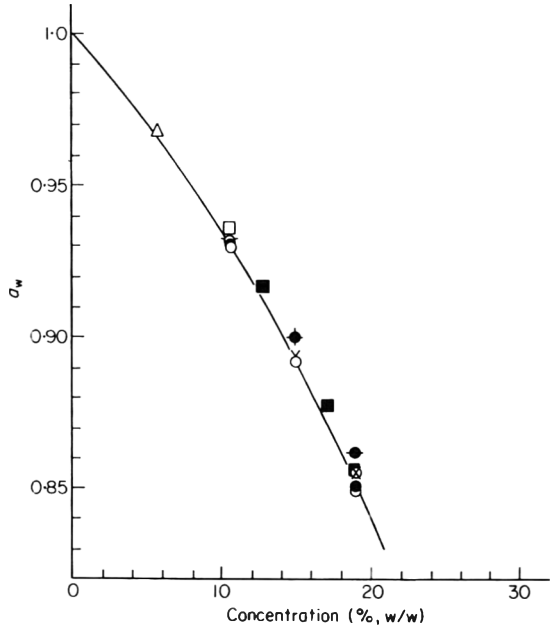

Figure 5. Comparison of a_w values calcuated from freezing and boiling point data with values measured at other temperatures, for sodium chloride solutions —, 25°C, Robinson & Stokes (1965); ⊕ (−10.81°C FP), ● (−15.14°C FP), □ (−6.90°C FP), △ (−3.37°C FP), ○ (60°C), ● (80°C) ⊹ (102.04°C BP), × (103.23°BP), ⊗ (104.5°C BP), *International Critical Tables* (1926); ■ (0°C) Platford (1973)

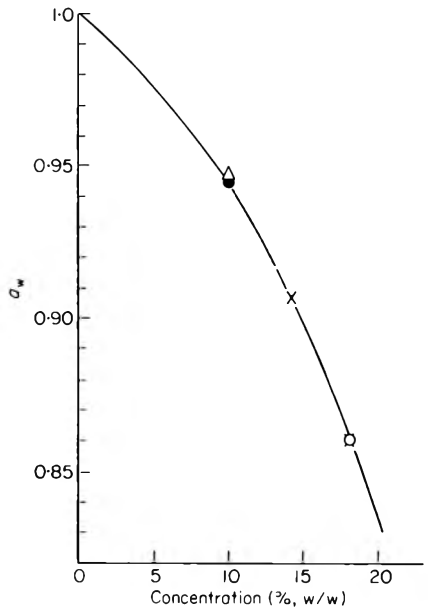

Figure 6. Comparison of a_w values calculated from freezing and boiling point data, with values measured at other temperatures, for calcium chloride solutions — (25°C), Pitzer & Mayorga (1973); ● (−5.85°C), ○(−15.36°C), △ (101.55°C), *International Critical Tables* (1926); × (0°C), Platford (1973).

ture differences involved, the a_w values calculated from freezing or even boiling measurements are not very different from values measured at 25°C. In fact, in the approximate range of a_w, 0.96–0.85, the differences are not greater than about 0.01 a_w units, which as mentioned before, may be considered as an acceptable level of accuracy in most food-related applications.

Shapero *et al.* (1978) studied the effect of ethanol on *Staphylococcus aureus* inhibition at limited a_w. They noted that as ethanol is volatile it would interfere in any present method of a_w measurement and consequently calculated its a_w lowering effect using Raoult's law. Figure 7 compares the a_w lowering behaviour of ethanol calculated from Raoult's law and from freezing point data in the literature. Figure 8 compares the a_w of ethylene glycol solutions calculated from freezing point data with corresponding values at 25°C. The a_w values at 25°C were theoretically predicted using Norrish's (1966) equation as shown by Chirife *et al.* (1979) and assuming a value of $K = 1$ for the correlating constant in Norrish's equation.

Although the observed variations of a_w with temperature are relatively small, they may perhaps be relevant for some accurate work. In this case the correction from the freezing point (T_F) to some other temperature (e.g. 25°C, T_R, is given by

$$\ln \frac{a_w(T_R)}{a_w(T_F)} = -\bar{L}_A(T_R)\left(\frac{T_R - T_F}{R\,T_R\,T_F}\right) + J_A\left[\frac{T_R}{R}\frac{T_R - T_F}{T_R\,T_F} - \frac{l}{R}\ln\frac{T_R}{T_F}\right]$$

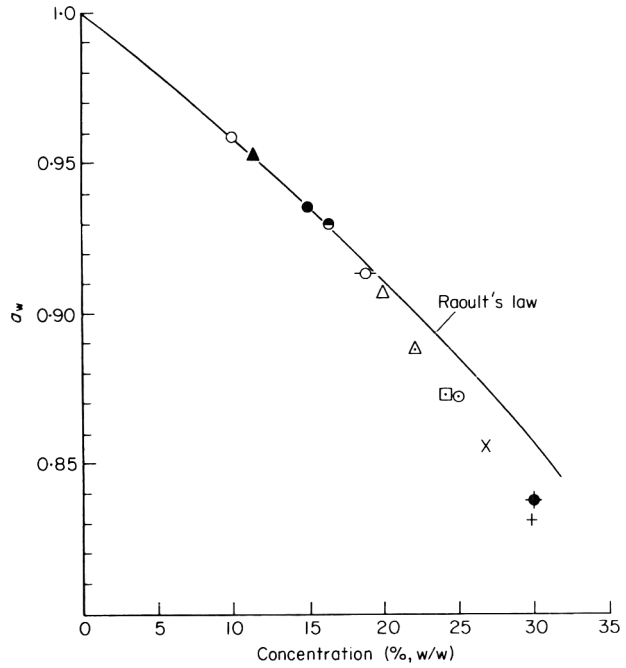

Figure 7. The a_w of aqueons ethanol solutions calculated from freezing point depression. ✦ (−18.1°C FP), ● (−6.9°C FP), △ (−10°C FP), ⊙ (−14°C FP), ○ (−4.3°C FP), Perry (1963); ▲ (−5°C FP), ◓ (−7.5°C FP),-○-(−9.4°C FP), ◬ (−12.2°C FP), × (−16°C FP), + (−18.9°C FP), ⊡(−14°C FP), Hodgman (1944).

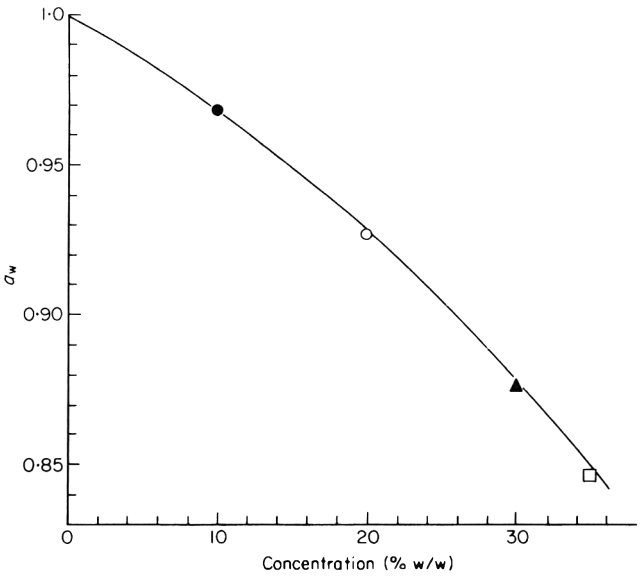

Figure 8. The a_w of aqueous ethylene glycol solutions calculated from freezing point depression as compared with predicted data at 25°C. —, predicted at 25°C; ● (−3.3°C FP), ○ (−7.8°C FP), ▲ (−13.5°C FP), □ (−17.1°C FP), Perry (1963).

where $\bar{L}_A$ is the relative partial molal heat content at T_R- and $\bar{J}_A$ is the relative partial molal heat capacity of water. Equation (7) may be written (Robinson & Stokes, 1965)

$$\log \frac{a_w(T_R)}{a_w(T_F)} = -\bar{L}_A(T_R)Y + \bar{J}_A Z \tag{8}$$

where

$$Y = \frac{T_R - T_F}{2.303\, R\, T_R T_F}, \quad Z = T_R Y - \frac{l}{R} \log \frac{T_R}{T_F}$$

In order to calculate $a_w(T_R)/ a_w(T_F)$ it is necessary to evaluate the relative partial molal heat content and heat capacity which is not easy because these properties are not only temperature but also concentration dependent, and its evaluation requires tedious calculations. In addition, the necessary data at different temperatures and concentrations are not always available for a given solute.

The relatively small variations of a_w with temperature observed in this work may be explained with the aid of equation (8): the relative molal properties are not big enough to overcome the small magnitude of Y and Z (in the temperature range of interest to this study, e.g. -4 to -17°C) leading to variations in a_w greater than 0.01 a_w units.

Conclusions

It has been shown that, in the approximate range of a_w 0.96–0.85, the a_w of solutions calculated from freezing point depression does not differ very much from the corresponding value at 25°C. It appears that it is safe to say that for solutes of present or potential interest for a_w control in foods, the a_w calculated from freezing point depression is not likely to differ in more than 0.01 a_w units of its value at 25°C.

The determination of a_w of aqueous solutions through freezing point measurement may be valuable for some situations in which a_w can not be measured using current techniques. This includes solutions containing highly volatile substances, like ethanol, or relatively volatile ones, like some glycols. For instance, electric hygrometers, which are widely used for a_w measurement in the food area, can not be used for systems containing volatile glycols like propylene glycol, or other highly polar substances, because they may contaminate the sensor leading to erroneous results (Sloan & Labuza, 1975; Troller, 1977).

Acknowledgments

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

References

Ayerst, G. (1965) *Int. Biodeter. Bull.* **1,** 13.
Benmergui, E.A., Ferro Fontán, C. & Chirife, J. (1979) *J. Fd Technol.* **14,** 625
Chirife, J., Ferro Fontán, C. & Benmergui, E.A. (1979) *J. Fd Technol.* **15,** 59.
Chirife, J. & Ferro Fontán, C. (1979) *J. Fd Sci.*, (in press).
Denbigh, K. (1964) *The Principles of Chemical Equilibrium,* Cambridge University Press, Cambridge.
Dunning, W.J., Evans, H.C. & Taylor, M. (1951) *J. Am. chem. Soc.* 2363
Hodgman, C.D. (1944)– *Handbook of Chemistry and Physics.* Chem. Rubber Pub. Co., Cleveland, Ohio.
International Critical Tables (1926) McGraw Hill, New York.
Labuza, T.P., Tannenbaum, S.R. & Karel, M. (1970) *Fd Technol., Champaigne,* **24,** 543.
Labuza, T.P., Acott, K., Tatini, S.R., Lee, R.Y., Flink, J.M. & McCall, W. (1976) *J. Fd Sci.* **41,** 910.
Newman, A.A. (1968) *Glycerol* P. 41. *C.R.C. Press, Cleveland.*
Norrish, R.S. (1966) *J. Fd Technol.* **1,** 25.
Perry, J.H. (1963) *Chemical Engineers's Handbook,* 3rd ed. McGraw Hill Book Co., New York.
Pitzer, K.S. & Mayorga, G. (1973) *J. phys. Chem. Wash.* **77,** 2303.
Platford, R.F. (1973) *J. Chem.* Engng. Data, **18,** 215.
Robinson, R.A. Stokes, R.M. (1965) *Electrolyte Solutions,* 2nd edn, revised Butterworths, London.
Scott, W.J. (1957) *Adv. Fd Res.* **7,** 83.
Shapero, M., Nelson, D.A. & Labuza, T.P. (1978) *J. Fd Sci.* **43,** 1467.
Sloan, A.E. & Labuza, T.P. (1975) *Fd Prod. Dev.* **9,** 75.
Strong, D.H., Foster, E. & G. Duncan, (1970) *Appl. Microbiol.,* **19,** 980.
Teng, T.T. & Lenzi, F. (1974) *Can. J. chem. Engng.* **52,** 387.
Troller, J. (1977) *J. Fd Sci.* **42,** 86.
Troller, J. & Christian, J.H.B. (1978) *Water Activity and Food.* Academic Press, New York.
Wodzinski, R.J. & Frazier, W.C. (1960) *J. Bacteriol.* **79,** 572.

(Received 29 May 1980)

J. Fd Technol. (1981) **16**, 31–38

Water activity and salt content relationship in moist salted fish products

H. M. LUPÍN, R. L. BOERI, AND S. M. MOSCHIAR

Summary

A linear relationship between water activity (a_w) in moist salted fish products and NaCl molality was found: $(a_w)_{SF} = 1.002–0.042\ m$; where m is the NaCl molality considering it to be in true solution in the total water content of the product.

The linear approximation was analyzed with reference to the a_w values of pure NaCl solutions and to a thermodynamic approach for a_w prediction; it was found consistent and appropriate for a_w estimation in moist salted fish products, with errors within the accepted range for intermediate moisture foods (IMF).

Introduction

Moist salted fish is one of the oldest intermediate moisture foods (IMF) developed by man and moist salted-fish products are available in the markets of the world. In European countries this type of product is produced using herring (*Clupea harengus*), anchovy (*Engraulis encrasicholus*) and sprat (*Clupea sprattus*); in South America, anchovy (*Engraulis anchoita*) is used in Argentina and Uruguay and sardine (*Sardinella aurita*) in Brazil and other countries. Moist salted-fish products are also produced in Africa and Asian countries.

As is known, salt produces various effects which contribute to the preservation of food (Ingram & Kitchell, 1967). The most important of them and one that is easily quantified and related to spoilage is the decrease in water activity (a_w).

Taking into account that a_w in moist salted-fish products remains between 1 and around 0.75, and because of the known difficulties in measuring a_w in its higher range (just below 1 down to 0.95–0.80) (Vos & Labuza, 1974) it is not

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0022–1163/81/0200–0031 $02.00

surprising that there have been comparatively few experimental data published on such products.

For this reason it was thought to be of interest to analyze the a_w values that appear in the literature on moist salted-fish products and to relate them to the NaCl concentration.

Results and discussion

The data found in literature which were considered in this work are: forty-one experimental results on salted cod (*Gadus morhua*) in the interval corresponding to moist salting reported by Fernandez Salguero Carretero (1973); thirty-three experimental measurements on salted anchovy (*Engraulis encrasicholus*) reported by Baldratti *et al.* (1977) and twenty-eight experimental results on salted mullet (*Mugil cephalus*) roe reported by Hsu, Deng & Cornell (1980) twenty determinations) and Hsu & Deng (1980) (eight determinations).

A satisfactory linear correlation between a_w and molality of salt in the product was found. Molality was determined considering NaCl to be in true solution in the total water content of the product.

Experimental data and the straight line found by the least squares method are plotted in Fig. 1. Confidence limits for 95% probability are also shown (Himmelblau, 1970). The line corresponds to the equation.

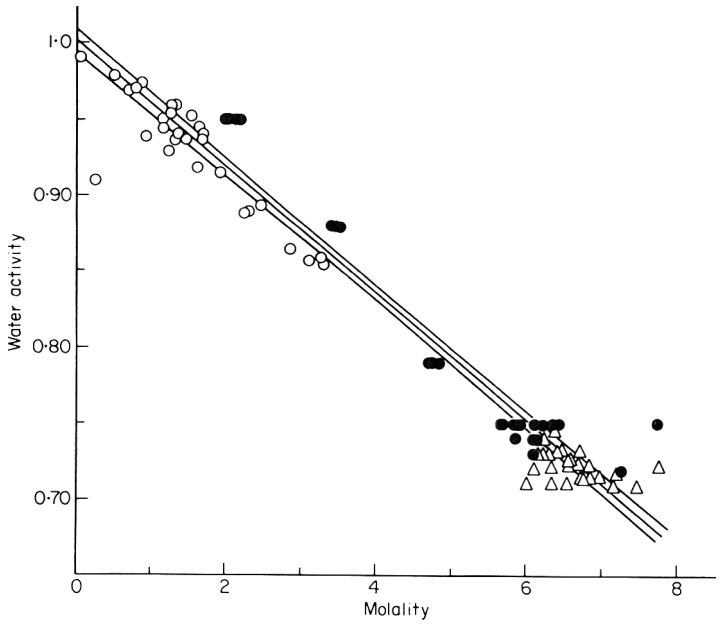

Figure 1. Water activity *vs* NaCl molality in fish products: (●) Salguero Carretero (1973);(○) Hsu, Deng & Cornell (1980), and Hsu & Deng (1980); (△) Baldratti *et al.* (1977)

$$(a_w)_{sf} = a_w^{i0} - b\,m = 1.002 - 0.042\,m \quad (1)$$

where:

$(a_w)_{sf}$: water activity of salted fish product
m : molality of solute.
a_w^{i0} : water activity at zero molality.
b : straight line slope.

Although the values given here are rounded to the third decimal figure, only the second decimal figure is significant in practical calculations as was pointed out by Labuza *et al.* (1976).

The relation between a_w and concentration of NaCl in pure solutions over the same range has also been studied for comparison.

Values at 25°C (Robinson & Stokes, 1968) and at 100°C (Weast & Astle, 1979) were found to adjust equally well to a straight line analogous to the one given by equation (1). The parameters for those lines are presented in Table 1.

Although 100°C is a temperature with no practical importance in moist salted fish it is useful to point out the linear behaviour over a wide range of temperatures and that the slope does not vary with temperature in a significant way.

Norrish (1966) reported that the influence of temperature on a_w is small and in the case of saturated solutions of NaCl Wexler & Hasegawa (1954) found changes of less than 1.5% in a_w over the 0–50°C range.

From the comparison of equation (1) and Table 1 it is evident that, in practice.

$$(a_w)_{sf} = (a_w)_{NaCl} \quad (2)$$

The estimation of a_w by means of a straight line over certain portions of the absorption of desorption isotherms in foods has been observed by different authors. In a review of equations for fitting absorption isotherms of foods Chirife & Iglesias (1978) have pointed out the differences that sometimes appear in the literature with regards to the application of this model.

The multilineal empirical model for fitting experimental data of a_w was also used by Teng & Lenzi (1974) for various non-electrolyte binary systems.

Table 1. Values of the parameters of the straight line correlations rounded to the third decimal figure.

System	T (°C)	a_w^0	b	r	n
Salted fish	2–38	1.002±0.009	0.042±0.002	0.978	102
NaCl – H_2O	25	1.007±0.003	0.040±0.001	0.998	35
NaCl – H_2O	100	1.008±0.007	0.038±0.002	0.999	7

However, the linear relationship can be based on the following known thermodynamical relation, valid for binary solutions:

$$a_w = \exp(-\psi\, m_1\, m\, \nu) \tag{3}$$

where:

ψ : osmotic coefficient.
m_1 : number of Kg per mol of water (0.018).
m : molality of solute.
ν : number of moles of all species which give 1 mol of solute in solution (ν = 2 for NaCl).

If expression (3) is expanded in series

$$a_w = 1 - (\psi\, m_1\, \nu)\, m + \frac{(\psi\, m_1\, \nu)^2\, m^2}{2\,!} - \frac{(\psi\, m_1\, \nu)^3\, m^3}{3\,!} + \ldots \tag{4}$$

and thus, within a certain range i.e., if $(\psi\, m_1\, \nu) \ll 1$, the following holds true:

$$a_w \cong 1 - (\psi\, m_1\, \nu)\, m \tag{5}$$

The case of salted fish conditions, for which equation (5) is a satisfactory approximation, can be analyzed.

The osmotic coefficient varies with molality, however, the changes it undergoes must be either small or influence linearity of equation (3) in such a way that it may be considered approximately constant between 0 molality and saturation.

The values of ψ can actually be calculated to apply them in the estimation of a_w in foods from the theoretical expressions given by Pitzer (1973) and Bromley (1973), as has been studied recently by Benmergui, Ferro Fontan & Chirife (1979) and Ferro Fontan, Chirife & Benmergui (1979). They can also be obtained from published experimental values.

In the present paper the experimental values reported by Robinson & Stokes (1968) were used to estimate the limits for the linear approximation and the mean osmotic coefficient ($\bar{\psi}_{R\&S}$) for the entire solubility range.

The optimal mean value, found by the least squares method is:

$$\bar{\psi}_{(R\,\&\,S)} = 1.111 \pm 0.028$$

This value can be compared to the apparent osmotic coefficient corresponding to salted fish, found as

$$\bar{\psi}_{sf} = \frac{k}{m_1 \nu} \tag{6}$$

When calculated from expression (1) $\bar{\psi}_{sf}$ is is found to be:

$$\bar{\psi}_{sf} = 1.167 \pm 0.056$$

The values $\bar{\psi}_{R\&S}$ and $\bar{\psi}_{sf}$ can be taken as equivalent because of the experimental errors involved.

The absolute values of the differences between experimental values of a_w for pure solutions of NaCl reported by Robinson & Stokes (1968) and the linear approximation for salted fish, equation (1), and the straight line for pure solutions of NaCl at 25°C (Table 1) can also be determined.

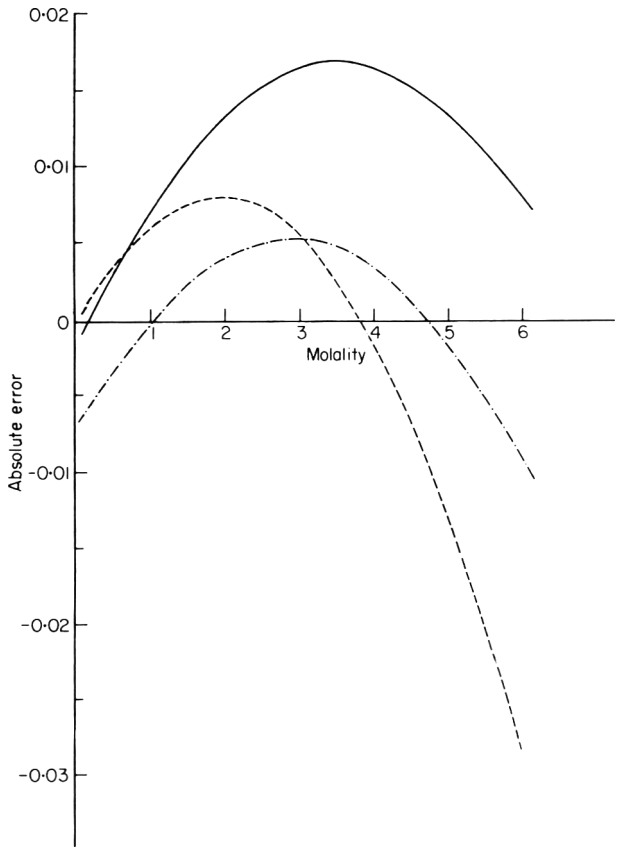

Figure 2. Absolute error in a_w estimation versus NaCl molality for: straight line approximation for salted fish data (——), straight line approximation for pure NaCl solutions (·—·—·), exponential approximation with constant osmotic coefficient (---); refered to experimental values of NaCl pure solutions (Robinson & Stokes, 1968).

The differences existing with the approximation

$$a_w = \exp(-\bar{\psi}_{R\&S}\, m_1\, m\, v) \tag{7}$$

have also been calculated and are plotted in Fig. 2.

According to Ferro Fontan, Benmergui & Chirife (1980), it is desirable and sufficient to predict a_w within about + 0.01 a_w for an IMF. In Fig. 2 it is possible to see that for pure solutions of NaCl the straight line approximation will predict values within this range. For salted fish products the straight line prediction will show absolute differences less than 0.02 with reference to the experimental values of a_w in pure NaCl solutions; a slight influence of the medium must not be disregarded.

It is interesting to point out that the deviation from 'ideality' is always in the same direction; this means that the straight line prediction for salted-fish products will give values slightly lower than those predicted for pure NaCl solutions.

In practice experimental values of a_w measured on salted fish products can deviate significantly from predicted ones, independently of the model that has been utilized. In such a situation the straight line approximation may be useful for a quick assesment if new measurements are necessary.

However, approximation (7) results in greater differences especially at high concentrations, this being the region of practical importance.

It is obvious that the absolute differences with the estimation will always be within the range of experimental errors commonly accepted for the determination of a_w because they will only affect the second decimal by a unit or less.

The apparently contradictory result that a larger error is obtained when using approximation (7) than with the linear approximation can be easily explained if the fact that a slight increase in ψ with molality will tend to straighten expression (3) over a range wider than the one corresponding to the simple linearization is taken into account.

There is no totally satisfactory explanation to justify the experimental points in which the molality is larger than that corresponding to saturation and a_w is less than what could be expected for saturation.

Most of these points correspond to moist salted anchovy heavily pressed; thus, on a same quantity of muscle base, the brine is less than that corresponding to fish either lightly pressed or not pressed at all. Under these conditions it may be accepted that the medium exerts some influence on the a_w values; and disregarding the possibility that solid NaCl has been included in the samples that were analyzed that would equally have influence on the amount of salt in 'solution' in the muscle. Pressing would have in practice a drying effect on fish.

Experiments carried out in our laboratory to answer industry's demands to find the causes of spoilage in salted anchovy and salted and cold-smoked patagonian haddock have always resulted in a substantial decrease in the content of NaCl with reference to the normal values for saturation and, therefore, an increase in the water activity. The low values for NaCl content can be

the result of several factors: not enough salting (insufficient amount of salt or too short salting time); excesive washing of salted fillets (to eliminate crystals of solid salt); skinning of the fillets in hot brine, etc.

There will exist the possibility for a quick estimate of the a_w value by determining either water content or NaCl. As determined for cod by Crean (1961) and extended for other species by Zugarramurdi & Lupín (1980), there is a linear relationship between the water and NaCl contents in moist salted fish. Using this information together with expression (1), a_w can be estimated.

Conclusions

An expression to estimate a_w in moist salted fish is presented. This expression can be adequately related to published data on moist salted fish products and to a current thermodynamic approach to estimate water activities in aqueous solutions.

In practice this relationship may be useful to estimate a_w in moist salted fish when specific equipment is not available or to check individual measurements of a_w.

Acknowledgments

The work was supported in part by the Consejo Nacional de Investigaciones Científicas y Técnicas of Argentina and the Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (Argentina).

References

Baldrati, G., Guidi, G., Pirazzoli, P. e Porretta, A. (1977) *Industria Conserve. Parma,* **52,** 221.
Benmergui, E.A.; Ferro Fontan, C. and Chirife, J. (1979) *J. Fd Technol.* **14,** 625.
Bromley, L.A. (1973) *AICHE Journal,* **19,** 313.
Chirife, J. & Iglesias, H.A. (1978) *J. Fd Technol.* **13,** 159.
Crean, P.B. (1961) *J. Fish. Res. Bd. Canada,* **18,** 833.
Fernandez Salguero Carretero, J. (1973) *Archos de zootecnia,* **22,** 241.
Ferro Fontan, C.; Benmergui, E.A. & Chirife, J. (1980) *J. Fd Technol.* **15,** 47.
Ferro Fontan, C.; Chirife, J. & Benmergui, E.A. (1979) *J. Fd Technol.* **14,** 639.
Himmelblau, D.M. (1970) *Process Analysis by Statistical Methods.* J. Willey and Sons, N.Y.
Hsu, W.H. & Deng, J.C. (1980). *J. Fd Sci.* **45,** 97.
Hsu, W.H., Deng, J.C. & Cornell, J.A. (1980) *J. Fd Sci.* **45,** 102.
Ingram, M. & Kitchell, A.G. (1967) *J. Fd Technol.* **2,** 1.
Labuza, T.P. Acott, K., Patini, S.R., Lee, R.Y., Flink, J. & Mc Call, W. (1976) *J. Fd Sci.* **41,** 910.
Norrish, R.S. (1966) *J. Fd Technol.* **1,** 25.
Pitzer, K.S. (1973) *J. of Physical. Chem.* **77,** 268.
Robinson, R.A. & Stokes, R.H. (1968) *Electrolyte Solutions,* 2nd. Edn. Butterworths, London.

Teng, T.T. & Lenzi, F. (1974) *J. Chem. Eng.* **52,** 387.
Vos, P.T. and Labuza, T.P. (1974) *J. Agr. Food Chem.*, **22,** 326.
Weast, R.C. & Astle, M.J. (1979) *CRC Handbook of Chemistry and Physics*. 60th Ed. CRC Press, Inc. U.S.A.
Wexler, A. & Hasegawa, S. (1954) *J. Res. natn. Bur. Stand.* **53,** (1).
Zugarramurdi, A. & Lupín H.M. (1980) *J. Fd. Sci.* **45,** 1305.

(Received 20 June 1980)

J. Fd Technol. (1981) **16**, 39–50

Modification of tempeh with the addition of bakla (*Vicia faba* Linn)

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Summary

Bakla (*Vicia faba* Linn), an indigenous pulse, was subjected to fermentation by the strains of *Rhizopus oligosporus* either alone or blended with soybean (*Glycine max*). Mycelial growth as viewed on the surface of the fermented mass was best obtained when strain NRRL 3271 was used. Increase in moisture content and pH during fermentation was highest in the case of strain NRRL 2710 irrespective of composition of bakla-soybean mixture. The tempeh, the fermented product, in each case had a mushroom-like odour, which was independent of the strain used. Bakla and bakla-soybean (up to 1:1 ratio) tempeh was free of beany flavour but this flavour increased as the soybean content of the blend was further raised and was perceptibly high when soybean content reached 75%. Bakla tempeh was more crisp than soybean tempeh. The crispness decreased with increasing soybean content.

Introduction

Wide-spread protein deficiency and rampant shortage of edible oils have been responsible for the introduction of high yielding varieties of soybean in India. However, its beany flavour has not permitted soybean to play a vital role in our diet, although the protein in it is better balanced than in most other legumes in respect of amino acid content. Whereas its Western style food products like soybean 'meat' etc. are beyond the reach of the common man in India, oriental foods based on it may hold some promise.

Several mould fermentation products from soybean are known in the East. One such product is tempeh, a common food in Indonesia. According to

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0022–1163/81/0200–0039 $02.00

Boedijn (1958) the principal species used in Indonesia for tempeh production is *Rhizopus oligosporus*. Hesseltine (1965) reported twenty-five strains of *R. oligosporus* Saito, four of *R. stolonifer* (Ehren) Vuill, three of *R. arrhizus* Fischer, three of *R. oryzae* Went and Geerligs, three of *R. formosaensis* Nakazawa, two of *R. achlamydosporus* Takeda – in all forty strains, which produce acceptable tempeh when soybean is fermented with their pure cultures.

It was considered that tempeh might be more liked by inhabitants of the Indian sub-continent if its flavour were further modified. With this in view, we have studied the effect of bakla (*Vicia faba* Linn) on the properties of tempeh produced by fermenting soybean (*Glycine max*) blended with it. Bakla, unlike soybean, does not possess any beany flavour and has a high protein content in comparison to other pulses but it is not easily digestible and is, therefore, not as popular as dal (pulse soup). Dal, one of the necessary dishes in the Indian diet, is a soup-like preparation made from cotyledons of a pulse.

Materials and methods

The three mould strains used for the preparation of tempeh or tempeh-like products were *Rhizopus oligosporus* NRRL 514, NRRL 2710 and NRRL 3271, kindly supplied by Dr C.W. Hesseltine of the Northern Regional Research Laboratory of Northern Utilization Research and Development Division, Peoria, Illinois, U.S.A. The cultures were maintained on potato dextrose agar medium. The inoculated slants were incubated at 33±1°C for 5 days to obtain profuse sporulation and later stored at 4±2°C in a refrigerator. Monthly transfers were made so as to keep the cultures viable. Soybean variety Bragg and locally available bakla seeds were used throughout this investigation. The tempeh and similar products were prepared by the method described by Djien & Hesseltine (1961) with certain modifications. Whereas the above authors soaked the soybean seeds in water over-night, we soaked soybean and bakla grains separately in water for 1 hr at room temperature (33±1°C). The beans were sun-dried and dehulled in a hand grinder. This also resulted in separation of the cotyledons. This is a common method for dehulling the pulses in India. The cotyledons were separately washed in tap water to remove dirt and adhering seed coats from the beans. The washed cotyledons of the two beans, either separately or in appropriate proportions, were placed in three times their dry weight of tap water. Glacial acetic acid was added at the rate of 7.5 ml/1000 g dry weight of beans. The addition of acid is also a departure from the method of Djien & Hesseltine (1961). This was found necessary to prevent the development of spoilage bacteria during mould fermentation (Steinkraus *et al.*, 1960). Although Hesseltine and colleagues (Djien & Hesseltine, 1961; Hesseltine *et al.*, 1963; Martinelli & Hesseltine, 1964) did not encounter bacterial growth during mould fermentation in the absence of acid, in our preliminary experiments we could not get tempeh in its absence, but a product with a putrid

odour accompanied by a ropiness in flavour. Soybean cotyledons in acidified water were autoclaved at 15 lb pressure (121°C) for 4–5 min. Cooking of bakla cotyledons for more than 5 min resulted in bursting which made them useless for tempeh production. Cooked beans were washed twice with tap water and collected in a muslin cloth and placed over a wire bottomed tray to drain out water and cooled for 15–20 min. To inoculate with mould, a slant with profuse sporulation was taken and 5 ml sterile water was added to it and the spores suspended. Two ml of this suspension were used to inoculate autoclaved cotyledons whose original dry weight was 100 g. Inoculated dry beans were packed tightly in sterile petri dishes (15 × 100 mm) and covered with the lids. Each petri dish held about 40 g dry weight of beans. The filled petri dishes were incubated at 33±1°C in an incubator containing a dish of water in its lower compartment. Incubation was allowed to continue for 19–22 hr. The temperature of incubation was 2°C higher than that used by Djien & Hesseltine (1961). This resulted in a saving of 2 hr on the incubation time that was allowed by them.

Tempeh was also prepared in plastic bags using the method as described by Martinelli & Hesseltine (1964). Thin polythene bags of 500 g capacity (18 × 21 cm) were taken and perforations were made at 1 cm distance on both sides with the help of a sewing needle (diam. 0.6 mm). About 200 g of inoculated beans were filled into the bags which were then sealed. The maximum thickness of bean layer, which was uniform as far as possible, was not more than 2.5 cm in the bags. The incubation temperature and period were kept as above.

The following method was used to determine the pH level of the fermenting beans at different stages.

Fermenting beans (2 g) at regular intervals were removed from the petri dishes or bags and mashed in 4 ml of sterile, distilled water and centrifuged. The pH of the supernatant as measured by a Toshniwal pH meter was considered the pH of the fermenting beans. Moisture content of the cotyledons and tempeh was determined according to the AOCS Official Method Ac 2–41 (1964). An accurately weighed 10-g sample was taken in a previously heated and weighed petri dish and heated in the oven at 130±2°C for 3 hr, after which the sample was cooled in a desiccator and weighed. The loss in weight × 10 was considered as moisture percentage.

The oil content of the dehulled cotyledons of bakla and soybean was determined by AOCS official method Ac 3–44. An accurately weighed, previously dried, 2-g powdered soybean sample (60 mesh) or 5-g bakla sample was taken in a filter paper thimble (Whatman No.2) and placed in the extraction tube of Soxhlet apparatus and 150 ml of petroleum ether (BP 60–80 C) was taken in a previously weighed soxhlet flask and the soxhlet assembly was fitted. The flask was heated in such a way that the solvent dropped from the condenser at the rate of 150 drops per minute. The extraction was continued for 8 hr after which time the solvent from the flask was evaporated, cooled and weighed. The heating and cooling continued till constant weight was obtained. Gain in flask weight gave the weight of oil in the sample.

Crude protein content in the dehulled seed cotyledons was determined by AOCS official method Ac 4–41. The weighed sample (1.7032 g) was digested in a Kjeldahl flask with recommended quantities of mercuric oxide, sodium sulphate and concentrated H_2SO_4. The clear digested mixture was cooled, diluted with water, a few granules of Zn added along with sodium thiosulphate followed by the addition of in excess of 50% NaOH solution and heated to distil out the ammonia. The distillate (150 ml) was collected in a known volume of 0.5 N H_2SO_4, containing a drop of methyl red. Excess H_2SO_4 was titrated against 0.25 N NaOH. A blank with sucrose was also run along with it. Crude protein was calculated as below:

Protein percentage = Percentage $NH_3 \times 5.14$

For extraction of oligosaccharides from bakla, a finely ground, dehulled 1-g pulse sample was shaken in a water bath shaker at 60°C with 15 ml of 70% alcohol for 15 min. After allowing it to stand for some time, the clear supernatant was collected. The above procedure was repeated 5 times with fresh alcohol samples, the supernatant pooled and 2 ml of 10% lead acetate solution was added to deproteinize it. After centrifugation, the clear liquid was flash evaporated at 50–55°C to reduce its volume to 1 ml.

The oligosaccharides from this extract were separated by a descending chromatographic method. An appropriate volume of the concentrated extract was loaded on Whatman no.1 filter paper strips (57 × 16 cm) side by side with 25 μl of a mixture of galactose, sucrose, melibiose, raffinose and stachyose (5 mg/ml of each) whose Rgal values were predetermined. The solvent system used was a mixture of Butanol, acetic acid and water in the ratio of 20:3:8 v/v. After running the chromatogram for 40 hr, it was dried and sprayed with aniline-diphenyl amine reagent which was prepared by mixing 10 ml each of 4% alcoholic solutions of aniline and diphenyl amine and 2.6 ml of orthophosphoric acid (Bailey & Bowne, 1960). The paper was then heated at 80°C for 5 min. Brown spots indicated the presence of individual oligosaccharides.

To analyse the lowest spot which could not be matched with any of the standard spots, a second filter paper strip (57 × 32 cm) was loaded at 3 spots with 25 μl of stachyose (0.5% solution), 50 μl and 200 μl of concentrated extract respectively whereas stachyose and 50 μl of the extract were loaded side by side on a round spot, 200 μl of the extract were loaded on 1 cm line. After developing as above, the paper was dried and the strip was longitudinally cut into halves, one containing stachyose and a small spot of the sugar extract and the other containing a large spot of the sugar extract. The first half was sprayed with aniline-diphenyl amine reagent and the spot once again identified after heating as before. On matching it with the unsprayed half, the area containing the sugar spot was identified and cut out from the strip. The sugar was eluted from it with distilled water and the resulting solution concentrated. The concentrated solution was acid hydrolyzed and chromatographed by ascending chromatography on Whatman no.1 filter paper using benzene, butanol, pyridine and water in 3:10:5:4 v/v ratio. The spots were detected as before. Galactose, glucose and fructose were used as standards.

Results

On fermentation of bakla (*Vicia faba* Linn) and soybean in the ratio of 1:0, 3:1, 1:1, 1:3, 0:1 with the three strains of *Rhizopus oligosporus*, tempeh was ready for consumption in about 19–22 hr depending on the strain and composition of the tempeh. The appearance of profuse and uniform mycelial growth on the surface of the bean mass in petri dishes or bags was considered as completion of tempeh fermentation. Table 1 indicates that the strain NRRL 2710 produced the tempeh from bakla soybean and their mixtures faster than strains NRRL 3271 and NRRL 514. It is also noteworthy in each case that pure bakla tempeh production required a longer period for completion than soybean or soybean-bakla tempehs.

Figures 1, 2 and 3 show the photographs of surface and transverse sections of different preparations obtained from petri dishes. As the photographs taken from bags were similar, they have not been shown here. A comparison of the petri dish photographs demonstrates that surface growth of strain NRRL 3271 on all samples appeared best, which was followed by that of strain NRRL 514 and NRRL 2710. Mycelial binding of bakla cotyledons by the strains NRRL 3271 also appeared best when compared with others which can be seen in the photograph of the slide in Fig. 1a.

A comparison of dish photographs in Fig. 1a, 2a and 3a with those in Fig. 1c, 2c and 3c shows that mycelial growth in pure bakla tempehs in each case was more than that in soybean preparations. Increased incubation of soybean preparations did not result in improved growth. Mycelial bindings in soybean-bakla mixture was also satisfactory in each case, however, the strain effect was not clearly discernible. It was observed that the tempeh produced by mould strains NRRL 3271 and NRRL 2710 started blackening due to the commencement of sporulation after 26 hr, whereas the tempeh prepared by using the strain NRRL 514 remained white for a longer period and the sporulation started after 36 hr. The sporulation started at the rim and prick points in the petri dishes and bags, respectively.

Moisture content

Table 2 gives the moisture content of unfermented and petri dish fermented samples containing bakla and soybeans in varying proportions. Unfermented

Table 1. Period required for production of tempeh at 33±1°C

S.No	Composition	NRRL 514 *	NRRL 2710 *	NRRL 3271 *
1.	Bakla 100%	22	21.0	21.5
2.	Bakla:soybean (3:1)	22	20.5	21.0
3.	Bakla:soybean (1:1)	21	20.0	21.0
4.	Bakla:soybean (1:3)	21	20.0	20.5
5.	Soybean 100%	20	19.0	20.0

*Period given in hr.

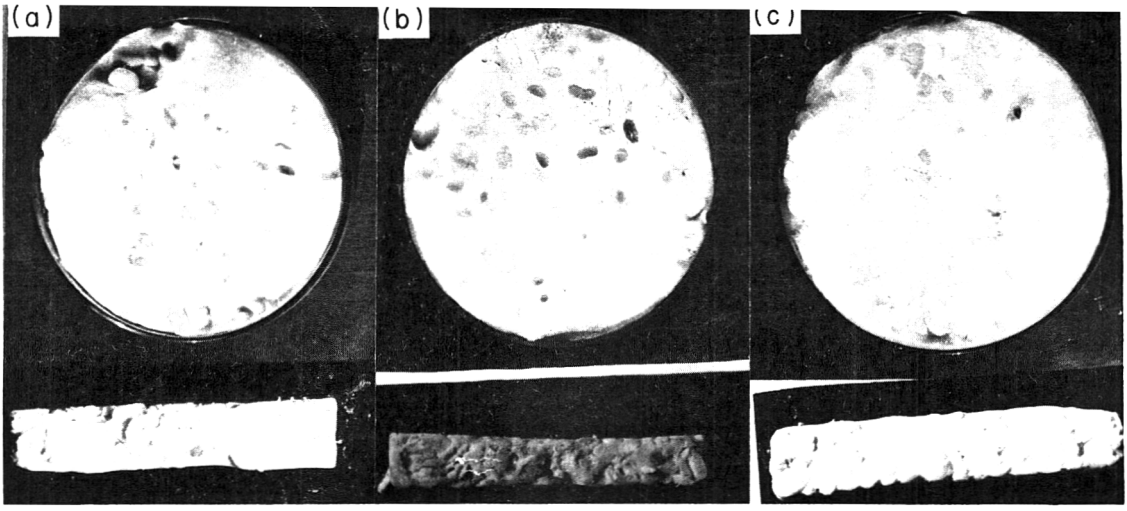

Figure 1. Tempeh produced by *Rhizopus oligosporus* NRRL 3271 (a) Bakla tempeh; (b) bakla:soybean mixture tempeh (1:1); (c) soybean tempeh.

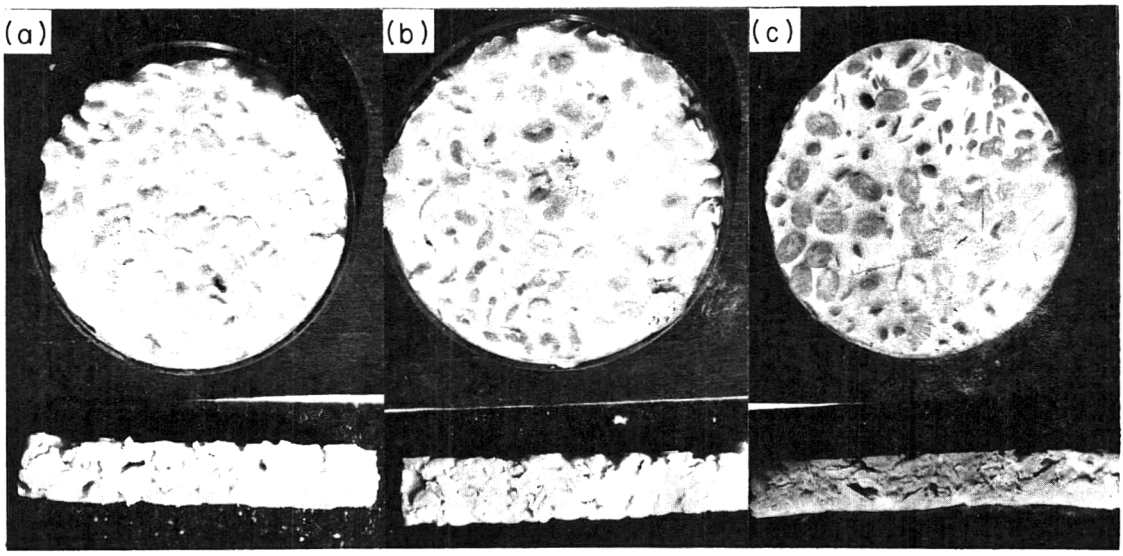

Figure 2. Tempeh produced by *Rhizopus oligosporus* NRRL 514 (a) Bakla tempeh; (b) bakla:soybean mixture tempeh (1:1); (c) soybean tempeh.

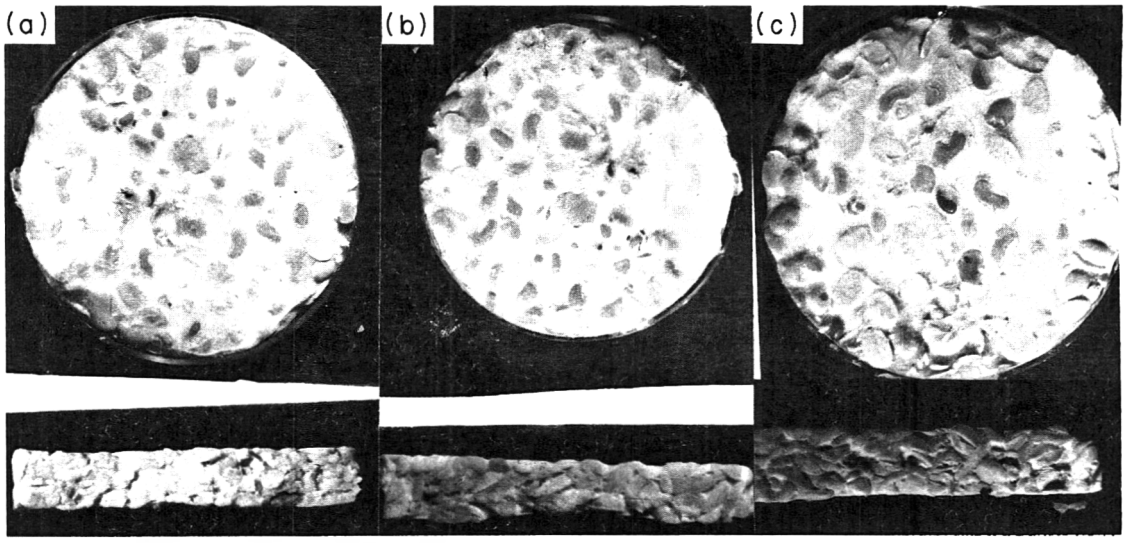

Figure 3. Tempeh produced by *Rhizopus oligosporus* NRRL 2710 (a) Bakla tempeh; (b) bakla:soybean mixture tempeh (1:1); (c) soybean tempeh.

Table 2. Moisture content of the unfermented and fermented bakla and/or soybean tempeh using different strains of *R. oligosporus*

Mixture composition	Moisture percentage in unfermented and fermented beans			
		Tempeh		
	Unfermented	NRRL 514	NRRL 2710	NRRL 3271
Bakla 100%	54.238	55.678	56.082	55.529
Bakla 75%–soybean 25%	55.496	58.045	58.721	57.917
Bakla 50%–soybean 50%	57.522	60.342	60.528	60.218
Bakla 25%–soybean 75%	60.690	63.477	62.094	63.274
Soybean 100%	63.509	64.124	64.764	64.081

and fermented samples of bakla showed lower water content than that of its mixtures with soybean. The moisture content increased with increasing soybean content in each case and was highest in unfermented and fermented soybean. Moisture content in fermented products was highest in the products where *R. oligosporus* NRRL 2710 was used followed by *R. oligosporus* NRRL strains 514 and 3271. The same trend was noticed in bag samples.

pH changes

The course of pH curve during fermentation was found to be the same in case of bakla, soybean and their mixtures for the same organism. However, the effect of fermenting organism on pH was noted. The pH increase at the end of fermentation was found to be highest when strain NRRL 2710 was used and lowest in case of strain NRRL 3271 as is evident from Fig. 4 for the bakla sample. In each case, the pH rise was slow up to 8 hrs, signifying the spore

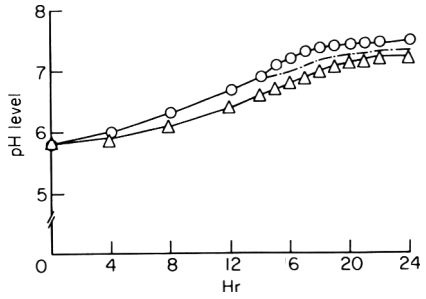

Figure 4. pH changes during the fermentation of bakla by *Rhizopus oligosporus* strains ○, NRRL 514, NRRL 2710, △, NRRL 3271.

Table 3. Oil and protein content of unfermented bakla and soybean cotyledons in 100 g dry weight

Observation no.	Cotyledon ratio (bakla:soybean)	Oil content (%)	Crude protein content (%)
1	1:0	4.68	28.90
2	3:1	8.57	32.12
3	1:1	13.25	35.27
4	1:3	17.50	38.47
5	0:1	20.56	41.36

germination and slow increase in biomass, then picked up due to vigorous mycelial development, and after 18 hr the curve became flat once again. The last stage signified that the organisms were preparing themselves for sporulation.

Oil and protein content of cotyledon mixtures

The oil and protein content of unfermented bakla and soybean cotyledons mixed in various proportions is given in Table 3. The oil content of bakla (4.68%) is much less than that of soybean (20.56%) and mixtures of them have intermediate values according to the ratio of the two legumes in the mixture. Similar is the case with the protein content of cotyledon mixtures which varied from 28.90% to 41.36% for 100% bakla to 100% soybean. The intermediate values are within the limits of experimental error.

Qualitative analysis of oligosaccharides present in bakla by paper chromatography indicated the presence of sucrose (O-α-D-glucopyranosyl-(1 → 2)-β-fructofuranoside); raffinose (O-α-D-galactopyranosyl-(1 → 6)-O-α∫ glucopyranosyl-(1 → 2)-β-D-fructofuranoside); stachyose (O-α-D-galactopyranosyl-(1 → 6)-O-α-D-galactopyranosyl-(1 → 6)-O-α-D-glucopyranosyl-(1 → 2)-β-D-fructosefuranoside and an unidentified sugar. The rate of movement of this oligosaccharide on the paper was lowest and its spot lying just below that of stachyose (Fig. 5). Since this sugar on hydrolysis gave galactose, glucose and fructose, it also belongs to the family of galactooligosaccharides (Fig. 6). This may be verbascose [(O-α-D-galactopyranosyl-(1 → 6)]$_3$ -O-α-D-glucopyranosyl-(1 → 2)-β-D-fructofuranoside which is fourth member of an homologous series of galactooligosaccharides, melibiose, raffinose, stachyose being the other members. As the pure verbascose was not available its confirmation could not be possible.

The dish or bag tempeh prepared from bakla and soybean in described proportions with either of the three strains could be cut into slices easily without breaking, indicating that binding was uniform and complete. The binding remained firm even after boiling for 3 min in 2% brine, sun drying and deep fat frying as with soybean tempeh which served as a control. The organism effect

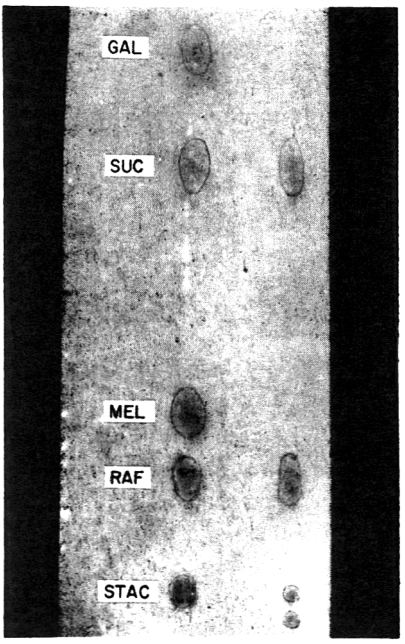

Figure 5. Paper chromatogram showing the presence of galactooligosaccharides in bakla (*Vicia faba*).

could not be noted in any of the above cases. The products became uniformly brown after frying.

Bakla tempeh as expected was free of beany flavour and was very crisp and tasty after frying. The crispness decreased with increasing proportion of soybean in tempeh. Similarly, bakla and bakla-soybean mixtures with up to 50% soybean had better palatability than pure soybean or 3:1 soybean bakla tempeh.

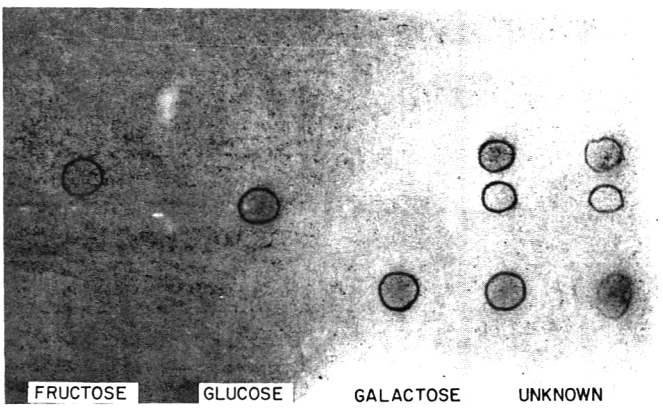

Figure 6. Paper chromatogram showing the production of monosaccharides on hydrolysis of unknown galactooligosaccharide.

Discussion and conclusions

It has been observed that bakla, grown in the hill region of Uttar Pradesh, has a higher protein (28.9%) and a higher oil (4.68%) content than common pulses in which they vary from 17.1 to 25.1% and 0.6 to 1.7%, respectively (Gopalan *et al.*, 1971). Unlike soybean, it has no beany flavour. In spite of these qualities, it is not commonly used because of its flatulent nature and probably low protein efficiency ratio (PER). Jha & Verma (1980) have shown that pea (*Pisum sativum*) and black gram (*Phaseolus mungo*) fermented by *R. oligosporus* are considerably less flatulent than unfermented beans. Earlier, Steggerda (1968) & Kawamura (1967) had shown the existence of a positive relationship between galactooligosaccharide content of beans and intestinal gas produced on their ingestion. In soybean, it has been shown that loss of the flatulent nature due to fermentation by the mould is caused by a decrease in galactooligosaccharide content during tempeh production (Calloway *et al.*, 1971). This may be true also for other pulses since Sadhu (1979) and Goyal (1979) have shown the presence of galactooligosaccharides in black gram (*Phaseolus mungo*) green gram (*Phaseolus aureus*), lentil (*Lens esculenta*), pigeon pea (*Cajanus cajan*) and Bengal gram (*Cicer arietinum*) which can be removed by fermentation with lactobacilli. The results on bakla indicate that it also contains raffinose, stachyose and an unidentified oligosaccharide, possibly verbascose, for which standards are not available. Therefore, the mould fermentation from bakla is expected to yield a product less flatulent than the unfermented cotyledons in each case. The non-flatulent nature of fermented products could also be attributed to the presence of an inhibitor of intestinal gas-producing microflora according to Wang, Ruttle & Hesseltine (1969) who have demonstrated that *R. oligosporus* produces an antibacterial substance which is very active against gram-positive bacteria. This substance, which should be normally present in tempeh, would inhibit the growth of Clostridia which are known to produce gas in the intestines (Richards, Steggerda & Murata, 1968).

According to Gyorgy (1961), tempeh has a better PER than the beans due to the better availability of amino acids liberated from the soyprotein during fermentation. Stillings & Hackler (1965) have shown that the tempeh protein is richer in methionine which is limiting in legume proteins. Murata, Ikehata & Miyamoto (1967) have observed increased concentration of some of the essential amino acids. Therefore, synthesis of new proteins with improved amino acid balance is indicated. According to Steinkraus *et al.* (1960, 1965) better digestibility of tempeh can be attributed to an increase in water-soluble solids. Murata *et al.* (1967) have reported that amounts of riboflavin, vitamin B_6, nicotinic acid and pantothenic acid are much higher in tempeh than in unfermented soybeans. Therefore, it can be concluded that bakla and bakla-soybean tempeh would have a higher PER and better digestibility than the corresponding beans.

Increase in moisture content during mould growth indicates utilization of mainly seed carbohydrates, and to a lesser extent, lipids and amino acids. Synthesis of mycelial constituents and extracellular enzymes does require

energy for which seed carbohydrates and lipids are readily available sources. However, according to Wagenknecht *et al.* (1961), lipid content does not decrease during the early stages of fermentation. Maximum increase in moisture content takes place during the fermentation by *R. oligosporus* NRRL 2710. But we find more profuse growth of mycelium in *R. oligosporus* NRRL 3271. Therefore, higher energy utilization does not necessarily mean higher growth of the organism. The synthesis of extracellular enzymes secreted in the seeds may utilize part of the energy.

The rise in the pH level is also greater during *R. oligosporus* NRRL 2710 growth in all substrates indicating maximum proteolysis. But since no foul smell or evolution of H_2S has been observed, the sulphur-containing amino acids are not lost. These amino acids, which may become available as a result of seed protein degradation, may find a place in mould proteins. This may also explain the increase in the proportion of cysteine and methionine in tempeh protein (Murata *et al.*, 1967).

Protein and oil contents of soybean Bragg cotyledons as determined here fall within the range obtained for this variety in different countries (Whigam, 1975). According to Whigham the protein content of this soybean variety varies from 28.9 to 45% and that of oil from 18.4 to 28.47% depending on soil and climatic conditions. Pantnagar soil being moderately rich and climatic conditions not hostile, our values appear to be justifiably correct. The oil and protein contents of bakla found here are considerably higher than those of other pulses. It appears therefore, that the protein and oil contents in legumes are closely related. In this regard soybean should top this list with a protein content of about 40% and an oil content of about 20%. Murata *et al.* (1967) have reported increased protein content after fermentation of soybean by *R. oligosporus*. This may be due to loss in the total weight of beans which is invariably due to mould respiration. However, total crude protein present in the beans can only be either modified or reduced during fermentation. Wagenknecht *et al.* (1961) have reported that there is no loss in total fat content (petroleum ether extract) when soybean is fermented by *R. oryzae* for up to 30 hr. However, lipase activity which does not affect the concentration of linoleic acid, an essential fatty acid has been noted. With this in view the oil analysis of tempeh has not been conducted since the fermentation is not permitted beyond 19–22 hr. Furthermore, beany flavour has not been noted in the tempeh prepared out of mixtures of soybean and bakla (up to 1:1 ratio) containing up to 13.25% oil in unfermented mixtures, above which a perceptible beany flavour, characteristic of the soybean, is observed. Since oil deterioration results in off-flavour, this is understandable. Bakla-soybean tempeh in the above ratio has been found to be crisper and more palatable than those having a higher soybean content. Mushroom-like odour was uniformly present in all fermented products.

Tempeh from bakla alone and bakla-soybean blend (up to 1:1 ratio) has been found to be crisp and palatable with no perceptible beany flavour. Bakla-soybean blend (1:1) tempeh, had sufficiently high protein content (~35%) and about half the oil content of soybeans, and, thus could be included in the diet without prejudice existing against soybean.

Acknowledgments

The authors express their gratitude to Dr C. W. Hesseltine, Northern Regional Research Laboratory, Peoria, Illinois, U.S.A. for providing cultures of *Rhizopus oligosporus* strains. The assistance of Krishna Jha in chromatographic analysis of oligosaccharides is acknowledged. They are also thankful to Director, Experiment Station and Dean, College of Basic Sciences and Humanities, G.B. Pant University of Agriculture and Technology, Pantnagar, India for financial assistance to the senior author (I.M.D.) and other facilities.

References

AOCS (1964) Official and Tenetative Methods of the American Oil Chemists Society. 2nd edn. Ed by E.H. Salle. Chicago, Illinois.

Bailey, R.W. & Bowne, E.J. (1960) *J. Chrom.* **4,** 206.

Boedijn, K.B. (1958) *Sydowia,* **12,** 321.

Calloway, D.H., Hickey, C.A. & Murphy, E.L. (1971) *J. Fd Sci.* **36,** 251.

Djien, K.S. & Hesseltine, C.W. (1961) *Soybean Digest,* **22,** 14.

Goel, R. (1979) *Identification of Flatulence Factors of some Pulses and Their Removal by Microbial Fermentation.* M.Sc. thesis. G.B. Pant University of Agriculture and Technology, Pantnagar.

Gopalan, C., Sastri, B.V.R. & Balasubramanian, (1971) Tables of food composition. In: *Nutritive Value of Indian Foods,* p. 60. National Institute of Nutrition, Hyderabad.

Gyorgy, P. (1961) *The Nutritional Value of Tempeh. Meeting Protein Needs of Infants and Children.* p. 281. Pub. 843. National Academy of Science – National Research Council, Washington, DC.

Hesseltine, C.W. (1965) *Mycologia,* **57,** 149.

Hesseltine, C.W., Smith, M., Bradie, B. & Djien, K.S. (1963) *Dev. Ind. Microbiol.,* **4,** 275.

Jha, K. & Verma, Jitendra (1980) *Ind. J. exptl. Biol.* **18,** 658.

Kawamura, S. (1967) Review of PL–480 work on soybean carbohydrates. In: *Proceedings International Conference on Soybean Protein Foods, Peoria, Illinois.* May 1967. ARS 71–35 U.S.D.A.O. 249.

Martinelli, A.F. & Hesseltine, C.W. (1964) *Food Technol., Champaigne,* **18,** 167.

Murata, K., Ikehata, H. & Miyamoto, T. (1967) *J. Fd Sci.,* **32,** 580.

Richards, F.A., Steggerda, F.R. & Murata, A. (1968) *Gastroenterology,* **55,** 502.

Sadhu, C. (1979) Removal of galactooligosaccharides from green gram (*Phaseolus aureus*), Bengal gram (*Cicer aeritinum*) and red gram (*Cajanus cajan*) by bacterial fermentation. M.Sc. thesis. G.B. Pant University of Agriculture and Technology, Pantnagar.

Steggerda, F.R. (1968) *Ann. NY. Acad. Sci.* **150,** 57.

Steinkraus, K.M., Van Buren, J.P., Providenti, M.I. & Hand, D.B. (1960) *Fd Res.,* **25,** 777.

Steinkraus, K.M., Van Buren, J.P., Heckler, L.R. & Hand, D.B. (1965) A pilot plant process for dehydration of tempeh. *Fd Technol.* **19,** 63.

Stillings, B.R. & Hackler, L.R. (1965) *J. Fd Sci.* **30,** 1043.

Wagenknecht, A.C., Mattick, L.R., Lewin, L.M., Hand, D.B. & Steinkraus, K.M. (1961) *J. Fd Sci.* **26,** 373.

Wang, H.L., Ruttle, D.I. & Hesseltine, C.W. (1969) *Proc. Soc. exp. Biol.,* **131,** 579.

Whigam, D.K. (1975) *International Soybean Variety Experiment.* First Report of Results. International Agricultural Publications. Intsoy series No. 8 pp. 144–160. College of Agriculture, University of Illinois, Urbana, Champaigne.

(Received 27 March 1980)

J. Fd Technol. (1981) **16,** 51–58

Production and acceptability testing of fish crackers ('keropok') prepared by the extrusion method

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Summary

'Keropok', a traditional Malaysian snack food composed of fish and flour, was successfully prepared by extrusion. The degree of expansion of the dried product on frying was measured as a function of extruder temperature and the ratio of fish to tapioca flour in the product. It was found that expansion decreased with increasing fish content.

Organoleptic evaluation indicated that the extruded products were as acceptable as those prepared using traditional methods.

Introduction

Crackers (in Malaysia known as 'keropok') are popular snack foods in Malaysia and the ASEAN countries. In the west, they would be classified as 'half-products' or 'intermediates' (Lachmann, 1969) and expanded snack products (Cumminford & Beck, 1972).

Traditionally 'keropok' is prepared by forming a dough from a mixture of flour, comminuted fish and water. The ratio of flour to fish varies but is generally within the range 70:30 to 50:50. The dough is shaped, boiled to gelatinize the starch and then cut into thin slices prior to sun drying. The dried slices are cooked in hot oil by the housewife whereupon they puff to give a porous low density product.

'Keropok' making in Malaysia is mainly confined to the areas along the eastern coast of the peninsula where it is an important cottage industry. The ingredients and methods used vary widely among processors. Because of the lack of knowledge the products are often of poor quality, with uneven expansion characteristics, dark objectionable colours and varying shapes, sizes and

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0022–1163/81/0200–0051 $02.00

thickness. In an attempt to upgrade product quality Siaw & Idrus (1979) have introduced mechanization and standardization into 'keropok' making. They claim their process is less time consuming and gives a better-quality product compared to the traditional methods. In particular their product had a higher expansion ratio and was found to be more acceptable.

Siaw, Yu & Chen (1979) made an evaluation on Malaysian fish crackers using six types of combinations from two types of fish and three types of flour. They found that 'keropok' made from tapioca flour and a highly-flavoured fish e.g. *Clupea leiogaster*, was preferred by taste panellists. In agreement with Siaw & Idrus (1979) they reported that crispness was the most important factor governing product acceptability. Expansion on frying was much higher when tapioca flour was used. The types of fish did not contribute to the expansion properties.

The object of the present study was to find out if 'keropok' could be made by an continuous extrusion process and to compare the organeleptic qualities of the extruded product with traditionally-prepared 'keropok'.

Materials and methods

Materials

Finely-ground tapioca (*Manihot esculentus*) flour (mesh size No. 350) was used. The fish, *Chirocentrus dorab*, was obtained fresh from the market, deboned, finely minced and stored frozen (−17°C) for a period up to a week prior to use.

Methods

Preparation of feed for extruder. Fish: flour ratios of 20:80, 30:70, 40:60, 50:50 and 60:40 were used. This range corresponds to that employed by traditional cottage industry operations in Malaysia. The minced fish and flour were mixed at room temperature (~24°C) employing a Kenwood mixer equipped with a paddle beater. During the mixing stage 2% NaCl was added to each formulation.

Preparation of keropok by extrusion. A Brabender (model 20DN) laboratory extruder was employed fitted with a spiral screw with 1:1 compression ratio. A ribbon type die with a 23 mm × 0.5 mm discharge slit was used. A screw speed of 120 rev/min was employed. The material was fed through a feed hopper equipped was a continuous agitator. For all experiments no heating or cooling was applied to the first stage of the barrel and the die section was maintained at a temperature of 100°C. The temperatures for the second stage was varied from 60 to 140°C. After extrusion the product was cut into pieces approximately 2.3 cm square and then dried in a forced-air cabinet drier (Apec, U.K.) at 70°C for 6 hr to give a final moisture content of 8–9%.

Preparation of the control sample. The control sample was prepared by the modified traditional method developed by Siaw *et al.* (1979). The comminuted fish and flour were mixed thoroughly to a ratio of 40:60 using a blender and 1.5% salt and 25% water (based on total formulation weight) were added. The mixture was then stuffed, using a machine, into 50 mm diam. fibrous casing and tied at both ends. The stuffed rolls were steamed for 60 min at atmospheric pressure and subsequently cooled at room temperature. They were then chilled at 5–10°C overnight until firm and sliced mechanically to a thickness of 1.25 mm. The slices were then cut into squares of approximately 2.3 cm × 2.3 cm and dried at 70°C.

Measurement of linear expansion. The linear expansion was obtained on deep frying the dried keropok in palm oil at 200°C. The unpuffed 'keropok' were ruled with five lines across using a fine oil pen. Each line was measured before and after puffing. The percentage linear expansion was calculated as follows:-

$$\% \text{ linear expansion} = \frac{\text{Length after puffing} - \text{Length before puffing}}{\text{Length before puffing}} \times 100$$

Organoleptic evaluation. The samples were evaluated after frying at 200°C by twenty-one experienced panellists who were asked to rate the colour, crispness, flavour and overall acceptability of the products using a rating test of 5 for excellent to 1 for poor. The results were analysed using the Duncan's multiple range test (DMRT).

Chemical analysis. Crude protein (N × 6.25) was determined using the semi-micro Kjeldahl method (Pearson, 1970) using the Hoskins (1944) distillation apparatus.

Moisture content was determined using the Brabender rapid moisture tester at a temperature of 130°C for 1 hr.

Results

Linear expansion on frying

Figure 1 shows the percentages of linear expansion of 'keropok' containing a fish:flour ration of 40:60 as a function of varying temperatures at the second stage of the extruder barrel. Expansion at 60°C was negligible (<5%) but increased with increasing temperature up to a maximum at 100°C. Further increases of temperature did not affect expansion levels. The control sample, however, showed an expansion of 101% which some 20% greater than the maximum obtained with the extruded samples. As can be been from Fig. 2 the linear expansion at an extrusion temperature of 100°C decreased with increasing fish content.

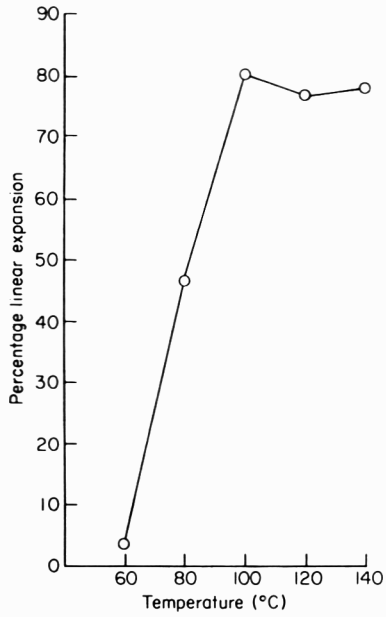

Figure 1. Effect of extruder temperature on percentage linear expansion of 'keropok' on frying. (40% fish: 60% tapioca flour)

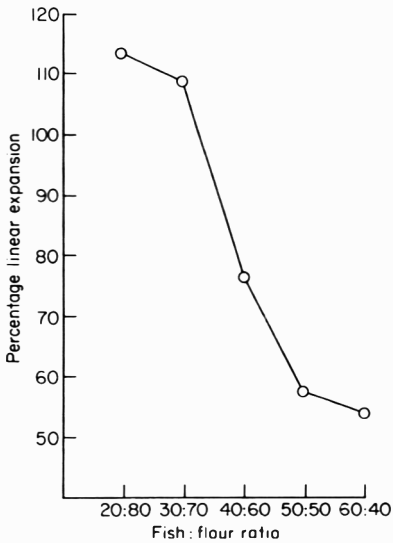

Figure 2. Effect of fish:flour ratio on percentage linear expansion of 'keropok' on frying – extrusion temperature, 100°C.

Table 1. Effect of extruder temperature on colour, crispness, flavour and overall acceptability of 'keropok' (Ratio of fish to flour 40:60)

Temp. (°C) at 2nd stage of extruder	Colour	Crispness (mean values)	Flavour (mean values)	Overall acceptability
60	1.76b	2.19b	2.24b	1.67b
80	2.10b	2.38b	2.24b	1.86b
100	3.05a	3.24a	2.95a	2.95a
120	2.76a	3.29a	3.10a	3.00a
140	2.90a	3.29a	2.85a	3.10a
Control (modified traditional method)	2.86a	3.29a	2.76a	3.10a

Figures with the same letter are not significantly different at the 0.05 level using the DMRT.

Organoleptic evaluation

The results of the organoleptic evaluation are shown in Tables 1 and 2. From Table 1 it can be seen that provided the extruder temperature was 100°C or greater the taste panellists detected no significant difference in colour, flavour, crispness or overall acceptability between the extruded product and 'keropok' prepared by the traditional method. Although the ratio of fish to flour significantly affected the panellists rating for flavour, with the samples containing higher fish levels being ranked higher (Table 2) the level of fish did not significantly effect the overall acceptability of the product.

Table 2. Effect of fish:flour ratios on colour, crispness, flavour & overall acceptability of 'keropok' (extruder temperature 100°C)

Fish:flour ratio	Colour	Crispness (mean values)	Flavour (mean values)	Overall acceptability
20:80	2.67a	3.29a	2.00c	2.48a
30:70	2.48a	3.14a	2.05c	2.43a
40:60	3.05a	3.33a	2.62b	3.05a
50:50	2.86a	3.25a	2.81a	2.90a
60:40	2.43a	3.05a	2.81a	2.95a
40:60 (control modified traditional method)	2.86a	3.19a	2.29b	2.76a

Figures with same letters are not significantly different at the 0.05 level using the DMRT

Table 3. Protein Content of 'keropok'

Fish–flour ratio	Protein* (%)
20:80	5.34
30:70	6.99
40:60	10.76
50:50	15.29
60:40	22.06
Control (40:60)	11.04

*Expressed as a percentage of product weight after drying

Chemical analysis

Chemical analysis (Table 3) indicated that the extruded samples containing 40% fish had similar protein contents as the control sample. All the samples were dried to a moisture content of about 8–9% (Table 4).

Discussion

The increase in expansion ratio with extruder temperature shown in Fig. 1 indicates that prior starch gelatinization is necessary if the product is to expand on frying. Experiments using marker dyes have shown that the residence time in the extruder is of the order of 70 sec. It would appear that this is sufficient to ensure enough starch gelatinization for extensive expansion on frying provided the extruder barrel temperature is 100°C or higher.

It has been reported that in the range of moisture contents corresponding to

Table 4. Moisture contents of 'keropok' at various stages during processing

	Moisture			
Fish:flour ratio	Temp. (°C)	Feed	After extrusion	After drying
40:60	60	40.3	28.3	8.3
40:60	80	"	29.5	8.4
40:60	100	"	30.7	8.5
40:60	120	"	30.4	8.4
40:60	140	"	30.2	8.4
20:80	100	25.4	22.4	8.5
30:70	"	34.3	27.4	8.5
50:50	"	46.1	37.0	9.2
60:40	"	55.0	–	8.3
40:60	Control by modified traditional method			8.4

the extruder feeds (Table 4) the gelatinization temperature of starch decreases with increasing moisture content. (Lelievre, 1973, Blanshard, 1979). It therefore seems unlikely that the decrease in expansion with increasing fish content can be attributed to differences in the degree of starch gelatinization. A more probable explanation is that the protein interacts in some way with the starch to inhibit expansion. Since Siaw *et al.* (1979) found that the presence of wheat protein also reduced expansion it seems possible that this effect may be independent of the type of protein present. Another factor which the referee has suggested might be important is the oil in the fish. It is known that the presence of fat and oils when added to extruded products tends to weaken the resultant dough and reduce product strength (Harper, 1978). The oil content of the fish employed in these experiments was 1.1%.

Extrusion appears to be a promising alternative method for preparing 'keropok'. For the product extruded at temperatures of 100°C and above panellists found no significant difference between the traditionally prepared control and the extruded samples. From the point of view of manufacture the extrusion method has several advantages. No extra water need be added to the original fish and flour mixture thus reducing the subsequent drying time. This variant of the process also cuts down labour requirements because the extensive manual mixing of the dough, shaping and subsequent boiling or steaming is no longer necessary. The process is faster and can be operated on a continuous basis as opposed to the traditional batch process. The product produced by extrusion should be more hygenic and homogenous.

In recent years there has been considerable interest in the development of low cost extrusion processes for the manufacture of traditional foods in developing countries (Maxwell, 1978). The manufacture of Malaysian 'keropok' by extrusion is another example of such a process.

Although 'keropok' is normally made with good quality fish, it should be possible to produce a variety of nutritionally valuable snack foods by extrusion using different protein sources. It would also be valuable to utilize cheaper varieties of fish and fish products in the manufacture of 'keropok'.

Acknowledgments

The authors are indebted to the Director of the Agricultural Product Utilization Division, Malaysian Agricultural Research and Development Institute for the provision of the facilities required for the study. We also gratefully acknowledge the assistance provided by Mr A. H. Hor.

References

Blanshard, J.M.V. (1979) In: *Polysaccharides in Food*, p. 129 ed by J.M.V. Blanshard and J.R. Mitchell. Butterworths, London.

Cumminford, P.D. & Beck, C.I. (1972) U.S. Patent No. 3703379.
Harper, J.M. (1978) Crit. Rev. Fd Sci. Nutrit. **11,** 204.
Hoskins, J.L. (1944) *Analyst,* **69,** 271.
Lachmann, A. (1969) *Snack and Fried Products,* p. 143. Noyes Data Corporation, New Jersey.
Lelievre, J. (1973) *J. appl. Polymer Sci.* **18,** 293.
Maxwell, D.L. (1978) *AIChE Symp. Ser.,* **74,** 189.
Pearson, D. (1970) *The Chemical Analysis of Foods,* 6th edn. Churchill-Livingstone, London.
Siaw, C.L. & Idrus, A.Z. (1979) *Proceedings of the Symposium on Protein-rich Foods in ASEAN Countries,* p. 81 Kuala Lumpur, Malaysia.
Siaw, C.L., Yu, S.Y. & Chen, S.S. (1979) *Proceedings of the Symposium on Food and Nutritional Biochemistry in Asia and Oceania.* Kuala Lumpur, Malaysia (In press).

(Received 10 July 1980)

J. Fd Technol. (1981) **16**, 59–66

The fluidization characteristics of potato chips

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Summary

The fluidization characteristics of potato chips and potato cuboids have been investigated and the results compared with correlations obtained for the fluidization of plastic cuboids.

It was found that potato chips and cuboids behaved in a similar manner to the plastic cuboids and that the plastic cuboid correlations may be used to predict the performance of potato chips and cuboids.

Certain fluidization parameters may be used to predict the minimum transportation velocity of potato chips in vertical pipelines.

Introduction

Fluidization is defined as the process by which solid particles are transformed into a fluid-like state through contact with a gas or liquid. In the food industry fluidization is used in a number of processes and new applications are continuously being developed. A number of techniques which have been applied to the food industry are listed.

Drying	– e.g. the fluidized drying of salt and grain using hot air.
Heat exchange	– e.g. the cooking of canned foods in fluidized beds of hot sand, the blanching of vegetables is a fluidized bed of air and steam.
Freezing	– e.g. the fluidized freezing of peas using cold air.
Mixing	– e.g. the mixing of fine powders of different solids using air.
Transportation	– e.g. the pneumatic transport of salt using air, the hydraulic transport of fruit and vegetables using water.

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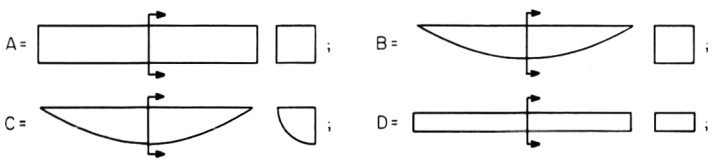
0022-1163/81/0200–0059 $02.00

Table 1. Size and shape distribution of a typical sample of potato chips obtained from the factory.

Shape classification*	Number of particles in each length band (length in mm)														Number of particles in group	Percentage of particles in group
	0–9.9	10–19.9	20–29.9	30–39.9	40–49.9	50–59.9	60–69.9	70–79.9	80–89.9	90–99.9	100–109.9	110–119.9	120–129.9	130–139.9		
A				4	13	14	11	12	13	1	6	3	4	1	82	56
B			1	4	2	10	8	5	3						33	22
C				1	4	2	3	3	1						14	10
D					2	3			3						8	5
E			1	1	2	3	1	2							10	7

*Shape classification



E = irregular shapes.

Despite the large amount of research which has been carried out on fluidized systems it is not possible to predict precisely the behaviour of a system completely in terms of the physical properties of the solid particles and the fluid and the operating conditions (Davidson & Harrison, 1971).

The specific problem investigated in this paper arose out of the need for hydraulic transportation of potato chips in a plant producing partically prepared potato chips for catering estabilshments. The initial study investigated the fluidization behaviour of chipped potatoes. From this the minimum fluid velocity for vertical transportation could be obtained, and the quality of fluid/solid mixing could be observed. It was found that established theory did not predict the minimum fluidization velocity or the terminal velocity at which entrainment occurs (McLain & McKay, 1979). Since potatoes are a variable material it was decided to use model potato chips made of a range of plastics of different density in order to establish the fluidization characteristics of this shape and size of particle. In the samples of chipped potatoes received from the processing factory the cuboid shape predominated, and Table 1 shows the size and shape distribution of a typical sample obtained from the factory. Hence the model chips were made to the cuboid shape with a range of typical lengths.

It was found (McKay & McLain, 1980) that a modified Ergun (1952) expression could be derived correlating the friction factor to the minimum fluidization velocity for cuboids. The terminal velocity at which vertical transportation occurs could be determined from a resistance coefficient which was related to the particle shape.

This present paper tests the relationships derived for plastic cuboids against the behaviour of chipped potatoes as received from the processing factory and a range of potato cuboids selected from the factory samples.

While these results were obtained as a preliminary to a transportation investigation they could be applied to a purely fluidized system, for example the blanching of potato chips.

Materials and methods

The fluidization tests were carried out in a continuous flow system in which water was continuously circulated through the bed of particles. The water flow rate through the bed and the pressure drop across the bed were measured. The quality of fluidization was observed through the glass wall of the test section.

A line diagram of the flow system is shown in Fig. 1. Water was circulated through the vertically mounted glass working section A by a centrifugal pump C. Flow valve E and by-pass valve F controlled the water flow rate through the working section. The water flow rate was measured in the flow measuring section B and the overflow from the top of the working section was collected in G.

The range of potato chips and cuboids tested is given in Table 2.

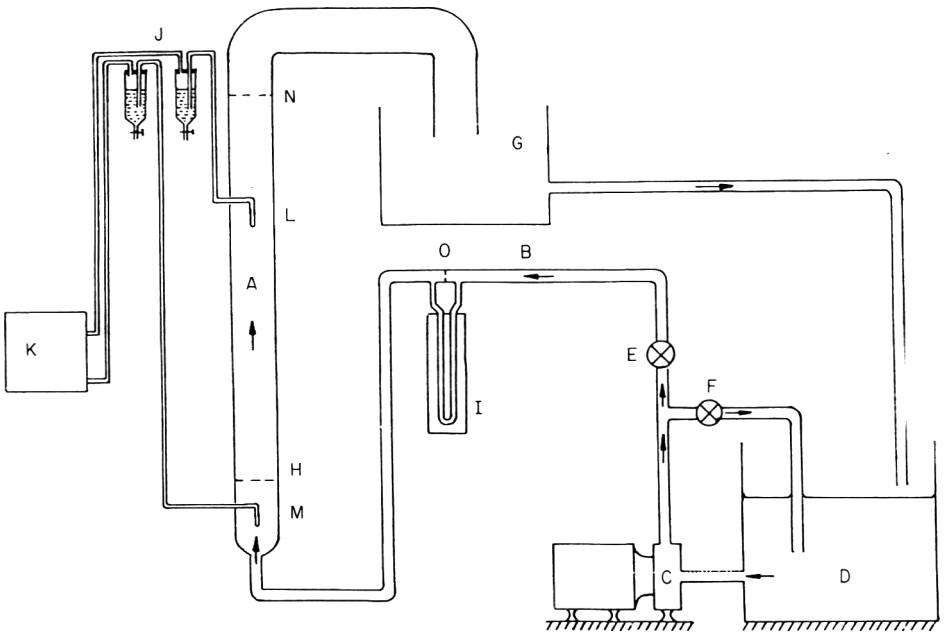


Figure 1. Schematic diagram of apparatus. Key: A, 150 mm diameter glass section; B, flow measuring section; C, centrifugal pump; D, water reservoir tank; E, flow control valve; F, by-pass valve; G, collection tank; H, perforated zinc screen; I, mercury U-tube manometer; J, pressurized flasks; K, Furness micro manometer; L & M, static pressure probes; N, retention plate; O, sharp-edged orifice plate.

Table 2. Size range of potato particles tested

Type	Specific gravity	Cross section (width × thickness) (mm)	Length (mm)
Cuboids	1.07–1.09	12.5 × 12.5	70, 50, 25, 12.5
Chips	1.07–1.09	Mixture of various cross-sections e.g. Table 1	Mixture of various lengths e.g. Table 1

Table 3. Material: potato (S.G. = 1.09). Cross-sectional dimensions: as received from factory

$\bar{d}_e$	$\bar{\theta}_s$	U_m (mm s^{-1})	U_t (mm s^{-1})	ΔP_m (mm water)	ε_m	L_m (mm)	f_v	Re_d	$\frac{Re_d}{1-\varepsilon_m}$	C_r
15.68	0.62	31	233	3.76	0.64	140	3707	426	1184	0.55
16.05	0.60	36.5	270	3.91	0.63	141	3076	514	1389	0.43

Results and theory

The results for the potato chips are given in Table 3.

For non-spherical particles like those tested the size is usually specified in the form of an equivalent diameter, d_e, (Davidson & Harrison, 1971).

d_e = diameter of a sphere with the same surface area to volume ratio as the particle

For potato chips mean values of effective diameters, $\bar{d}_e$, were calculated.

The sphericity factor, θ_s is used as a measure of the degree of deviation of non spherical particles from the spherical shape (Wadell, 1934).

θ_s = the ratio of the surface area of a sphere to the surface area of the particle, both of the same volume.

For potato chips mean values of sphericity factor, $\bar{\theta}_s$, were calculated.

The pressure drop across the bed, Δp, was obtained over a range of flow velocity, U, from a plot of log Δp against log U, the minimum fluidization velocity, U_m, was obtained from the point of intersection of the two linear correlations.

The terminal velocity, U_t, which represents the minimum velocity for vertical transportation was obtained from a plot of log U against log ε at a value of log ε equal to unity. The voidage, ε, is the ratio of the volume of the void space between the particles to the total volume of the bed.

Δp_m = pressure drop at minimum fluidization velocity.
ε_m = voidage at minimum fluidization velocity
L_m = bed height at minimum fluidization velocity.

A friction factor, f_v, representing the ratio of the pressure drop to the viscous energy term has been proposed by Ergun (1952).

$$f_v = \frac{\Delta p_m}{L_m} \frac{d_e^2}{\mu} \frac{\varepsilon_m^3}{U_m} \frac{}{(1-\varepsilon_m)^2}$$

$$\text{Reynolds number } Re_{de} = \frac{\rho U_m d_e}{\mu}$$

$$\text{Resistance Coefficient } C_r = \frac{2V(\varphi_p - \rho)g}{A_p U_t^2 \rho}$$

where ρ = density of fluid
ρ_p = density of solid particles
μ = viscosity of fluid
g = gravitational constant
V = volume of particles in bed
A_p = total surface area of particles

Discussion

Minimum fluidization

Ergun produced a linear correlation of friction factor, f_v, against the term $Re_{de}/(1-\varepsilon_m)$ for small spherical particles. McKay & McLain (1980) found that large plastic cuboids did not conform to the Ergun equation and they produced a modified form of this equation, equation (1), which correlated the results for large cuboids of sphericity factor, $\theta_s \geq 0.66$:

$$f_v = 478 + 2.51 \frac{Re_{de}}{1-\varepsilon_m} \tag{1}$$

Figure 1 shows the results for potato cuboids and potato chips compared with equation (1) and the Ergun equation. The potato cuboids conform to equation (1) though there is some scatter due to their poor fluidization performance. The broken line shows the assumed digression of the potato chip results from equation (1). This is based on the plastic cuboid experiments which indicated that as the sphericity factor reduced from 0.66 the results digressed from equation (1) in this manner.

Resistance coefficient

At the terminal velocity the resistance coefficient may be defined by equation (2):

$$C_r = \frac{2V(\rho_p - \rho)g}{A_p U_t^2 \rho} \tag{2}$$

If the resistance coefficient C_r is known the terminal velocity, U_t, can be calculated from equation (2).

For plastic cuboids McKay and McLain (1980) correlated the resistance coefficient, C_r, with sphericity factor, θ_s, to produce equation (3):

$$\log C_r = 5.78 \log \theta_s + 0.84 \tag{3}$$

Figure (3) compares the results for potato cuboids and potato chips with

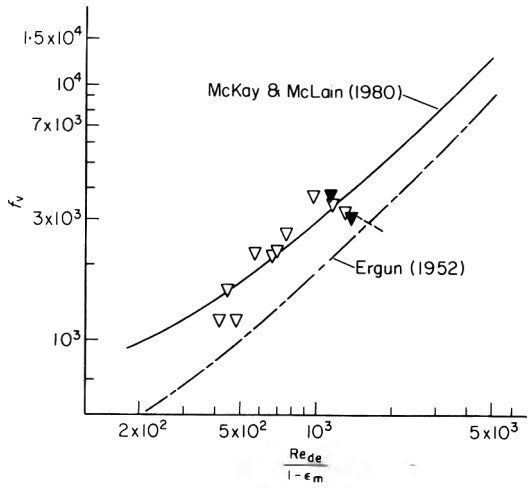


Figure 2. Friction factor, f_v, against $Re_{de}/1 - \varepsilon_m$ for potato chips and potato cuboids. ▼, Potato chips; ▽, potato cuboids.

equation (3). It can be seen that very good agreement is obtained. This indicates that equation (3) may be used to calculate the resistance coefficient, C_r, of potato chips and hence enable the terminal velocity U_t, to be calculated from equation (2). U_t is the minimum velocity at which transportation will occur in a vertical pipe. For potato chips mean values of particle dimensions and sphericity factor must be used in equations (2) and (3).

Quality of fluidization

The quality of fluidization can be influenced by many factors e.g. bed geometry, particle size and shape, distributor plate design and the vessel internals such as screens or baffles (Volk *et al.*, 1962). When potato chips and cuboids were tested in the fluidized bed two anomalies from ideal behaviour were observed, namely channelling and slugging. Channelling occurs when the particles collect to one side of the bed allowing an open channel for the fluid to flow past. Slugging occurs when the particles form a clump which rises up the column until the particles eventually separate and fall back through the fluid. Channelling and slugging occurred frequently with potato chips and the long potato cuboids. It is considered that these phenomena produced the scatter in the results of Fig. 2.

Conclusion

The characteristics of potato cuboids and potato chips have been compared with the correlations obtained by McKay & McLain (1980) for plastic cuboids.

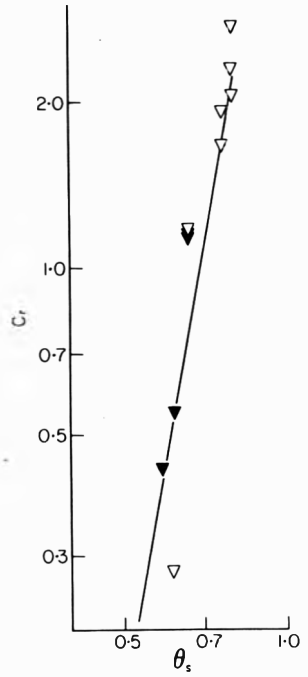


Figure 3. Coefficient of resistance, C_T, against sphericity factor, θ_s, for potato chips and potato cuboids. Key: see Fig. 2.

The friction factor, f_v, for potato cuboids of sphericity factor, $\theta_s \geqslant 0.66$ conformed to the equation:

$$f_v = 478 + 251 \frac{Re_{de}}{1 - \varepsilon_m}$$

Potato chips whose mean sphericity factors were less than 0.66, gave results which digressed from this equation in the same manner as had been observed for the plastic cuboids.

Potato chips were prone to slugging and channelling during fluidization. Both these phenomena tend to reduce the performance of a fluidized bed.

At the terminal flow velocity, U_t, the equation, $\log C_T = 5.28 \log \theta_s + 0.84$, may be used for both potato chips and cuboids. It is proposed that U_t should be calculated when designing a hydraulic transportation system for potato chips is vertical pipelines since below this velocity transportation will not occur.

References

Davidson, J.F. & Harrison, D., (1971) *Fluidisation.* Academic Press, London and New York.
Ergun, S., (1952) *Chem. Eng. Progr.* **48,** 89.
McKay, G. & McLain, H., (1980) *Trans. I. Chem En* **58,** 107.
McLain, H. & McKay, G., (1979) *Trans. Am. Soc. Agric. Engs.* **22,** 671.
Volk, W., (1962) *Chem. Engr. Progr.* **58,** 44.
Wadell, H. (1934) *J. Franklin Inst.* **217,** 459.

(Received 5 July 1980)

J. Fd Technol. (1981) **16,** 67–72

Correlation between iodine number and proton relaxation times in maize oil

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Summary

We have found a good correlation between the iodine number of maize oil and an empirical parameter-related to the proton T_1 relaxation time determined by means of low resolution pulsed nuclear magnetic resonance spectroscopy. This result opens a way to fast and reliable determination of the iodine number during oil hydrogenation.

Introduction

Pulsed low resolution nuclear magnetic resonance (NMR) is becoming a standard technique for determination of solid fat index (Van Putte & Van den Enden, 1973, 1974; Jansson&Andersson, 1976). A series of other applications has also been developed and found a positive response in the food industry; this includes studies of fat polymorphism (Brosio *et al.,* 1980), studies of fatty emulsions (Trumbetas, Fioriti & Sims, 1976), determination of oil content of seeds (Tiwari, Gambhir & Rajan, 1974), determination of humidity in a variety of foodstuffs (Hester & Quine, 1976; Brosio *et al.,* 1978; Brosio *et al.,* 1979), etc.

All these techniques are based exclusively on the analysis of the free induction decay (FID) after a single-pulse excitation and are possibly due to a marked difference between so-called transverse relaxation times (T_2) of solids and liquids. It is, however, well known (Farrar & Becker, 1971) that certain NMR techniques based on two-pulse excitation provide a different parameter called the longitudinal relaxation time (T_1). Like T_2, this parameter is also related to molecular mobility but in a quite different way.

In this paper we will show that T_1 of maize oil varies considerably upon hydrogenation and the experimental T_1 values correlate with the iodine number

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0022–1163/81/0200–0067 $02.00

(NI) of the oil. Let us repeat that the iodine number is defined as the weight of iodine absorbed by 100 parts (by weight) of the sample and that it is a widely used measure of the degree of unsaturation. The determination of iodine number is today rarely made according to the original definition (the Wij's method).

A more common procedure is to calculate it from fatty acid composition of the oil as determined by gas chromatography after a hydrolysis. Even so, it is a rather lengthy process. The T_1 values, on the other hand, can be measured in a matter of seconds, an important factor in industrial hydrogenation monitoring. The fact that the iodine number correlates with T_1 is perhaps not too surprising considering the marked effect of hydrogenation upon the melting point of oils a clear indication that molecular mobility is affected.

It should be noted that the iodine number can be determined also by means of high resolution proton NMR (Johnson & Shoolery, 1962; Nielsen, 1976). The disadvantage is that automated (and therefore fast) high resolution spectrometers are currently much more expensive than the low resolution versions and require a trained operator.

Experimental

The measurements were made on a low resolution pulsed NMR instruments 'Minispec P20' produced by Bruker Analytische Messtechnik GmbH (Karlsruhe, Germany). The definition of the longitudinal relaxation time T_1 and the various methods for its determination are described in the literature (Farrar & Becker, 1971) as well as in the company manual. We will only briefly summarize the principle of the method of inversion recovery which was employed in this study.

The sample, located within a permanent magnet, is first subject to a 180° RF pulse (i.e. a pulse which 'flips' the equilibrium nuclear magnetization M_0 by 180° with respect to the magnetic field direction). After a variable delay t, a 90° RF pulse is applied which gives rise to a transverse nuclear magnetization with a component M_t detected in the direction of M_0, by the transmitter/receiver coil wound around the sample. T_1 is then determined as the slope of the semi-logarithmic plot of $(M_0 - M_t)$ against t. The 90 and 180° pulses are adjusted empirically by varying their respective widths (about 4 μsec and 8 μsec on our instrument).

The time dealy t between the pulses was varied from 10 msec to 1000 msec. Each measurement was repeated 30 times, the time between two consecutive measurements was kept to 3–5 sec to allow the nuclear magnetization to return to its equilibrium value.

The oil samples were measured without any pretreatment in sample tubes of 10 mm external diameter.

During measurement the sample temperature was kept constant by means of

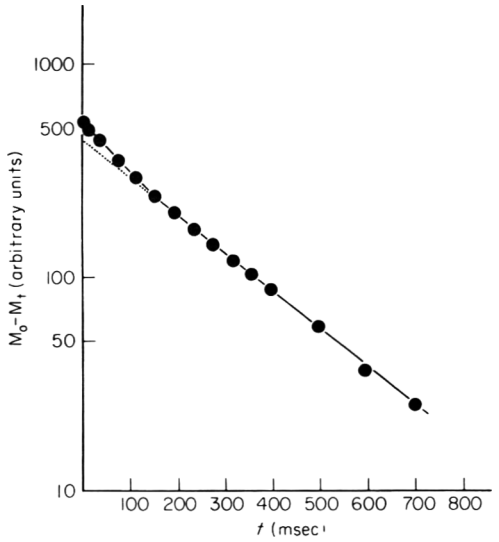

Figure 1. Longitudinal magnetization decay of a maize oil sample (iodine number = 123.2) measured at T = 41°C.

a thermostated water bath with a precision of ± 0.1°C. Its absolute value was checked by a mercury thermometer.

The untreated and partially hydrogenated maize oil samples were kindly supplied by STAR S.p.A. (Agrate, Brianze, Italy).

Results and discussion

Fig. 1 shows a typical 'decay' (i.e. return to equilibrium value) of the proton longitudinal magnetization of a maize oil sample (NI = 123.2) at the temperature T = 41°C. Unfortunately, the semilogarithmic plot of $(M_0 - M_t)$ against t does not yield a straight line. This can be caused by chemically distinct protons in the sample, which have different relaxation times T_1, due to different mobility of the double bond segments and the saturated chains.

The decay may be well approximated ('fitted') by a combination of two exponentials of the type

$$M_0 - M_t = C_1 \exp(-t/T_{1F}) + C_2 \exp(-t/T_{1S}), \quad (1)$$

T_{1F} and T_{1S} are the relaxation times of a fast and a slow decaying component, respectively, while C_1 and C_2 are the relative concentration of the two components.

There is however no reason why only two components should be present; the curves may in fact be fitted by formulae similar to eqn (1), but with practically any number of terms.

Moreover, the uncertainties in the parameters derived by least square fitting of eqn (1) to the experimental curves are large enough to make the whole procedure questionable.

Rather than insisting on a detailed interpretation of the decay curves, we have therefore decided on a purely empirical parameter which would be related to overall decay rate.

In order to be practical, such a parameter should also be easy to determine by means of only a few measurements and a simple calculation suitable for any programmable pocket calculator.

The parameter we have chosen is defined as

$$\bar{T}_1 = (t_2 - t_1)/\ln\,(M_t - M_{t1})/(M_t - M_{t2}), \tag{2}$$

where t_1 and t_2 are fixed delays equal in our case to 10 and 200msec., respectively, and t is long enough to insure that M_t is practically equal to M_0 (in our case $t = 2$ sec.). $\bar{T}_1$ may therefore be calculated from only three measurements.

In Table 1 are reported $\bar{T}_1$ values for maize oil samples with different iodine

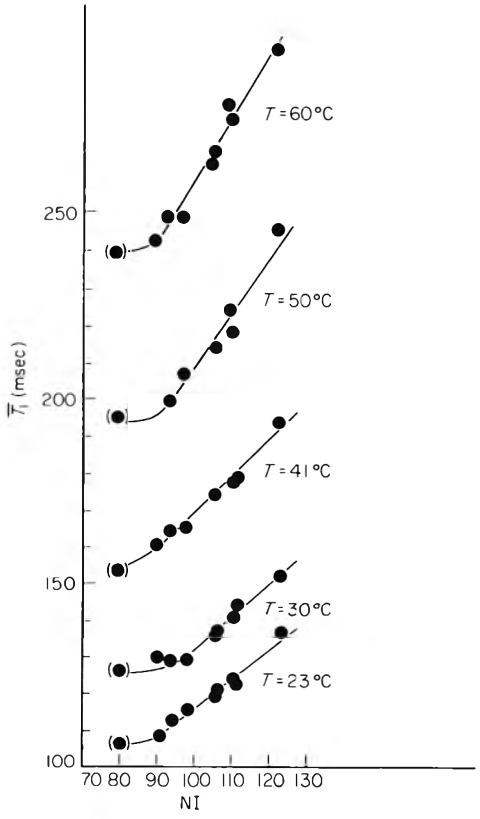

Figure 2. Correlation between $\bar{T}_1$ [see equation (2) in the text] and iodine number values at different temperatures. The experimental points in parenthesis have not been taken into account in the linear regression.

Table 1. Correlation between iodine number and $\bar{T}_1$ values at different temperatures

NI	$\bar{T}_1 \pm 1.5$ (msec)				
	$T = 23°C$	$T = 30°C$	$T = 41°C$	$T = 50°C$	$T = 60°C$
123.2	136	152	193	245	293
111.4	122	144	179	217	275
110.4	123	140	177	224	279
106.3	120	136	175	214	266
106.0	119	135	174	213	263
97.9	115	129	165	207	249
94.0	112	128	164	199	249
90.4	108	129	160	195	243
80.2	106	126	154	195	240
r^2	0.961*	0.969*	0.984*	0.951*	0.968*

*excluding the sample with NI = 80.2

numbers measured at temperature $T = 23, 30, 41, 50$, and 60°C. The respective plots are presented in Fig. 2. It is apparent that there is a good linear relationship between $\bar{T}_1$ and NI: the linearity breaks down only for the most hydrogenated sample (NI = 80.2) which is close to the liquid-to-solid transition.

The correlation coefficients reported in Table 1 were calculated excluding this particular sample. It must be pointed out, however, that there is no reason why the relationship between $\bar{T}_1$ and NI should be linear. A non linearity also does not invalidate the practical value of this study as long as calibration curves can be prepared *a priori* for each type of oil.

We want to point out that the technique requires a good control of the sample temperature T. $\bar{T}_1$ in fact depends quite strongly upon T and an error of 1°C in sample temperature would result in an error of about 3.5 in iodine number.

The present study indicates that the NMR phenomenon of longitudinal relaxation can be exploited for fast determination of the iodine number of partially hydrogenated oils. Further work is needed in order to see the behaviour of different types of oil. It is clear, however, that the method is viable and could become valuable for industrial production monitoring.

By automating the instruments, it is certainly possible both to increase the precision and to develop the method to a fully operator independent level.

References

Brosio, E., Conti, F., Lintas, C. & Sykora, S. (1978) *J. Fd Technol.* **13,** 107.
Brosio, E., Conti, F., Paci, M., Sykore, S. (1979) *J. Magn. Res.* **34,** 593.
Brosio, E., Conti, F., Di Nola, A. & Sykora, S. (1980) *J. Am. Oil Chem. Soc.* **57,** 78.
Farrar, T.C. & Becker, E.D. (1971) *Pulse and Fourier Tansform NMR.* Academic Press. N.Y.

Hester, R.E. & Quine, D.E.C. (1976) *J. Fd Technol.* **11,** 331.
Jansson, H. & Anderson, K. (1976) *Milchwissenschaft,* **31,** 593.
Johnson, L.F. & Shoolery, J.N. (1962) *Anal. Chem.* **34,** 1136.
Nielson, L.V. (1976) *Milchwissenschaft,* **31,** 598.
Tiwari, P.N.; Gambhir, P.N. & Rajan, T.S. (1974) *J. Am. Oil Chem. Soc.* **51,** 1049.
Trumbetas, J., Fioriti, J.A. & Sims, R.J. (1976) *J. Am. Oil Chem. Soc.* **53,** 722.
Van Putte, K. & Van den Enden, J. (1973) *J. Phys. E.: Sci. Instr.* **6,** 910.
Van Putte, K. & Van den Enden, J. (1974) *J. Am. Oil Chem. Soc.* **51,** 316.

(Received 22 July 1980)

J. Fd Technol. (1981) **16,** 73–80

Some factors affecting oxygen transmission rates of plastic films for vacuum packaging of meat

I. J. EUSTACE

Summary

Film composition, temperature and humidity affect the oxygen transmission rates of some films used for the vacuum packaging of meat.

Of the transparent films suitable for meat included in the investigation, those which include polyvinyl alcohol or polyvinylidene chloride offer the greatest barriers to oxygen. Oxygen transmission rates (OTR) at 3.5°C (i.e. near the recommended storage temperature for vacuum packed chilled meat) are only 5–15% of those at 25°C.

High humidity increases the OTR of nylon based films, but the effect is less marked at 3.5°C than at 25°C. At 25°C the OTR reduces to rates expected at 75% r.h. within 50 hr of a change in relative humidity from 98 to 75%. Humidity has a negligible effect on the OTR of films containing PVDC.

Shrinking of heat shrinkable films reduces the oxygen transmission rate approximately in proportion to the reduction in surface area.

The practical implications of the findings are discussed. Relative humidities near 100% r.h. for OTR measurements probably closely simulate the r.h. conditions within cartons of vacuum packaged meat. OTR measured at 20–25°C do not indicate the performance of a film at 0°C, although they do provide a guide to the performance of a given film relative to alternative films of similar composition.

Introduction

Vacuum packaging dramatically changes the gaseous environment at the meat surface. The oxygen in the small quantity of air remaining in a pack after the bag has been evacuated and sealed is depleted by continuing metabolic activity of the meat and of the bacteria at the surface of the meat. Carbon dioxide is

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0022–1163/81/0200–0073 $02.00

produced by the respiration activity. Eventually, the oxygen concentration within the pack falls below 1% while the carbon dioxide concentration rises to about 20% or more. This environment severely restricts the growth of normal aerobic spoilage organisms and storage life is therefore extended. Permanent discolouration of the meat surface by the irreversible oxidation of myoglobin to metmyoglobin is avoided.

As well as having the necessary gas barrier properties, a vacuum packaging film must have mechanical toughness, particularly a high resistance to puncture and abrasion, plus the ability to form reliable seals even in the presence of contamination (e.g. meat juice, and fat) and film overlap. The films which best meet all these requirements are composites which utilize the properties of two or more individual film materials to provide a good package.

The composite films are either laminated where the various layers are bonded with an adhesive, or the layers may be co-extruded, or a combination of both lamination and co-extrusion techniques may be involved.

If the pack is intact (i.e. good seals and no punctures) the amount of oxygen reaching the interior of the pack will be determined by the permeability of the packaging materials. Newton & Rigg (1979) found that storage life of vacuum packaged meat as assessed by discolouration and development of putrefactive odours, was inversely related to film permeability. Meat industry experience suggests that long storage life is enhanced by use of films of very low oxygen permeability (less than 100 ml m^{-2} $24h^{-1}$ atm^{-1} at 25°C and 75% r.h.). Use of these films seems to have reduced discolouration problems, including those due to brown metmyoglobin and green sulph-myoglobin. Consequently emphasis is placed upon the permeability properties of a film to be used for vacuum packaging.

Composition has a great influence on the gas transmission characteristics of a vacuum packaging film. All the film products currently used in Australia for vacuum packaging fresh meat have either nylon or PVDC included as the primary barrier to oxygen.

The permeability/transmission rate data quoted in the literature and by film manufacturers and distributors have usually been measured at temperatures in the range 20–25°C. Relative humidities used were frequently in the range 0–75%. As with most polymer penetrant systems, the transmission of gases and vapours through polymer films obeys the Arrhenius relationship (Rogers *et al.*, 1956). For permanent gases, permeability increases with temperature. Davis (1971) states that gas transmission rates of different types of film determined at one specific temperature may not be in the same relative order at other temperatures. Rigg (1979) reports a marked increase in the permeability of nylon film above 70% r.h.

Clearly, temperatures of vacuum packages containing meat would never exceed 10–12°C at the time of packing in the boning room and they would reduce to 0°C in holding chillers. Further, relative humidities within the pack will always be near 100%, and the relative humidity on the outside will, on occasions, be considerably in excess of 75%.

This report presents data to show how oxygen transmission rates are affected by the composition of the material, by temperature, and by short term and long term exposure to high relative humidities.

Experimental

The oxygen transmission rates (OTR) of film samples were determined by the concentration increase principle using equipment, the design of which was described by Davis & Huntington (1977). The gases (oxygen as the test gas, nitrogen as the inert gas) were humidified to 75% r.h. by passing them through saturated sodium chloride solution or to near 100% by passing them through deionized water. The RH of the gases was monitored with a Lufft relative humidity meter which was modified to fit a flow through cell which was in turn connected to the measurement cell.

Measurements were made in constant temperature rooms maintained at 25 or 3.5°C.

Oxygen concentrations were estimated with a Fisher-Hamilton Model 29 gas chromatograph, fitted with an aluminium column (1,850 × 6 mm) filled with DEHS on Chromosorb P and an aluminium column (1,950 × 5 mm) filled with Molecular Sieve 13×.

Where possible, measurements at the two temperatures and the two relative humidities were made on a single sample of each film material (area 0.0294 m^2) to avoid the variations which may be expected among different samples of the same type of film material (Karel *et al.*, 1963). Duplicate determinations were made on each film under each set of conditions.

The effect of heat shrinking was determined by shrinking EVA/PVDC/EVA material by known amounts. The amount of shrink was controlled by placing a sheet of aluminium of predetermined size in a bag of known dimensions. The bag was heat sealed, and then shrunk by immersion for approximately one second in water at 90°C. Samples of film were thus prepared which had been reduced to 50, 60, 70, 80 and 90% of the original area.

Results and discussion

With composite films it is more meaningful to express permeability data as gas transmission rates (GTR) rather than permeabilities or permeability coefficients which include a thickness term. The GTR of a film may be defined as the amount of gas passing through a unit area of the film in unit time under a unit partial pressure difference across the film. Data presented for oxygen in this paper are termed oxygen transmission rates (OTR). Conditions of temperature and relative humidity are defined and average thickness of the test film is specified.

Table 1. Oxygen transmission rates for packaging materials at 25°C and 75% r.h.

Material composition	Average thickness (mm)	Oxygen transmission rate*
Polyethylene (high density)	0.031	3140
Polyester/polyethylene	0.046	100.0
Nylon/polyethylene	0.110	60.6
Nylon/ionomer	0.090	91.2
" "	0.080	44.5
Nylon/ionomer/polyethylene	0.100	46.5
Metallised nylon/EVA†	0.090	1.0
EVA/PVDC‡/EVA	0.060	37.5
PVDC/PVA1§/PVDC/EVA	0.077	2.1
PVDC/polypropylene(PVDC/EVA	0.100	18.4
Polyester/Alfoil/polyester/ polypropylene copolymer	0.123	<0.2

*Units: ml m^{-2} $24h^{-1}$ atm^{-1}; †EVA, ethylene vinyl acetate copolymer; ‡PVDC, polyvinylidene chloride; §PVA1, oriented polyvinyl alcohol.

Effect of composition

The composition of a film has a major influence on oxygen transmission rate (Table 1). Polyethylene offers little resistance to the passage of oxygen and will not maintain an environment which will inhibit aerobic spoilage organisms. On the other hand a laminate film which contains aluminium foil has great resistance. However, the foil laminate is opaque and hence is unsuitable for packaging fresh meat. Of the transparent films, those which include PVA1 and/or PVDC have the lowest oxygen transmission rates.

All the film products currently available in Australia for vacuum packaging fresh meat have either nylon or PVDC included as the primary barrier to oxygen. As shown in Table 1, the OTR of one of the five nylon based films was considerably higher than the others. It has been found (E. G. Davis, personal communication) that the thickness of nylon in some samples of nylon/polyethylene film varies considerably from the nominal thickness (usually 0.020–0.025 mm), and it is possible that the thickness of nylon in this sample was less than that in other samples. Unfortunately, inability to delaminate the ionomer material from the nylon made it impossible to confirm this.

Effect of temperature

Data presented in Table 2 show the effect of temperature on the oxygen transmission rates of several films which contain nylon or PVDC. The oxygen

Table 2. Effect of temperature and relative humidity on the oxygen transmission rates of several films

	Thickness (mm)	Oxygen transmission rate* 25°C 75% r.h.	3.5°C 75% r.h.	0°** 75% r.h.	25°C 98% r.h.	3.5°C 98% r.h.	0°C** 98% r.h.
EVA/PVDC/EVA	0.060	37.5	3.0	1.8	40.0	3.4	2 2
Nylon/ionomer	0.090	91.2	12.2	8.5	156	15.4	10.5
Nylon/ionomer/pe	0.100	46.5	4.9	3.3	110	8.2	5.3
PVDC/PVA1/ PVDC/EVA	0.077	2.1	0.1	0.05	4.2	0.1	0.05

*Units, As for Table 1, **Values estimated using Arrhenius' equation.

transmission rate of each type of film at 3.5°C was generally less than 10% of the rate at 25°C.

With most polymers oxygen permeability is related to temperature according to the Arrhenius relationship which means that the effect of temperature on OTR may be studied conveniently from a plot of $\log_{10}$ OTR *vs* the reciprocal of the absolute temperature. Frequently such a plot produces a straight line which makes it possible to estimate OTR values with reasonable accuracy at temperatures other than those studied. The relationship was used to obtain the estimated values at 0°C which are presented in Table 2. At 0°C, the recommended storage temperature for chilled vacuum packaged meat, OTRs were less than 10% of those at 25°C.

Seventy-five percent and 98% are near the extremes of r.h. likely to be experienced by packages containing vacuum packaged meat. The effect of temperature is significant at both relative humidities. From Table 2 it may be calculated that the percentage reduction in OTR for the four samples of film tested at 25°C and then at 3.5°C at 98% r.h. range from 90.1 to 97.6%. At 75% r.h. the percentage reductions for the same four films range from 86.6 to 95.2%.

The relative humidity of the atmosphere influenced the oxygen transmission rates of nylon based barrier films (Table 2). Increases in relative humidity from 75 to 98% increased the OTRs significantly at 25°C. Rigg (1979) also reported a rapid increase in the oxygen transmission rate (at 25°C) at conditions above 70% r.h. for nylon film suitable for meat packaging. In contrast, changes in r.h. had a negligible effect on films in which PVDC is the gas barrier. Polyvinyl alcohol is hydrophilic. Its barrier characteristics were affected by high r.h. at 25°C, even when sandwiched between PVDC. However, there was no measurable effect of humidity at 3.5°C. From Table 2 the effect of high r.h. on nylon films at the lower temperature of 3.5°C is again evident but much less pronounced. The magnitudes of the increases were very small when compared with those at 25°C. It is likely that the characteristics of nylon could be improved if it was similarly isolated from water by, say, PVDC.

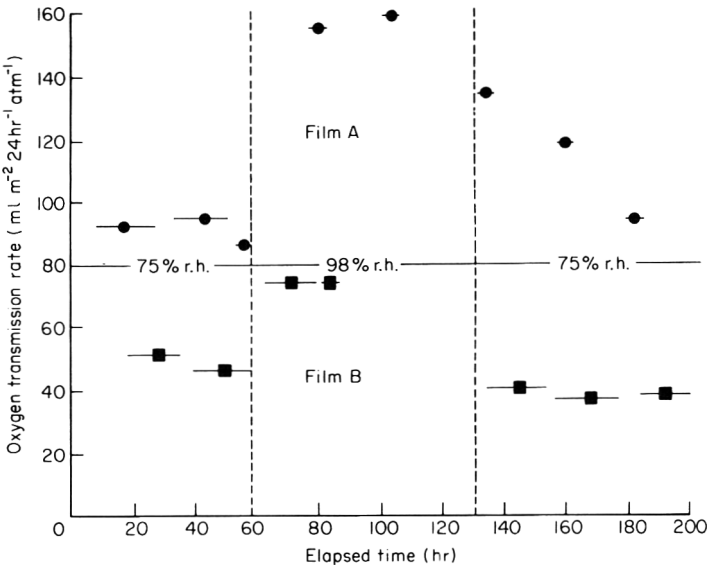

Figure 1. Oxygen transmission rates of two nylon-containing films as affected by short exposure to high humidity. On the figure lengths of lines denote measurement times.

In an effort to determine whether a short exposure of meat packaging films to high humidity had a lasting effect on the OTR values of nylon based film, two samples of film were subjected to changes in r.h. from 75% to 98%, and finally back to 75%. Sufficient time was allowed for equilibration to occur at each r.h. The data presented in Fig. 1 show the increases in OTR after the r.h. was raised from 75 to 98%. When the r.h. was changed back to 75%, reductions in the OTR back to the rates originally measured at 75% r.h. resulted. This reduction occurred over a period of approximately 50 hr for film A and 32 hr (or less) for film B. This indicates that the effect of high humidity on nylon based films is reversible.

Bags made of EVA/PVDC/EVA film are used extensively for vacuum packaging meat. This film is heat shrinkable. Heat shrinking reduces the total surface area of the film and increases the film thickness. It is apparent from Table 3 that there is a close relationship between OTR and degree of shrink.

Practical implications

The films most widely used for vacuum packaging are either nylon laminated to polyethylene or an equivalent heat sealable material, or PVDC co-extruded between EVA. Both these films perform well in commercial practice. Although there are films which have better barrier properties (e.g. the film containing PVDC and PVA1), it has yet to be shown that these films give better performance.

The OTR of the films tested were much lower at temperatures near 0°C than

Table 3. Effect of heat shrinking on oxygen transmission rate of EVA/PVDC/EVA film

Percentage of original area	Average thickness (mm)	Oxygen transmission rate*	
		At 25°C	At 3.5°C
100 (not shrunk)	0.060	37.5	3.0
90	0.074	34.8	–
80	0.080	30.4	–
70	0.083	30.0	2.0
60	0.095	23.3	2.0
50	0.110	20.4	2.0
47.2 (unrestrained)	0.140	17.6	–

*Units: As for Table 1.

at 25°C. Oxygen permeability/transmission rate values which are measured and quoted at temperatures of 20–25°C therefore do not indicate the performance of a film at 0°C, the recommended storage temperature for chilled vacuum packaged meat. At best, those films with the lowest OTR at 25°C generally have the lowest OTR at the lower temperature. This relationship cannot always be assumed, however (Davis, 1971).

The effect of humidity on the oxygen transmission rate of nylon containing film is marked at 25°C. High humidities (near 100%) result in elevated OTRs. However, at a temperature near the recommended storage temperature for chilled vacuum-packaged meat (0°C) the influence of humidity is far less in magnitude. Humidity has a negligible effect on the OTR of films which contain PVDC as the primary barrier.

In normal commercial packaging practice the outer surfaces of packs are most unlikely to be at ambient humidities near 100% for long periods. Nylon pouches will normally only be at high ambient humidity while they are in boning rooms, i.e. a short term exposure at a temperature of around 10°C. It should be appreciated, however, that vacuum packaged meat will provide water to the polyethylene laminate (inner layer) of nylon polyethylene pouch. Moisture will diffuse through the polyethylene into the nylon. In the confined spaces of a carton it is likely that the rate of evaporation from the nylon will be slow. A possible nett result is that nylon will be maintained in a state which permits a high oxygen permeation rate.

In most vacuum packaging establishments the size of the bag is tailored to the size of the particular cut of meat. For bags which may be heat shrunk the OTR is reduced approximately in proportion to the reduction in surface area. From limited observations I have estimated that in practice the area of a shrunk pack is usually no less than 70–80% of the original area.

OTR measurements at low temperature (near 0°C) and at high humidity (near 100% r.h.) probably provide the best estimates of the quantity of oxygen

which will permeate through a film to be used for the vacuum packaging of chilled meat. It is recognized, however, that measurements at ambient temperature are much more convenient with the type of measuring equipment used to obtain the data for this report, because duplicate measurements on one sample of film at low temperature require approximately 2 weeks. If measurements are made at ambient temperature, the relationships discussed in this report will prove useful in the estimation of the approximate OTRs at 0°C.

Acknowledgments

Mr E. Davis, CSIRO Food Research Laboratory, North Ryde, provided considerable technical advice. The author also wishes to thank the suppliers of the test films.

References

Davis, E.G. (1971) *Fd Pres. Q.* **31,** 47.
Davis, E.G. & Huntington, J.N. (1977) *CSIRO Fd Res. Q.* **37,** 55.
Karel, M., Issenberg P., Ronsivalli, L. & Jurin, V. (1963) *Fd Technol., Champaigne,* **17,** 327.
Newton, K.G. & Rigg, W.J. (1979) *J. Appl. Bacteriol.* **47,** 433.
Rigg, W.J. (1979) *J. Fd Technol.* **14,** 149.
Rogers, C., Meyer, J.A., Stannett, V. & Szwarc, M. (1965) *Tappi,* **44,** 741.

(Received 11 August 1980)

J. Fd Technol. (1981) **16**, 81–91

Preparation of blood globin through carboxymethyl cellulose chromatography

Y. SATO, S. HAYAKAWA AND M. HAYAKAWA

Summary

A simple and inexpensive procedure for globin preparation from blood of various animals was developed in order to use globin as human food. Carboxymethyl cellulose was most effective among some cation exchangers tested, although amounts of haemoglobin applied to 1 g of the exchanger should be limited to less than 70 mg. The behaviour of adsorbing haem or haemin to the exchanger could not be explained only by a general theory of ion exchange chromatography. Yields and Fe contents of globin preparations from various haemoglobins by this procedure ranged from 67 to 80% and from 0.008 to 0.03% respectively, except for a chicken haemoglobin sample.

Introduction

Blood collected in a slaughter house is a good source of edible protein. Blood serum or plasma proteins have been prepared industrially and used successfully as an ingredient of sausage (Pedersen, 1979). Although Delaney (1977) and Pedersen (1979), taking notice of the nutritive effect of Fe, have developed a new treatment to use red blood cell or haemoglobin as feed, it would most likely still be unsuitable for human food because of its disagreeble colour and odour. For this reason, in Japan at present, it is either used as a nitrogenous fertilizer or disposed of even at some expense.

Globin has successfully been prepared from blood using acid-acetone (Rossi Fanelli, Antonini & Caputo, 1958), but a procedure for globin preparation without organic solvents has not been established. If globin could be prepared from blood by a simple, inexpensive procedure without an organic solvent, it would probably be judged as suitable for food or feed purposes. This study is aimed at finding a new method of globin preparation without the use of an organic solvent.

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0022–1163/81/0200–0081 $02.00

Materials and methods

Haemoglobin samples

Human, bovine, ovine and porcine haemoglobin, which were crystallized twice, were purchased from Sigma Chemical Co. (St Louis). Purchased haemoglobins were used without further purification. Chicken and a bovine haemoglobin prepared from blood in our laboratory were also used as examples of haemoglobin industrially applied. The preparation method of crude haemoglobin from blood is as shown in Fig. 1. Bovine haemoglobin prepared in our laboratory and a purchased one were designated as bovine haemoglobin L and P, respectivley.

Screening method of ion exchangers

SP-sephadex C-50, Amberlite CG-120, Dowex 50W-x4, as strongly acidic exchangers, and Amberlite CG-50 and Carboxymethyl cellulose (CMC), as weakly acidic exchangers, were tested.

Each ion exchanger (about 0.05 g, unless otherwise stated) was swollen in 10 ml of 0.01 N HCl (pH 2). To each ion exchanger suspension 1 ml of 1% bovine haemoglobin P solution adjusted to pH 2 was added and the mixture was shaken and centrifuged. The optical densities at 375 and 280 nm of the supernatant were measured. The ratio of the optical density at 375 and 280 nm of 1% haemoglobin of pH 2 is 1.12.

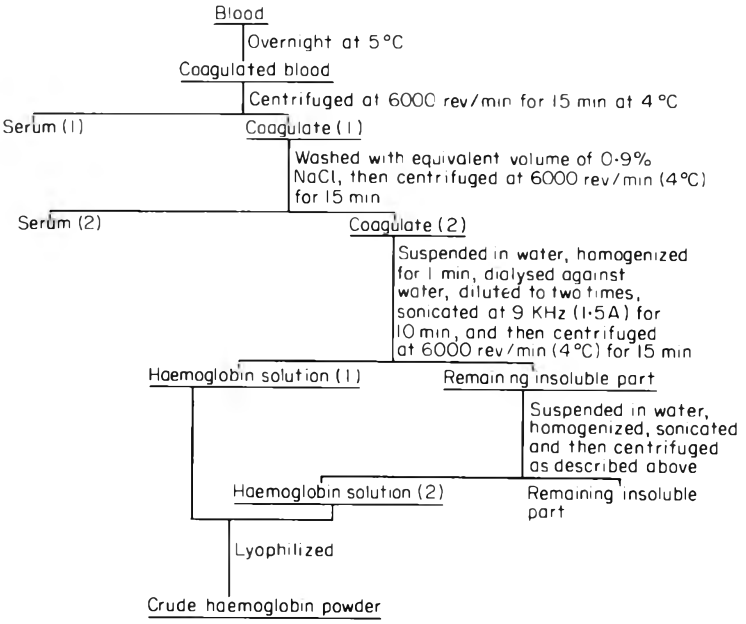

Figure 1. Procedure of haemoglobin preparation from blood.

When either haem or globin is adsorbed, the optical density ratio of the supernatant should be lower or higher than 1.12. When both globin and haem are adsorbed to an exchanger and optical densities at 375 and 280 nm decrease simultaneously, the exchanger may be undesirable for the removal of haem.

Electrophoresis

Slab gel electrophoresis was performed on 7.5% polyacrylamide gel (pH 4.3) containing 0.006 M KOH, 0.378 M acetic acid and 5 M urea. After setting the sample solution into a slot, the electrophoresis was conducted at 15 mA/cm^2 and for 3 (for haemoglobin L) or 4 hr (for haemoglobin P) using the running buffer (pH 4.5) consisting of 0.35 M β-alanine and 0.14 M acetic acid.

Sodium dodecylsulphate (SDS) polyacrylamide gel electrophoresis was performed on slab or tube of 8% polyacrylamide gel (pH 8.9) containing 0.378 M Tris, 0.06 M HCl and 0.1% SDS. The electrophoresis was run at 5 mA/cm^2 for 16 hr (for slab) or 3 mA/tube for 3.5 hr (for tube) using 0.2 M Tris HCl (pH 8.6) solution as running buffer. The internal diameter of the tube was 5 mm.

After these gel electrophoreses, gels were stained with 0.25% Coomassie brilliant blue R 250.

Yields of globin preparations

Yields of globin preparations were calculated as a percentage of globin nitrogen on the basis of total nitrogen of the haemoglobin sample.

Analysis of globin preparations

Analysis of iron content in the prepared globin. A sample in a crucible was burnt in an electric muffle to ash and the ash was dissolved in 1 N HCl and diluted with distilled water. Iron content in the solution was determined with an Atomic Absorption Spectrophotometer using $FeCl_2$ as the standard. The iron content was expressed as μg Fe per mg of each sample.

Analysis of amino acids. After the protein solution was hydrolyzed with 6.0 N HCl at 110°C in an evacuated and sealed tube for 24 hr, the amino acid composition was analysed with a JEOL auto amino acid analyser (JLC-6AH).

Results

Selection of effective ion exchanger

Table 1 shows that a weakly acidic ion exchanger, especially CMC, is promising for the preparation of globin. On the basis of this finding, a procedure for separating globlin by CMC was investigated through this study.

Table 1. Screening of ion exchanger for the separation of globin

Ion exchangers	Optical density at 280 nm in the supernatant	The ratio of optical density at 375 nm at 280 nm in the supernatant
SP-Sephadex C-50	1.17	0.9
Amberlite CG-120 type 1	1.92	1.0
Dowex 50W-x4	1.90	1.1
Amberlite CG-50 type 2	1.83	1.0
Carboxymethyl cellulose	1.67	0.9
Carboxymethyl cellulose (200 mg)	1.64	0.7
Control (haemoglobin)	1.88	1.1

Effect of NaCl concentration on the chromatographic separation of globin

CMC (about 7 g) was poured into a glass tube (2.3 × 18 cm) with 0.01 N HCl and equilibrated with the same solution. Forty five ml of 0.5% bovine haemoglobin P – 0.01 N HCl solution (180 mg of the protein) was applied to the column. Then the column was eluted with 1000 ml of 0.01 N HCl having a linear gradient from 0 to 0.1 M NaCl. Flow rate in the elution was approximately 2 ml per min. The result is shown in Fig. 2 with the gradient of NaCl concentration. The first peak monitored by optical density at 280 nm is likely to show a globin fraction with a slight absorption at 420 nm indicating the presence of a small

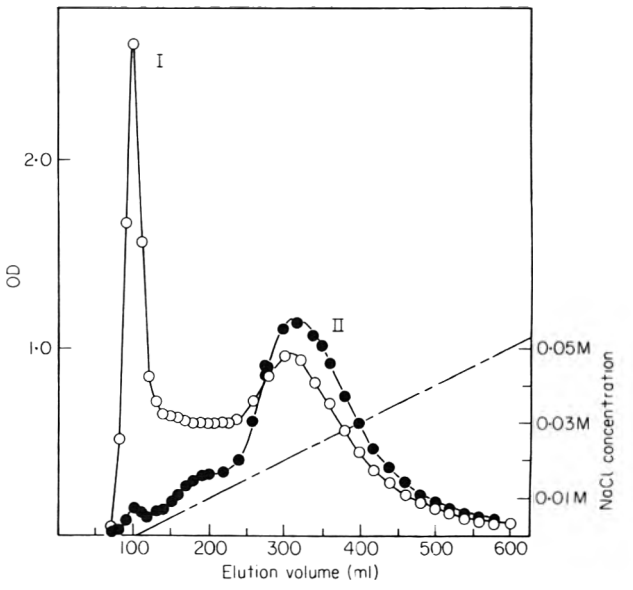

Figure 2. Effect of NaCl concentration on separation of haem from haemoglobin in the CMC column chromatography. I: the first peak, II: the second peak. ○, OD_{280}; ●, OD_{420}; ———, NaCl.

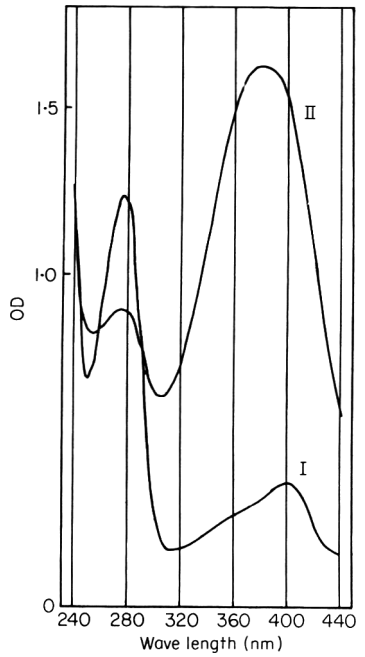

Figure 3. Spectra in the soret and ultraviolet region of the first and the second peak component in the CMC chromatogram of haemoglobin. I: the first peak (0.01 N HCl solution) II: the second peak (0.01 N HCl solution containing 0.1 M NaCl).

amount of haem or haemin. As optical density at 420 nm was increased with the increase of NaCl concentration, it appeared that haem was eluted more and more with the solution of higher NaCl concentration.

Generally speaking, there are two peaks in spectra of the soret region of the protohaem, and intensities of two peaks at 375 and 410 nm are changeable according to kinds of solvent (Clezy & Morell, 1963). As shown in Fig. 3, maximum absorption in spectra of the soret region of the first peak fraction appeared around 410 nm and that of the second peak fraction around 375 nm. In the column chromatography by CMC, haem was monitored at 420 nm for convenience sake in the experiment.

Chromatography for globin preparation

Thirty ml of 0.56–0.57% bovine haemoglobin L solution (170 mg of the protein) dialysed against 0.01 N HCl was applied to the column filled with CMC equilibrated with 0.01 N HCl. The column with sample was eluted with 300 ml of 0.01 N HCl. Then 0.01 N HCl solution containing 0.1 M NaCl was used for elution as a second step. After the elution of 500 ml in the second step, 0.1 N NaOH was used for elution as a third step. The elution pattern of the haemoglobin is shown in Fig. 4.

Almost the same results were obtained using bovine (P), chicken, ovine,

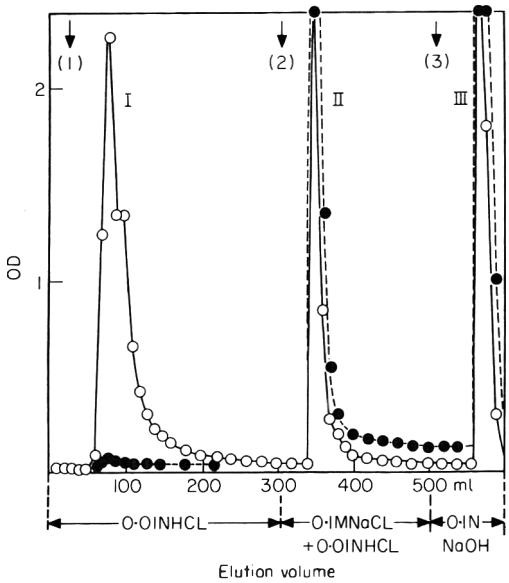

Figure 4. Elution profile in the CMC column chromatography of bovine haemoglobin L solution. I: the first peak eluted by 0.01 N HCl (equivalent to 69% of total nitrogen), II: the second peak eluted by 0.01 N HCl containing 0.1 M NaCl (equivalent 18% of total nitrogen), III: the third peak eluted by 0.1 N NaOH (equivalent to 3% of total nitrogen), Arrows (1), (2) and (3) express the beginning of elution by each solution. ○, OD_{280}; ●, OD_{420}.

porcine and human haemoglobin, although the eluted patterns of their chromatograms were omitted here.

Analysis of the peaks components

The eluates of the first and the second peaks in the column chromatography of bovine haemoglobin P were collected, dialysed against distilled water and then lyophilized, respectively. The lyophilized samples were dissolved into a small amount of the gel buffer. Electrophoresis of the solution on the slab gels was carried out at room temperature. From the result as shown in Fig. 5, the first peak component was assumed to be globin.

The electrophoretic patterns of the first and the second peak components in the column chromatography of bovine haemoglobin L are shown in Fig. 6. The result suggests that contaminating serum proteins are concentrated in the second peak and globin is mainly concentrated in the first peak.

Table 2 showed that amino acid composition of the first peak component from bovine haemoglobin P was almost the same with that of bovine original haemoglobin. Table 3 showed that amino acid composition of the first peak component from chicken haemoglobin was the same as that of chicken original haemoglobin.

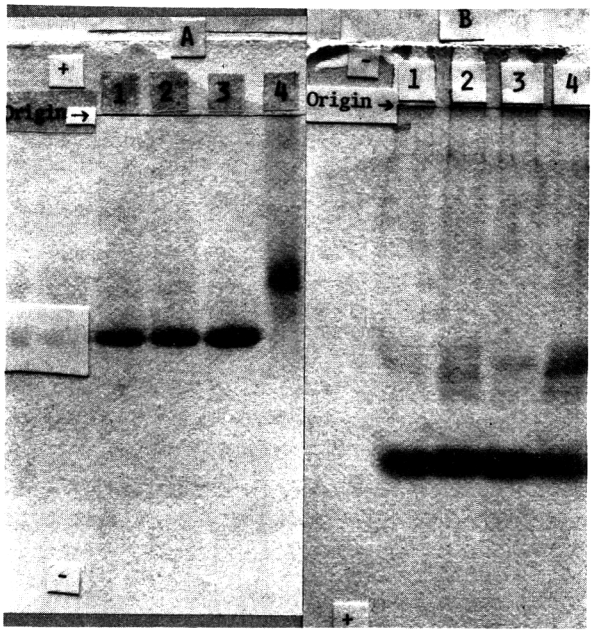

Figure 5. Electrophoretic patterns of the first and the second peak components in the CMC column chromatography of bovine haemoglobin P. (A) Slab polyacrylamide gel electrophoresis; (B) SDS polyacrylamide gel electrophoresis (slab), 1, haemoglobin, 2, globin preparation by acetone method, 3, the first peak component, 4, the second peak component.

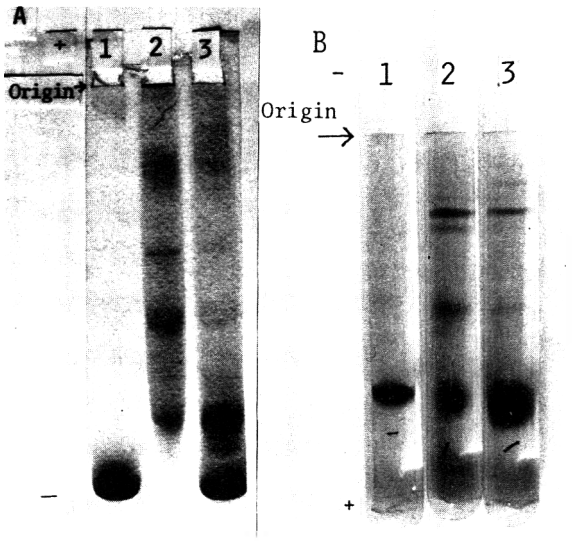

Figure 6. Electrophoretic patterns of the first and the second peak components in the CMC column chromatography of bovine haemoglobin L. (A) Slab polyacrylamide gel electrophoresis, (B) SDS polyacrylamide gel electrophoresis (Tube), 1, the first peak component (I in Fig. 4), 2, the second peak component (II in Fig. 4), 3, haemoglobin.

Table 2. Amino acid composition of bovine globin

Amino acid	Percentage in mol. of amino acid				
	Haemoglobin	Globin of I*, by CMC method	Globin of II *, by CMC method	Globin by acetone method	Haemoglobin (References†)
Asp	9.9	8.8	10.3	10.0	9.5–9.9
Thr	5.0	5.4	4.5	5.2	5.0
Ser	6.0	6.9	4.7	6.3	6.4–6.7
Glu	6.7	5.5	7.7	6.1	6.0
Pro	3.9	4.2	3.6	3.9	3.5
Gly	7.6	7.1	7.9	7.4	7.1–6.7
Ala	12.7	13.4	12.3	12.9	12.7
Cys/2	0.32	0.26	0.42	0.27	0.4
Val	8.4	8.7	11.2	8.7	10.6
Met	1.6	1.4	1.7	1.7	1.4
Ile	0.2	0.17	0.71	0.41	0
Leu	13.0	13.3	11.2	12.9	13.1
Tyr	1.9	2.3	1.6	2.1	1.8
Phe	5.7	5.4	6.6	5.6	6.0
His	5.8	6.5	4.6	5.9	5.7–6.0
Lys	8.6	8.2	8.1	8.2	8.5–7.8
Arg	2.7	2.5	2.8	2.7	2.5
Trp	ND	ND	ND	ND	ND
Total	100.0	100.1	99.9	100.3	100.2

*1, the first peak in Fig. 4, II, the second peak in Fig. 4, † Dayoff, 1972; ND, not determined.

The moving distance in the slab gel electrophoresis of the second peak component in the column chromatography of bovine haemoglobin P is different from that of the first peak component. The amino acid composition of the second peak component is slightly different from that of the first peak component. When electrophoresis was performed with SDS, the moving distance of a part of the second peak component became the same as that of the first peak component. These experimental findings may indicate that the second peak component contains a small amount of complex of α or β subunit of globin and contaminating proteins.

Yields and iron contents of various globin preparations

Yields and iron contents of globin preparations from haemoglobin of human and various domestic animals were shown in Table 4.

Yields of globin preparations ranged from 67 to 80% except for chicken globin. Lower yields of globin in chicken seems likely to be due to the different properties of avian, as opposed to mammalian, haemoglobin.

Iron contents of globin preparations ranged from 0.008 to 0.04%. Percen-

Table 3. Amino acid composition of chicken globin

Amino acid	Amino acid composition of globin (%)*		
	By CMC method†	By acetone method	Amino acid composition of haemoglobin
Asp	9.1	9.3	9.2
Thr	5.8	5.8	5.8
Ser	4.9	4.8	4.8
Glu	7.9	8.4	8.3
Pro	4.3	3.4	3.7
Gly	6.5	6.4	6.3
Ala	11.7	12.2	11.7
Cys/2	1.5	1.0	1.9
Val	7.4	7.9	7.3
Met	1.1	1.1	1.1
Ileu	4.3	4.2	4.2
Leu	11.3	11.7	11.4
Tyr	2.5	2.2	2.4
Phe	5.2	5.2	5.5
His	5.4	5.4	5.5
Lys	7.9	7.3	7.8
Arg	3.2	3.5	3.2
Trp	ND	ND	ND
Total	100.3	99.8	100.1

*Percentage in mol; †, the first peak only; ND, not determined.

Table 4. Yields and Fe content of the prepared globins

Sources of haemoglobin	Yields* of crude globin (%)	Fe content (%) cf crude globin	Fe removed from haemoglobin sample (%)
Human	80	0.024	93
Chicken†	52	0.041	85
Porcine	67	0.018	95
Ovine	78	0.0083	98
Bovine	69	0.0086	97
Bovine†	71	0.0096	97

*Percentage of the prepared globin nitrogen on the basis of total nitrogen of the haemoglobin sample. Average value in three trials.
†Prepared in our laboratory.

tage of removed iron, (1 – Fe content of globin preparation/Fe content of haemoglobin sample) × 100, ranged from 85 to 98%. These findings suggested that the method by CMC column chromatography is rather suitable for the preparation of blood globin used as food and feeds.

Discussion

It is widely accepted that imidazol groups of histidine residue and/or γ-or δ-carboxyl groups of aspartic or glutamic acid residue of globin participate in the binding between Fe of protohaem and globin (Wymann, 1948; O'Hagan, 1959). The bonds are probably broken in 0.01 N HCl solution to separate haem and globin, in that case globin becomes positively charged and haem or haemin may form simple salt with Cl ion. This is the reason why an anion exchanger was not tested in this study.

When haem is removed from haemoglobin by acidification, globin may be dissociated into α and β subunit. It is probable, therefore, that globin in the first and the second peak of the chromatogram consists of α and β subunits in a different ratio from that in original haemoglobin. The discrepancy in the mol percentage of several amino acids in globin fractions contained in the first and the second peak from bovine haemoglobin P, may be due to the different combination of two subunits. On the other hand, amino acid composition of globin in the first peak from chicken haemoglobin is somewhat comparable to that of original haemoglobin. It is reasonable to consider that the coincidence may be due to the similarity of amino acid composition of globin subunits of chicken haemoglobin (Dayhoff, 1972), and not due to the similarity of combination of globin subunits in the first and the second peak from chicken haemoglobin.

CMC equilibrated with 0.01 N HCl should be in the non-ionized H-form. Thus, in the column chromatography by the CMC, haem seems to loosely bind to the CMC, perhaps due to the formation of a co-ordinate bond between non-ionized carboxyl group and Fe of haem or haemin.

When simple cellulose was used in chromatography, neither haem nor globin was adsorbed. Thus, the carboxymethyl group of the ion exchanger might be indispensable for adsorption of haem or haemin. When a haemoglobin solution contains Na ions, haem or haemin is more incompletely adsorbed with the increase of the concentration of Na ion. Hence, absence of Na ion might also be indispensable for adsorption of haem or haemin. NaCl might interupt the co-ordination between Fe and carboxyl group, just as carboxyl groups co-ordinated for chromium in a chrome complex. For example, $[Cr{\cdot}3\ H_2O{\cdot}2\ COO]^+Cl^-$, is exchanged with Cl ion in the presence of NaCl. However, without NaCl the complex remains in itself.

In this chromatography, amounts of haemoglobin applied to 1 g of CMC should not be over 70 mg, because haem or haemin adsorbed to CMC seems to

be slowly eluted after globin elution and can not be explained only by the general theory of ion exchange chromatography. Further study on the procedure for increasing the applied amount of haemoglobin is now in progress.

Acknowledgments

The support of the Ryoshoku Kenkyukai in Japan for this project is gratefully acknowledged.

References

Clezy, P.S. & Morell, D.B. (1963) *Biochim. Biophys. Acta,* **71,** 165.
Dayhoff, M.O., (1972) *Atlas of Protein Sequence and Structure,* Vol. 5. National Biochemical Foundation, Washington D.C.,
Delaney, R.A.M. (1977) *J. Fd Technol.* **12,** 339
O'Hagan, J.E. (1959) *Nature, Lond.,* 183, 393.
Rossi Fanelli, A., Antonini, E. & Caputo, A. (1958) *Biochim. Biophys. Acta,* **30,** 608.
Tybor, P.T., Dill, C.W. & Landmann, W.A. (1975) *J. Fd Sci.* **40,** 155
Wismer-Pedersen, J. (1979) *Food Technol. Champaigne.* **33,** (8) 76.
Wymann, J. (1948) *Advances in Protein Chemistry,* eds. M.L. Anson and J.T. Edsall, Vol. 4, p. 407. Acad. Press Inc., N.Y.

(Received 18 August 1980)

J. Fd Technol. (1981) **16,** 93–100

Book reviews

Functionality and Protein Structure. (ACS Symposium Series, No. 92) Ed. by A. Pour-El.
Washington, D.C.: American Chemical Society, 1979. Pp xii + 243. ISBN 0–8412–0478–0 $24.50.

This book is based on a symposium sponsored by the American Chemical Society Division of Agriculture and Food Chemistry at the 175th Meeting of the ACS, Anaheim, California, March 13, 1978. It contains twelve chapters, many of which are written by authorities who are well known for their work on proteins and protein functionality. A summary of the contents is as follows: functionality of oilseed and soy proteins (four studies); gel formation, emulsifying properties, and formation of coacervate systems (three studies); enzyme-to-protein binding (one study); lysinoalanine formation (one study); milk proteins, wheat gluten proteins, and chemical modification of plant and yeast proteins (three reviews).

Editor Pour-El has defined functionality to be 'any property of a substance, besides its nutritional ones, that affects its utilization'. During the last decade, there has been an increasing awareness of the importance of such properties with respect to the proteins of foods and those proteins employed as food additives. Food scientists and technologists working in this area will find *Functionality and Protein Structure* a valuable source of relevant information.

The three reviews in this book are well-written introductions to the relevant literature: those on milk and gluten proteins being concise, yet informative; and that on the chemical modification of plant and yeast proteins (Kinsella & Shetty) being comprehensive and thought-provoking. It is also stimulating to have available, in one text, so many descriptions of food-related, physicochemical properties of proteins. As for the experimentalist, the various practical studies of functionality should be useful guides to the wide range of techniques that are currently available for this purpose.

This book is recommended to those who are directly concerned with the functional properties of proteins, although anyone with a more general interest in food proteins should find an opportunity to browse through it.

Alan L. Lakin

Effects of Heating on Foodstuffs. Ed. by R. J. Priestley.
London: Applied Science Publishers, 1979. Pp. ix + 417. ISBN 085334–797–2. £24.00.

In the Preface, the Editor describes this book as a 'collection of reviews to be used by researchers and processors'. These are mostly of a high standard and are well supplied with references. The chapter headings are as follows: Proteins (D. R. Ledward); Carbohydrates (C. T. Greenwood and D. N. Munro); Pigments (M. L. Woolfe); Vitamins (R. J. Priestley); Meat (D. A. Ledward); Poultry Meat (Ruth E. Baldwin and Owen J. Cotterill); Eggs (Ruth E. Baldwin and Owen J. Cotterill); Fisher (A. Aitken and J. J. Connell); Fruits (S. D. Holdsworth); Vegetables (S. D. Holdsworth); Milk and Dairy Products (R. L. J. Lyster); Cereals, Roots and Other Starch-Based Products (C. T. Greenwood and D. N. Munro).

In so far as commodities are concerned, the reviews provide valuable introductions to effects of the various heat treatments used during processing. The same can be said with respect to the effects of heat on proteins, carbohydrates and pigments, although the amount of space devoted to the basic chemistry of these food constituents is surprising; something not to be expected in a book with this title. Similarly, the chapter on meat contains a major section on meat structure and other background material which also seem out of place.

Inevitably, in a book of this kind, there is some duplication of content and there are always omissions. (Examples of the latter are the absence of adequate treatments of: pectic substances and the softening of vegetables on cooking; mechanisms associated with non-enzymic browning; thermal inactivation of enzymes and their reactivation.) The most significant omissions, however, are in relation to lipids and they occur because of an editorial decision, on account of reviews published elsewhere.

A more fundamental criticism of this book could be that it lacks homogeniety, being more like the proceedings of a symposium than a text with a unifying theme. Even so, it will be a useful asset to any library or reference collection because of its value as a 'timer saver' in that it is a collection of useful information taken from throughout the literature of food science and technology.

Alan L. Lakin

Soy Protein and Human Nutrition. Ed. by H. L. Wilcke, D. T. Hopkins and D. H. Waggle.
New York: Academic Press, 1979. US$25.00.

This book is the Proceedings of the Keystone Conference on Soy Protein and

Human Nutrition held in Keystone, Colorado, 22–25 May 1978. Despite its title, the subject matter is broad and contains a good deal of background information as well as up-dating aspects of soy-protein nutrition.

A description of the types of soy protein products in commercial use is given by D. Waggle and Kolar, followed by a chapter on the importance of functionality of vegetable proteins in foods by Wilda Martinez. This latter chapter follows the typically logical pattern of most of the presentations, dealing with the wide concepts of the meaning of functionality of foods and its measurement, before citing specific examples. From this chapter and later ones it is clear that not all soy isolates are the same, but that their specific properties very much depend on the extraction conditions used. On the whole the main components of isolated soy are the storage globulins, which have different properties from the structural proteins.

The most controversial paper in the Conference was that by Carroll, Huff and Roberts. Called 'Vegetable protein and lipid metabolism', it described experiments carried out in these authors' laboratory on the effect of animal and plant proteins on the serum cholesterol level of rabbits fed these materials. This paper elicited strong discussion, which is recorded in detail, making most interesting reading.

The chapter on the 'Effect of Soy Protein on Trace Mineral Availability' by O'Dell emphasises the role played by phytate, in particular phytate-protein interaction, in promoting loss of zinc by the body. Methods for removing phytate from foods are discussed. In 'Biological Active Substances in Soy Products' by Anderson and colleagues, they conclude that most toxic substances present in soy can be removed by proper processing, that flatulence causes discomfort and embarrassment but is not anti-nutritional and that increased consumption of soy protein will result in more cases of allergenicity, but this would be expected with any food product. Still on the toxicity aspects of soy, Struthers and colleagues stressed that the bound lysinoalanine formation during the production of soy concentrates and isolates was relatively unimportant, as rat studies have shown that only the free form of lysinoalanine has high potency in the causation of nephrocytomegaly in the rat. This disorder, which has only been demonstrated in the rat, and which has not been shown to lead to hyperplasia, is largely reversible, at least in short-term studies.

Human requirements for lysine and the sulphur-containing amino acids were described by Harper. Great emphasis was given in this Conference to methods of evaluating protein quality by animal and human assay, particularly those methods which should be used for the measurement of protein quality by regulatory agencies. Thus, despite the fact that two of the pages have been incorrectly assembled, this is a very valuable document, which should be of interest to workers in many fields.

Ann F. Walker

ICUMSA: International Commission for Uniform Methods of Sugar Analysis; Report of the Proceedings of the Seventeenth Session held in Montreal, 4–9 June 1978.

Peterborough: ICUMSA. 1979. xxi + 448 pp. Index. ISBN 0–905003–02–0. £20.00.

Scientists in the sugar industry are probably well aware of this series, and clearly many will have been connected in one way or another with the extensive collaborative trials which provide the base for the recommendations on standardization of methods adopted at the four-yearly meetings of the International Commission. For the reviewer, despite acquaintanceship with '*Sugar Analysis: ICUMSA Methods*', and involvement in standards work in other fields, perusal of this book was a revelation. Here, instead of mere instructions on methodology, we have meticulously detailed referee reports on thirty-one topics and a full account of the open discussion – even haggling – of experts from twenty-seven countries, leading to the final adoption of recommendations on methods and procedures. Many of the subject chapters contain extensive and useful tables, some resulting from collaborative physical measurements of the highest attainable accuracy and precision, such as those referring to temperature corrections for measured refractive index of sugar solutions, on polarimetry and density measurements, and on the rheological properties of molasses and massecuites. This is clearly an important volume of reports for all involved in sugar analysis, but it contains much that will be found instructive by those working in other fields of analytical chemistry, food quality evaluation. or methods standardization. In particular, the short referee's report on 'Sampling sugar and related products' (by E. G. Muller of Tate and Lyle Refineries Ltd) is a model of clarity in marrying statistical theory with 'practical politics'; the discussion on the extensive report on 'Specifications and tolerances for pure sucrose and reagents' (by G. Rens of Raffinerie Tirlemontoise SA, Belgium) illustrates the involvement of several countries in the final determination of accepted quantitative standards, while the discussion of the report on 'reducing sugars' (by R. Pieck of the same company) illustrates the importance of economics in methods selection – and, incidentally, a certain measure of irritation with the views of the EEC Commission on methods standardization. Later in the proceedings there was an outright rejection of the EEC proposed method for the determination of loss on drying of white sugars in recommendation 4 of the report on the 'Characteristics of white sugars' (by D. Hibbert of the British Sugar Corporation).

This book is, by no stretch of the imagination, to be considered as light reading – and I must admit I would probably have passed it by had I not undertaken to review it! Yet, on extensive perusal it continues to reveal more and more of interest and relevance far beyond the apparently narrow field to which it is dedicated. I complete my review with a heightened respect for ICUMSA and their style or work – which deserves, and will repay, study well outside their ranks.

E. C. Apling

Food Processing Waste Management. By J. H. Green and A. Kramer. West Port, Connecticut: AVI Publishing Company, 1979. Pp. xii + 629. ISBN 0–87055–331–3. $43.00.

A well written and readable book, giving comprehensive coverage to the whole range of American food processing waste management which, because of their similarity throughout the world, renders the volume of value in all English speaking countries. Its diversity and detail commends it to all specialist consultants and students of waste water treatment and management, whilst manufacturers will also find, not only points of interest in their particular processing, but a wealth of detail and general principles applicable to many different wastes.

The diversity of material does not detract from the logical and practical approach to waste management which is of use to every producer of food processing waste and also to pollution control staff of water, river or sewerage authorities.

Considerable space is devoted to the methods used to initiate and carry out waste surveys and the methods and equipment (American) used for sampling and flow measurement. Emphasis is placed upon the fact that the individuals best placed to understand how waste is produced are the staff employed in the processing industries themselves, albeit professional guidance is usually desirable to correlate the data and identify the options. The theory of flow measurement is well covered in practical terms without excessive detail of hydraulic and fluid mechanics theory and includes reference to more sophisticated techniques, such as ultrasonic instrumentation currently finding favour in this country.

The chapter on sampling could have been expanded, although the necessity for accurate and representative sampling is stressed and the advantages of different techniques discussed. More detail of the relative merits and findings of the different types of sampling apparatus would have been of value and here comparison of not only American but other commercially available equipment would have been extremely useful. Attention is wisely drawn to the storage and handling of samples dependent upon the different parameters under study.

Waste characterization by the use of laboratory and field equipment was a topic I found particularly well written and easy to understand. The diverse methods available to estimate waste organic matter and oxygen demand, together with the relationship between the results obtained by different methods, is especially well described. Correct emphasis is placed on the need for accurate and meaningful study of the individual processes before a comprehensive plan is formulated, a point often insufficiently stressed by other authors.

Regretfully, an omission in respect of these chapters on initial survey and characterization is a section or chapter dealing with the statistical handling of the data. Clearly to optimize the benefits of sophisticated sampling techniques and automated analytical processing requires correct statistical handling and interpretation of the considerable quantity of data which inevitably accrues.

The book strives to adhere to the logical route through the waste manage-

ment scheme of monitoring, in process modifications, treatment, regulations and economics, but I found the intrusion of specific examples of process modification relating to dry caustic peeling and whey treatment a little confusing. Similarly, whilst applauding the effort to provide additional reference value in limited space, the inclusion of examples by other authors tended to detract from the readability of the book. In my view these topics could have been covered more lightly or have been provided in an appendix.

A series of chapters deal with the general processes used to treat food waste authoratively and are well documented, particularly valuable to students and manufacturing staff not directly involved in waste management. Each of the more widely used techniques are fully described, although today the wider issues of total energy control might have warranted more detail and comparison of the relevant energy budgets of different options.

I found the chapters on U.S. legislation relating to the Food Industry of interest, if only for comparison with the advantages and shortfalls of British legislation. Similarly, the table of EPA analytical methods is useful for comparison rather than of direct value.

Hence I found the book informative and readily readable and worthy of a place on my reference shelves. My criticisms are of some inclusions which I have mentioned and which tend to break up the flow of the script, and a few omissions. It is well stocked with detail and well referenced, the use of tables in the appendices facilitating excellent cross reference. For the specialist, the text contains logical methods for waste management, a wealth of detail and references. For the student and non-specialist, a readable text which succinctly explains points coherently and in plain terminology, plus a comprehensive glossary. For me it highlighted the need for similar volumes covering the same and other industries which produce waste liquor, but related to the British and European views, legislation and fiscal arrangements of pollution control and waste management.

V. H. Lewin

Books Received

Hygienic Design and Operation of Food Plant. Ed. by R. Jowitt. Chichester, Sussex: Ellis Horwood, 1980. Pp. 292. ISBN 0–85312–133–8. £20.00 (paperback ISBN 0–85312–153–2, £7.50).

The proceedings of a symposium organized by the Food Engineering Panel of the Society of Chemical Industry on behalf of the Food Manufacturers' Federation, the Food Machinery Association and the Food Research Association, which was held at the National College of Food Technology, Weybridge on 19–20 April, 1978.

Cost Effective Quality Food Service: An Institutional Guide. By J. F. Stokes.
Germantown, Maryland: Aspen Systems Corp., 1979. Pp. xiv + 314. ISBN 0–89443–083–1. £15.50.

A manual designed to offer practical ways to contain costs in health care food service which covers such topics as purchasing, stores and inventory cost control; financial aspects of meal preparation; selection of energy-efficient equipment and energy cost management. Appendices, which comprise 137 pages, include representative menus and recipes for regular, soft/bland, low sodium, low fat and low residue diets.

Food Service Systems: Analysis, Design and Implementation. Ed. by G. E. Livingston and C. M. Chang.
New York: Academic Press, 1979. Pp. xv + 483. ISBN 0–12–453150–4. $27.50.

The 'updated' proceedings of a symposium organized by Food Science Associates Inc., and held in Framingham, Massachusetts during 1976. The book considers the problems of analysing and optimizing the major elements of food service systems, such as food, labour utilization, kitchen design, equipment selection, quality control, training, and microbiological and nutritional aspects.

World Review of Nutrition and Dietetics. Vol. 35. Ed. by G. H. Bourne.
Basel, Switzerland: S. Karger, 1980. Pp. x + 238. ISBN 3–8055–0442–X. Sw.Fr. 192.00/DM 230.00.

The topics reviewed in this volume comprise: anaemia during hard physical training; evaluation of Ile-Ife Nutrition Rehabilitation Centre; pancreatic lipase; organochlorine pesticide residues in food of animal origin in Italy; variations in the concentration of metabolites in the blood of cattle, and interpretation of metabolic profiles.

Food for All the Family. By M. Pyke.
London: John Murray, 1980. Pp. 256. ISBN 0–7195–3720–7. £6.50.

'A simple accessible book for anyone who has to plan meals for men and women, children, babies, old people, invalids and expectant mothers', as well as dogs, cats, rabbits, guinea pigs, and many other pets.

Planning for Cook-Chill, 2nd ed. By The Electricity Council's Project Planning Unit (Catering).
London: Electric Catering Centre, 1980. Pp. 59. Free of charge to caterers from Area Electricity Boards or from the Electricity Council, London.

A catering handbook which defines and describes cook-chill production methods in the context of the recommendations made by the Department of Health and Social Security.

Pesticide Residues and Food.
London: Consumers Association, 1980. Pp. 115. ISBN 0–85202–187–9. £25.00.

A study and commentary on the systems of control of pesticide residues in food in the U.K. and West Germany, discussed in the context of the EEC directive 76/895/EEC and draft directive COM(80)14.

The Genus *Penicillium* and its Teleomorphic States *Eupenicillium* and *Talaromyces*. By J. I. Pitt.
London: Academic Press, 1979. Pp. vii + 634. ISBN 0–12–557750–7. £40.00.

This book provides descriptions of 150 species of *Penicillium,* thirty-seven species of *Eupenicillium* and sixteen species of *Talaromyces*, based on colonial morphology after incubation under standardized conditions of medium, temperature and time, and microscopic morphology. Identification keys, and checklists of accepted species and of epithets are included.

JOURNAL OF FOOD TECHNOLOGY: NOTICE TO CONTRIBUTORS

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Typescripts (two complete copies) should be sent to the Editor, Dr H. Liebmann, c/o Institute of Food Science and Technology (U.K.), 105–111 Euston Street, London NW1 2ED. Papers should be typewritten on one side of the paper only, with a 1½ inch margin, and the lines should be doubled-spaced. In addition to the title of the paper there should be a 'running title' (for page headings) of not more than 45 letters (including spaces). The paper should bear the name of the author(s) and of the laboratory or research institute where the work has been carried out. The full postal address of the principal author should be given as a footnote. (The proofs will be sent to this author and address unless otherwise indicated.) The Editor reserves the right to make literary corrections.

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

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

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

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

SI UNITS

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

NON SI UNITS

inch	in	= 25·4 mm
foot	ft	= 0·3048 m
square inch	in^2	= 645·16 mm^2
square foot	ft^2	= 0·092903 m^2
cubic inch	in^3	= 1·63871 × 10^4 mm^2
cubic foot	ft^3	= 0·028317 m^3
gallon	gal	= 4·54611
pound	lb	= 0·453592 kg
pound/cubic inch	lb in^{-3}	= 2·76799 × 10^4 kg m^{-3}
dyne		= 10^{-5} N
calorie (15°C)	cal	= 4·1855 J
British Thermal Unit	BTU	= 1055·06 J
Horsepower	HP	= 745·700 W
Fahrenheit	°F	= 9/5 T°C + 32

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

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

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Journal of Food Technology
Volume 16 Number 1 February 1981

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Printed by Adlard and Son Ltd, Bartholomew Press, Dorking