VOLUME 3 NUMBER 2 JUNE 1968

JOURNAL OF FOOD TECHNOLOGY

PUBLISHED FOR

THE INSTITUTE OF FOOD SCIENCE AND TECHNOLOGY (U.K.)

BY

BLACKWELL SCIENTIFIC PUBLICATIONS

OXFORD AND EDINBURGH

JOURNAL OF FOOD TECHNOLOGY

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

Contributions and editorial correspondence should be sent to Dr E. C. Bate-Smith, A.R.C. Institute of Animal Physiology, Babraham, Cambs.

General correspondence should be sent to Dr. E. H. Steiner, British Food Manufacturing Industries Research Association, Randalls Road, Leatherhead, Surrey; and items for the Newsletter to Mrs J. V. Russo, Lyons Central Laboratories, 149 Hammersmith Road, London, W.14.

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

Business matters, including correspondence and remittances relating to subscriptions, back numbers, advertising and offprints, should be sent to the publishers: Blackwell Scientific Publications Ltd, 5 Alfred Street, Oxford.

The Journal of Food Technology is published quarterly, each issue consisting of 90–110 pages; four issues form one volume. The annual subscription is £5 ($17.50) post free; the price per issue is 30s ($5.00). Volumes 1 and 2 are still available at £6 ($20.00).

The Institute of Food Science and Technology of the United Kingdom was established in 1964. It is a professional qualifying organization having the following grades of membership: Fellows, Associates, Licentiates and Students. Application forms and information about the Institute can be obtained from the Honorary Secretary, Dr R. M. Johnson, Department of Food Science and Technology, Borough Polytechnic, Borough Road, London, S.E.1.

J. Fd Technol. (1968) **3**, 81–86.

Nutritional changes in proteins during heat processing

R. C. OSNER AND R. M. JOHNSON

Introduction

During the past few years considerable progress has been made in the study of protein changes, and some account of this has been included in general reviews on the nutritional effects of food processing (Rice & Beuk, 1953; Harris & von Loesecke, 1960; Bender, 1960, 1966). Inevitably under the conditions of food processing, some degradation of the protein occurs, but this is not necessarily harmful. In some cases protein breakdown is beneficial as it makes the food more digestible.

Nutritional losses occur when individual amino acids are destroyed or become metabolically unavailable. Interaction with carbohydrates or lipids present in foods is known to cause such losses, but amino acids may become unavailable even in the absence of these food components.

Interaction with carbohydrates

Many authors have reported losses in the nutritional value of proteins heated in the presence of carbohydrates, in cereals (Morgan & King, 1926; Morgan, 1931; Mitchell & Block, 1946; Rosenberg & Roldenburg, 1951; Halevy & Guggenheim, 1953; Hepburn, Calhoun & Bradley, 1966), in soybean meal (Evans & Butts, 1949a, b, 1951; Evans, Groschke & Butts, 1951; Iriarte & Barnes, 1966), in milk or milk products (McCollum & Davis, 1915; Cook *et al.*, 1951; Kraft & Morgan, 1951; Schroeder *et al.*, 1953), and in cake mixes (Block *et al.*, 1946). The protein efficiency ratio of a cake mix, for example, fell from 3·5 to 2·4 during baking for 15 min at 200°C and to 0·8 after toasting at 130°C for 1 hr. The growth potential of the cake protein was restored by the addition of lysine to the diet of rats. The fact that interaction between the proteins and the carbohydrates present is at least in part responsible for such losses has been confirmed by work on soybean and on milk and milk products (Mader, Schroeder & Smith, 1949; Lea & Hannan, 1949, 1950a, b, c; Henry & Kon, 1950; Evans & Butts, 1951; Evans, Butts & Bandemer, 1951; Evans *et al.*, 1951; Schroeder, Stewart & Smith, 1951; Halevy & Guggenheim, 1953; Schroeder *et al.*, 1953). The carbohydrates react with amino acid groups in the protein in typical Maillard reactions, and these have been reviewed elsewhere (Ellis, 1959; Reynolds, 1963, 1965). Several amino acids, particularly lysine, may become unavailable as a result of these interactions.

Authors' address: Department of Food Science and Technology, Borough Polytechnic, Borough Road, London, S.E.1.

Interaction with lipids

A reduction in the availability of certain amino acids may occur when meat or fish products are heated and this has been attributed to interaction with the lipids present. When fresh herring meal was processed for 30 hr at 100°C under nitrogen, no losses of available lysine occurred, but when oxidized fish meal was similarly treated, a 12% loss of the lysine was observed, accompanied by an increase in the bound lipids (Lea, Parr & Carpenter, 1960). Losses of lysine, arginine, histidine, threonine, valine, isoleucine, leucine and phenylalanine were reported when herring meal was flame-dried for 36 hr at 105°C (Clandinin, 1949), although methionine and tryptophane were unaffected. Miller (1956), however, heated dry fish for 24 hr at 105°C without nutritional loss. Pork processed at 110°C for 24 hr lost 44% cystine, 34% available lysine and up to 20% of other essential amino acids (Donoso *et al.*, 1962) although it was suspected that the nutritional losses were greater than the analytical figures would suggest. Beuk, Chornock & Rice (1948), however, reported that after processing at 112°C for 24 hr followed by enzymic hydrolysis, pork had lost 70% cystine and 50–65% of other essential amino acids. For beef, processed at 121°C for 1, 2, 3 and 4 hr, losses of available lysine of 13, 19, 21 and 35%, respectively, were reported (Dvorak & Vognarova, 1965).

In beef, pork or chicken processed at 121°C for 30 min, the amino nitrogen liberated was the same as for the unprocessed control (Schroeder, Iacobellis & Smith, 1961) but when pork was processed at 121°C for 4 hr, amino acids absorbed into the portal bloodstream of rats showed 33–95% increase compared with 101–340% increase for raw pork (Wheeler & Morgan, 1958). This suggests that the digestive enzymes *in vivo* cannot fully break down processed proteins. Mayfield & Hendrick (1949) reported that digestibility of beef processed at 121°C for 85 min decreased from 98 to 94% and the biological value from 86 to 79, but Morgan & Kern (1934) claimed that after 1 hr at 121°C, beef became slightly more digestible (99 compared with 97) although the biological value decreased. Pork, processed at 128°C for 3 hr had the same protein efficiency ratio as raw meat (Poling, Schultz & Robinson, 1944) and similarly treatment for 5½ min at 177°C produced a crisp bacon having the same nutritional value as raw bacon (Mcbride *et al.*, 1951).

The results of investigations so far do not present a clear picture on the interaction of proteins with fats during processing, although evidently both the digestibility and the biological value of proteins may be impaired. Some of the difficulties in interpreting and correlating results may be resolved if closer attention is paid to the nature and composition of the fats present, and especially their degree of oxidative rancidity.

When herring meal was stored in air for 2½ months at 25°C, the available lysine was reduced by 4% (Lea, Parr & Carpenter, 1958), although similar storage under nitrogen did not result in any loss. The peroxide value of the meal stored in air was higher than that under nitrogen. The nutritional value (as assayed with chicks) was not seriously impaired. It was further demonstrated that losses in available lysine occurred only when

the lipids were oxidized (Lea *et al.*, 1958). Subsequent research confirmed these results (Carpenter *et al.*, 1962). When oxidized meat meal replaced fresh meal in the diet of pigs, the growth rate was reduced significantly, but only in the 3rd to the 7th week (L'Estrange *et al.*, 1967). No significant difference in the growth rate for turkeys (Lea *et al.*, 1966) was caused by feeding them on white fish meal that had been mixed with anchovy oil and stored in air at 15°C for 3 months. Egg albumen complexes with oxidized linoleic acid, but not with unoxidized linoleic, oleic or lauric acids (Narayan & Kummerow, 1958), but the number of sulphydryl and hydroxyl groups in the complexed protein was reported to be the same as in the unreacted protein. It was inferred from this that a covalent bond is not formed and that the complex is held together by hydrogen bonding. Oxidized linoleic acid does not form a complex with lysine, glycine gelatine or sodium caseinate (Narayan & Kummerow, 1963). Egg albumen and lactalbumen each readily complex with oxidized corn oil at 60°C. Casein is much less reactive but fresh egg white reacts most readily. Increasing the temperature undoubtedly increases the action of oxidized unsaturated fats on proteins (Venolia & Tappel, 1958). The mechanism of this protein damage is not clear. One possible reaction, the Stricker degradation, has been reviewed elsewhere (Schonberg & Moubacher, 1952).

Proteins in the absence of carbohydrates or fats

Even in the absence of carbohydrates or fats, proteins may lose available amino acids and nutritional value during heat processing. The nutritive index of reconstituted casein, for example, was reduced after heating at 120°C for 1 hr (McCollum & Davis, 1915). The growth rate of rats fed gluten, previously toasted at 150°C for 30 min was lower than controls fed on raw gluten (Morgan, 1931), although the digestibility was similar. Nitrogen was lost mainly in the urine suggesting that the availability of the amino acids was lowered by the heat treatment. Rats fed with crystalline edistin previously heated at 121°C for 5 hr grew less than controls fed on unheated edistin (Waisman & Elvehjem, 1938). When lysine was added to the heat-treated edistin fed to the rats, growth improved. Rats fed on egg-white previously heated for 6 hr at 134°C maintained full growth (Hogan, 1917). In this food, lysine is not the limiting amino acid.

Beyond a certain point increasing the time or the temperature of heat-treatment usually increases the losses in the nutritive value of proteins. Thus although the nutritional qualities of casein heated at 100–105°C for 8 hr (Greaves, Morgan & Lovern, 1938) or cod heated at 100°C for 31 hr (Carpenter *et al.*, 1957) were not impaired, lactalbumen heated at 121°C for 30 min or 1 hr had nutritional indices of 100 and 85, respectively (Mader *et al.*, 1949), and digestibilities of 97 and 80. Furthermore, lactalbumen heated at 200°C for 1 hr lost more nutritive value than when heated at 140°C (Davis, Rizzo & Smith, 1949). The digestibility, 91 after 15 min at 140°C, was reduced to 30 after heating at 200°C.

Besides destroying amino acids, the action of heat may make certain amino acids unavailable. Lysine, present in casein, is damaged by heating at 130°C for 30 min (Greaves *et al.*, 1938), and at 140°C histidine is also damaged although cystine, tyrosine or tryptophane appear unaffected. Absorption of casein, processed at 140°C for 15 min, was reduced by at least 15% compared with an untreated control, and the protein index was reduced from 74 to 68 (Mabee & Morgan, 1951). The lysine liberated from casein by acid or alkaline hydrolysis was unaffected by processing the casein at 150°C for 65 min (Block, Jones & Gersdorff, 1934), but in samples treated for 70 min only 62–90% of the lysine could be detected after enzymic hydrolysis (Eldred & Rodney, 1946). With casein heated at 121°C for 20 hr, the available aspartic acid, cystine and proline as estimated after digestion *in vitro*, fell from 6·54, 0·50 and 10·69 to 6·17, 0·14 and 9·34% of the protein, respectively.

In cod muscle, the available cystine, methionine, lysine, tryptophan and leucine were reduced by heating at 85°C for 27 hr (Miller *et al.*, 1965). At 100°C the losses were greater and at 116°C more than half of the available cystine and tryptophan and at least one-third of the methionine, lysine and leucine were lost. When defatted herring presscake was heated for 27 hr at 130°C, losses of lysine, methionine, arginine and tryptophan occurred (Carpenter *et al.*, 1962). Although the methionine present was unaffected by heating soyprotein at 121°C for 4 hr, 5% cystine was destroyed (Evans *et al.*, 1951).

In general, provided the temperature does not exceed about 100°C and the period of heating is no more than 1 hr or so, little damage occurs, but as these conditions are exceeded, lysine, arginine, methionine, cystine, leucine, tryptophan and histidine may become metabolically unavailable to some degree.

The effect of moisture and pH

Although the nutritional value of cod muscle with 2–3% moisture was not reduced significantly by heating at 100°C for 31 hr, the same commodity with 11% moisture lost 28% of its nutritional value after heating at 105°C for 36 hr (Carpenter *et al.*, 1957). In defatted herring cake processed for 27 hr at 130°C, maximum amino acid binding occurred for lysine, methionine, arginine and tryptophan at a moisture level between 5 and 14% (Carpenter *et al.*, 1962). Cod muscle processed for 27 hr at 85°C lost more methionine, tryptophan and leucine with 50% moisture than with 14%, although the lysine was undamaged in both cases (Miller *et al.*, 1965). At 100–105°C, processing for 27 hr resulted in a greater loss of lysine with 14% moisture present than with 50% moisture although the reverse was true for methionine, tryptophan and leucine. Thus although moisture content undoubtedly has an effect, no simple generalizations emerge from the limited data available.

Little has been reported concerning the influence of pH upon amino acid losses. The amount of hydrogen sulphide produced when meat is heated is directly related to pH, whether the pH variation is artificial (i.e. due to the addition of acids or alkalis) or the

pH is increased by starvation (Johnson & Vickery, 1964). Excessive production of hydrogen sulphide is correlated with a relatively high pH. The production of hydrogen sulphide has been correlated with the decomposition of sulphur-containing amino acids in beef (Kugen, Golovina & Krasnaya, 1961) and it seems likely that increasing the pH of meat will therefore be accompanied by more destruction of the amino acids during processing. In the presence of 5% added glucose, pork or beef has been autoclaved at 121°C for 30 min at various pH values. Over the pH range 7–11 units, the amino nitrogen liberated after *in vitro* digestion decreased with increasing pH (Schroeder *et al.*, 1961). This indicates that more amino acids are bound at the higher pH values, but the influence of glucose under these conditions is not clear.

References

Bender, A.E. (1960) *Fd Process Packag.* **29,** 323.

Bender, A.E. (1966) *J. Fd Technol.*, **1,** 261.

Beuk, J.F., Chornock, F.W. & Rice, E.E. (1948) *J. biol. Chem.* **175,** 291.

Block, R.J., Cannon, P.R., Wissler, R.W., Steffe, C.H., Jr, Straube, R.L., Frazin, L.E. & Woolridge, R.L. (1946). *Arch. Biochem.* **10,** 295.

Block, R.J., Jones, D.B. & Gersdorff, C.E.F. (1934) *J. biol. Chem.* **105,** 567.

Carpenter, K.J., Ellinger, G.M., Munro, M.I. & Rolfe, E.J. (1957) *Br. J. Nutr.* **11,** 162.

Carpenter, K.J., Morgan, L.B., Lea, C.H. & Parr, L.J. (1962) *Br. J. Nutr.* **16,** 451.

Clandinin, D.R. (1949) *Poultry Sci.* **28,** 128.

Cook, B.B., Morgan, A.F., Weast, E.O. & Parker, J. (1951) *J. Nutr.* **44,** 51.

Davis, R.M., Rizzo, R. & Smith, A.H. (1949) *J. Nutr.* **37,** 115.

Donoso, G., Lewis, O.A.M., Miller, D.S. & Payne, P.R. (1962) *J. Sci. Fd Agric.* **13,** 192.

Dvorak, Z. & Vognarova, I. (1965) *J. Sci. Fd Agric.* **16,** 305.

Eldred, N.R. & Rodney, G. (1946) *J. biol. Chem.* **162,** 261.

Ellis, G.P. (1959) *Adv. Carbohyd. Chem.* **14,** 63.

Evans, R.J. & Butts, H.A. (1949a) *Science, N.Y.* **109,** 569.

Evans, R.J. & Butts, H.A. (1949b) *J. biol. Chem.* **178,** 543.

Evans, R.J. & Butts, H.A. (1951) *Fd Res.* **16,** 415.

Evans, R.J., Butts, H.A. & Bandemer, S.L. (1951) *Arch. Biochem. Biophys.* **32,** 300.

Evans, R.J., Groschke, A.C. & Butts, H.A. (1951) *Arch. Biochem.* **30,** 414.

Greaves, E.O., Morgan, A.F. & Lovern, M.K. (1938) *J. Nutr.* **16,** 115.

Halevy, S. & Guggenheim, K. (1953) *Arch. Biochem. Biophys.* **44,** 211.

Harris, R. E. & von Loesecke, H. (1960) *Nutritional Evaluation of Food Processing*, 1st edn, p. 522. Wiley, New York.

Henry, K.M. & Kon, S.K. (1950) *Biochim. biophys. Acta*, **5,** 455.

Hepburn, F.N., Calhoun, W.K. & Bradley, W.B. (1966) *Cereal Chem.* **43,** 271.

Hogan, A.G. (1917) *J. biol. Chem.* **30,** 115.

Iriarte, B.J.R. & Barnes, R.H. (1966) *Fd Technol., Champaign*, **20,** 835.

Johnson, A.R. & Vickery, J.R. (1964) *J. Sci. Fd Agric.* **15,** 695.

Kraft, R.A. & Morgan, A.F. (1951) *J. Nutr.* **45,** 567.

Kugan, I., Golovina, Zh. & Krasnaya, B. (1961) *Myas. Ind. SSSR*, **32,** 50.

Lea, C.H. & Hannan, R.S. (1949) *Biochim. biophys. Acta*, **3,** 313.

Lea, C.H. & Hannan, R.S. (1950a) *Biochim. biophys. Acta*, **4,** 518.

LEA, C.H. & HANNAN, R.S. (1950b) *Biochim. biophys. Acta*, **5,** 433.
LEA, C.H. & HANNAN, R.S. (1950c) *Nature, Lond.* **165,** 438.
LEA, C.H., PARR, L.S. & CARPENTER, K.J. (1958) *Br. J. Nutr.* **12,** 297.
LEA, C.H., PARR, L.S. & CARPENTER, K.J. (1960) *Br. J. Nutr.* **14,** 91.
LEA, C.H., PARR, L.S., L'ESTRANGE, J.L. & CARPENTER, K.J. (1966) *Br. J. Nutr.* **20,** 123.
L'ESTRANGE, J.L., CARPENTER, K.J., LEA, C.H. & PARR, L.J. (1967) *Br. J. Nutr.* **21,** 377.
MABEE, D. & MORGAN, A.F. (1951) *J. Nutr.* **43,** 261.
MADER, I.J., SCHROEDER, L.J. & SMITH, A.H. (1949) *J. Nutr.* **39,** 341.
MAYFIELD, H.L. & HEDRICK, M.T. (1949) *J. Nutr.* **37,** 487.
MCBRIDE, B.H., GUTHNECK, B.T., HOFFERT, E., KNICKEL, D. & SCHWEIGERT, B.S. (1951) *J. Nutr.* **45,** 393.
MCCOLLUM, E.V. & DAVIS, M. (1915) *J. biol. Chem.* **23,** 247.
MILLER, D.S. (1956) *J. Sci. Fd Agric.* **1,** 337.
MILLER, E.L., CARPENTER, K.J. & MILNER, C.K. (1965) *Br. J. Nutr.* **19,** 547.
MITCHELL, H.H. & BLOCK, R.J. (1946) *J. biol. Chem.* **163,** 599.
MORGAN, A.F. (1931) *J. biol. Chem.* **90,** 771.
MORGAN, A.F. & KERN, G.E. (1934) *J. Nutr.* **7,** 367.
MORGAN, A.F. & KING, F.B. (1926) *Proc. Soc. exp. Biol. Med.* **23,** 353.
NARAYAN, K.A. & KUMMEROW, F.A. (1958) *J. Am. Oil Chem. Soc.* **35,** 52.
NARAYAN, K.A. & KUMMEROW, F.A. (1963) *J. Am. Oil. Chem. Soc.* **40,** 339.
POLING, C.E., SCHULTZ, H.W. & ROBINSON, H.E. (1944) *J. Nutr.* **27,** 23.
REYNOLDS, T.M. (1963) *Fd Res.* **12,** 1.
REYNOLDS, T.M. (1965) *Fd Res.* **14,** 167.
RICE, E.E. & BEUK, J.F. (1953) *Advanc. Fd Res.* **4,** 233.
ROSENBERG, H.R. & ROHDENBURG, E.L. (1951) *J. Nutr.* **45,** 593.
SCHONBERG, A. & MOUBACHER, R. (1952) *Chem. Rev.* **50,** 261.
SCHROEDER, L.J., IACOBELLIS, M., LEES, H. & SMITH, A.H. (1953) *J. Nutr.* **50,** 351.
SCHROEDER, L.J., IACOBELLIS, M. & SMITH, A.H. (1961) *J. Nutr.* **73,** 143.
SCHROEDER, L.J., STEWART, R.A. & SMITH, A.H. (1951) *J. Nutr.* **45,** 61.
VENOLIA, A.W. & TAPPEL, A.L. (1958) *J. Am. Oil Chem. Soc.* **35,** 135.
WAISMAN, H.A. & ELVEHJEM, C.A. (1938) *J. Nutr.* **16,** 103.
WHEELER, P. & MORGAN, A.F. (1958) *J. Nutr.* **64,** 137.

J. Fd Technol. (1968) **3**, 87–89.

Rapid method for estimating degree of chlorination of cake flours by determination of soluble chloride

J. V. RUSSO AND A. D. SZCZEPANOWSKA

Introduction

Chlorine has been used as an improving agent in cake flours at levels of about 5–10 oz/sack (280 lb) for over 30 years (Montzheimer, 1931; Smith, 1931). In most milling laboratories the degree of chlorination is measured by the pH of an aqueous slurry of the flour. Chlorine addition leads to the formation of HCl, and thus a lowering of pH, through oxidation and substitution reactions with flour components. However, a statutory additive in flours is Creta Praeparata ($CaCO_3$; normally added after chlorine) and this buffers the pH (see Table 1). Hence it is difficult for a flour-using department to check on the degree of chlorination in flour after this addition of Creta without resorting to lengthy determination of lipid-bound chlorine (Kent-Jones, 1957; A.A.C.C., 1961).

TABLE 1. Effect of addition of Creta Praeparata on pH

Treatment level (ppm Cl_2)	Fruit cake flour A (pH)	Fruit cake flour A + Creta Praeparata (pH)
0	6·27	6·10
500	5·56	5·90
1000	5·41	5·80
1500	5·25	5·80
2000	4·92	5·70
3000	4·14	–

Treatment level (ppm Cl_2)	High-ratio cake flour (pH)	High-ratio cake flour + Creta Praeparata (pH)
0	6·0	5·95
500	4·45	5·8
1000	5·15	5·8
2000	4·4	5·8

Authors' address: Lyons Central Laboratories, 149 Hammersmith Road, London, W.14.

In order to overcome this problem we have recently developed a method for estimating the degree of chlorination by determination of soluble chloride using an EEL chloride meter and comparing results obtained with an appropriate calibration curve. This instrument measures trace levels by a coulometric method and was originally designed for measurements on blood.

Details of method

(1) Weigh 6 g of flour into a 100-ml beaker (in triplicate).

(2) Add 10 ml of de-ionized water and mix to a smooth paste then add further 50 ml of de-ionized water gradually while stirring so that no lumps are formed.

(3) Allow to stand for 10 min, then stir and filter through Whatman No. 5 filter paper.

(4) Use the filtrate to obtain chloride meter readings as outlined in meter instruction book.

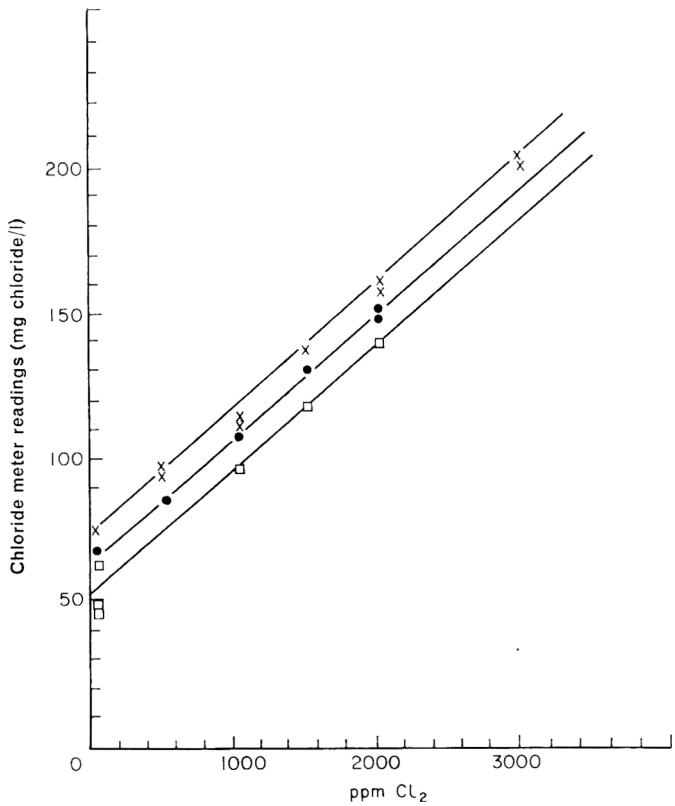

FIG. 1. Relationship between level of chlorination and chloride meter readings. ×, Fruit cake flour; ●, fruit cake flour + Creta Praeparata; □, high-ratio cake flour.

(5) Obtain also readings for blank (10 ml de-ionized water, instead of filtrate) and two standards (10 ml standard NaCl solution, 10 mg Cl/l) as a check on instrument calibration.

(6) Prepare calibration curves by chlorinating flours of different types in the laboratory. Three such curves are shown in Fig. 1.

(7) In subsequent work use calibration curves to assess degree of chlorination.

Discussion

It has been found that for a flour of a particular type the natural chloride content does not vary sufficiently to invalidate the results.

The method has been in use in our laboratories for 6 months and has been found to give consistently reliable results.

References

A.A.C.C. (1961) *Cereal Laboratory Methods*, 6th edn, p. 71. Lund Press, Minneapolis.

KENT-JONES, D.W. (1957) *Modern Cereal Chemistry*, 5th edn, p. 626. Northern Publishing Co., Liverpool.

MONTZHEIMER, J.W. (1931) *Cereal Chem.* **8,** 510.

SMITH, E.F. (1931) *Cereal Chem.* **8,** 425.

J. Fd Technol. (1968) **3**, 91–94.

Inhibition of clostridia by nitrite

J. A. PERIGO* AND T. A. ROBERTS†

Summary. An earlier report that sodium nitrite heated in a laboratory medium is highly inhibitory against *Clostridium sporogenes* has been extended to thirty clostridial strains including *Cl. botulinum* types A, B, E and F (fourteen strains) and *Cl. welchii* (eight strains).

Introduction

Microbiological investigations on the inhibitory effect of nitrite in laboratory media do not fully explain the stability of lightly processed cured meat products (Roberts & Ingram, 1966). To inhibit spores of *Clostridium sporogenes* in an agar medium it was necessary to add 800–1200 ppm sodium nitrite at pH 7·5 and 50–100 ppm at pH 6·5. Subsequent investigations using vegetative cells of *Cl. sporogenes* in a different system (Perigo, Whiting & Bashford, 1967) produced essentially the same order of inhibitory concentrations: 500 ppm at pH 7·5 and 160 ppm at pH 6·5. The latter authors also heated sodium nitrite in the growth medium before inoculation and found that heating at 90–130°C reduced the concentrations of sodium nitrite required to inhibit growth to as low as 3·5 ppm. This level was virtually undetectable chemically after the heat process, and suggested the formation of a potent growth inhibitor.

It was clearly of interest to determine whether this phenomenon was generally applicable to a wide range of clostridial species, particularly *Cl. botulinum* and *Cl. perfringens* (*welchii*).

Experimental

Vegetative inocula were obtained by subculturing in cooked meat medium, TPG (Roberts, 1965) or Reinforced Clostridial Medium (Oxo Ltd, London, S.E.1). Subcultures seen, by phase contrast microscopy, to contain spores were rejected. *Cl. botulinum* type E was incubated at 30°C; all other subcultures were incubated at 37°C. Five drops of vegetative cell culture, 18–42 hr old, were inoculated into a duplicate series of 1 fluid oz screwcap bottles containing the basal medium (Perigo *et al.*,

* Present address: The Metal Box Co. Ltd, Research and Development Department, Kendal Avenue, London, W.3.

† Present address: Meat Research Institute, Agricultural Research Council, Langford, Bristol.

1967) and increasing concentrations of sodium nitrite at pH 6 and 7, heated at 121°C for 20 min or unheated, as indicated in Table 1. Cultures were incubated for 7 days at 37°C (*Cl. botulinum* type E, 30°C) although in no case was inhibition delayed longer than 72 hr.

TABLE 1. Concentrations of sodium nitrite (ppm) used against clostridial species

pH	Treatment*	Concentration (ppm)									
7	Unheated	0	10	20	40	80	160	320	640	1280	2560
	Heated	0	1·25	2·5	5	10	20	40	80	160	320
6	Unheated	0	10	20	40	60	80	120	160	200	240
	Heated	0	1·25	2·5	5·0	7·5	10	15	20	40	60

* Treatment: Unheated = basal medium + filter sterilized nitrite solution; Heated = basal medium + nitrite solution heated at 121°C for 20 min.

Organisms used

The sources of most of the organisms used (Table 2) may be obtained by reference to Roberts (1967). *Cl. botulinum* G2A was isolated by and received from Dr D. Gimenez, Institute of Microbiology, Universidad Nacional de Cuyo, Mendoza, Argentina. *Cl. sporogenes* PA-R and 93-R were received from J. Baltzer, The Danish Meat Research Institute, Roskilde, Denmark.

Results and discussion

Results are summarized in Table 2, the tabulated nitrite concentrations being the highest concentration at which growth was observed in either of the duplicate bottles.

Unheated nitrite

At pH 7 the inhibitory concentrations were of the order which might have been anticipated from previous publications (Roberts & Ingram, 1966; Perigo *et al.*, 1967). The three strains of *Cl. botulinum* type E were among the most sensitive, and *Cl. perfringens* was most resistant, although one strain of *Cl. botulinum* type A, *Cl. botulinum* type F ('Langeland') and *Cl. bifermentans* were as resistant.

At pH 6, *Cl. perfringens* was again most resistant. Three strains of *Cl. botulinum* (62A, 7272A and 4318/63) failed to grow, even in the absence of nitrite.

TABLE 2. Range within which the inhibitory concentration (ppm) of unheated and heated nitrite at pH 6 and pH 7 falls

Species		Unheated†		Heated‡	
		pH 6	pH 7	pH 6	pH 7
Cl. sporogenes	532*	160–200	640–1280	5–7·5	10–20
	PA 3679	60–80	320–640	5–7·5	5–10
	PA–R	80–120	160–320	5–7·5	5–10
	93–R	80–120	320–640	5–7·5	10–20
Cl. botulinum					
Type A	62 A	0–10	640–1280	2·5–5·0	20–40
	1192 A	120–160	320–640	5–7·5	10–20
	3805* A	10–20	640–1280	0–1·25	10–20
	7272* A	0–10	1280–2560	0–1·25	10–20
	G2 A	20–40	320–640	15–20	10–20
Type B	751* B	80–120	640–1280	7·5–10	10–20
	213 B	10–20	640–1280	15–20	40–80
	3807* B	120–160	640–1280	7·5–10	10–20
(Non-proteolytic)	17 B	60–80	320–640	10–15	10–20
Type E	16/63	60–80	160–320	5–7·5	5–10
	1537/62	10–20	160–320	7·5–10	10–20
	4318/63	0–10	80–160	5–7·5	10–20
Type F	Langeland	60–80	1280–2560	5–7·5	40–80
(Non-proteolytic)	202F	80–120	320–640	7·5–10	10–20
Cl. perfringens (*welchii*)					
Classical	8237*	Above 240†	640–1280	10–15	10–20
	3181*	160–200	1280–2560	7·5–10	10–20
	8084*	Above 240†	1280–2560	7·5–10	10–20
	8503*	120–160	640–1280	5–7·5	10–20
	8081*	200–240	640–1280	10–15	5–10
'Food-poisoning'	8239*	Above 240†	1280–2560	5–7·5	10–20
	8797*	60–80	640–1280	7·5–10	10–20
	10240*	Above 240†	640–1280	7·5–10	10–20
Cl. septicum	547*	80–120	640–1280	2·5–5·0	5–10
Cl. tetani	5411*	40–60	160–320	7·5–10	5–10
Cl. bifermentans	506*	160–200	1280–2560	15–20	10–20
Cl. histolyticum	503*	160–200	640–1280	2·5–5·0	10–20

* N.C.T.C. catalogue number.

† Growth occurred in the highest concentration of nitrite used.

‡ Unheated and heated, see Table 1.

Heated nitrite

At both pH 7 and pH 6 growth was generally inhibited at an initial nitrite level of 5–10 ppm, only six cultures of the sixty tested showing growth at a higher level, the highest being 40 ppm (*Cl. botulinum* 213B and F 'Langeland'). Inhibitory levels at pH 6 were slightly lower than at pH 7.

The inhibitory levels tabulated for PA 3679 are consistent with those previously published (Perigo *et al.*, 1967).

Preliminary results with spores of *Cl. sporogenes* and *Cl. botulinum* type B indicate that the inhibitory concentrations of both unheated and heated nitrite are of the same order as demonstrated above for vegetative inocula.

Conclusion

The enhancement of the inhibitory effect of nitrite after heating in laboratory media is amply confirmed, and appears to be general to a wide selection of clostridia, and not restricted solely to *Cl. sporogenes*. It is, therefore, a matter of even greater interest to identify the inhibitory substance with a view to its possible use as a food additive.

References

Perigo, J.A., Whiting, E. & Bashford, T.E. (1967) *J. Fd Technol.* **2,** 377.
Roberts, T.A. (1965) *J. appl. Bact.* **28,** 142.
Roberts, T.A. (1967) *J. appl. Bact.* **30** (3), 430.
Roberts, T.A. & Ingram, M. (1966) *J. Fd Technol.* **1,** 147.

J. Fd Technol. (1968) **3,** 95–106.

The susceptibility to mould growth of white bread produced by different processes

DOROTHY M. COLLYER

Summary. Bread produced by the Chorleywood Bread Process (CBP), which is a mechanical development process, had a mean mould-free shelf life which was 14·5–19 hr shorter than that of bread made by a 3-hr bulk fermentation process, following natural inoculation by controlled exposure to a low mould-spore concentration in the air. Assessing mould susceptibility by comparing the time taken for half the exposed surfaces to show some mould growth, four-fifths or more of the deficiency in mould resistance of the CBP bread compared with the bulk-fermented could be made good by adding at the dough-mixing stage a fermented-out brew which had been started with one part of sugar for every twenty-eight parts of flour to be used in the dough.

Correlation of mould resistance with the strengths of fermented-out sugar brews indicated that the favourable effect was due to a fermentation product. Ethanol was found to be the substance responsible, products present in trace amounts evidently not playing any important part in the observed differences in rate of mould growth on CBP bread with and without added brews. The ethanol content of the bread accounted for most but not all of the mould resistance of bulk-fermented bread compared with CBP bread. Equilibrium relative humidity, pH, and propionic acid and moisture contents did not vary enough from one kind of bread to another to affect relative mould-free shelf life appreciably. Evidently, therefore, an unidentified mould inhibitor is present in the bulk-fermented bread.

The commercial significance of the findings is discussed.

Introduction

This work was prompted by observations that bread made by bulk dough fermentation processes had a tendency to become mouldy slightly less quickly than bread made by the Chorleywood Bread Process (CBP) (Chamberlain, Collins & Elton, 1965). In the CBP, mechanical development, use of ascorbic acid and certain other features together replace the effects of a long fermentation of the bulk dough (Chamberlain, Collins &

Author's address: Flour Milling and Baking Research Association, Chorleywood, Rickmansworth, Hertfordshire.

Elton, 1962). Although the difference in average mould-free shelf life had been estimated at only about 6–12 hr at 80°F, it was an observation of some interest, particularly as about half the white bread produced in Britain is now made by the CBP.

If the greater resistance to mould growth of bread made by longer fermentation methods is due to an inhibitory effect of the products of yeast fermentation, it was thought that it might be possible to increase the mould-free shelf life of CBP bread by adding a fermented sugar brew at the dough mixing stage. This possibility formed the basis of the investigation reported here.

Methods

Bread-making methods

Fourteen-ounce loaves were produced by the bulk-fermentation and CBP methods given in a previous paper (Collyer, 1966). All brews for the mould-resistance work were simple sucrose–calcium carbonate–yeast–water brews fermented at 98°F. The weight of extra water necessary for inversion was added with the sucrose when the brews were made up. Initial brew strength in relation to the flour used subsequently to make the dough is defined as the weight of invert sugar to a 280-lb sack of flour. CBP bread containing brew was made in exactly the same way as the normal CBP bread, except that the yeast and most of the water in the dough formula were replaced by brew, which had always completely fermented out when used. The amount of brew so used contained the quantities of yeast and water omitted from the formula, together with fermentation products. Ethyl alcohol equivalent to a brew was 47% of the weight of the fermented invert sugar. Bread containing added alcohol was made by the CBP with the required weight of ethyl alcohol mixed with the dough water. Inserting labels the full length of the loaves, before baking, ensured that the bread-type of every slice would be identifiable. The baked bread was allowed to touch only sterile or scrupulously clean surfaces.

Techniques for inoculation with mould spores

Bread was inoculated when approximately 18 hr old.

Using the method of Seiler (1962), four loaves of each kind were halved and the cut surfaces equally sprayed with a suspension of spores of *Aspergillus niger*. The bread was wrapped in moisture-resistant film, heat-sealed and incubated at 70°F. At intervals the amount of mould growth was compared with photographic standards.

A technique more closely related to the baker's practical problem, based on carefully controlled exposure of bread surfaces to the air for natural inoculation, was developed. This proved to be more suitable for measuring degrees of mould resistance when comparing different kinds of bread, as required by the present work. The method was to place out on a sterile sheet of glass slices of bread 1 in. thick. Equal numbers (usually five) of slices of all the types of bread under comparison, placed out in repeated sequence, were exposed together. After the set time (usually 5 min) a second sterile

glass sheet was placed on top, the whole carefully turned over, and the other surfaces exposed by removing the upper piece of glass for the same period. With negligible further exposure the slices were packed individually in bags made of MST 300 film. The whole batch was then made air-tight in a numbered polythene bag. The sequence of packing the samples aimed to equalize among the different types any loss of vapour due to permeability of these films. The effect of any diffusion which may have occurred would have been a tendency to make the types more similar to one another. Sample slices for chemical analysis were put into air-tight jars at the end of the second exposure period. The whole procedure was repeated several (usually seven) times, until about seventy surfaces of each kind of bread had been exposed in one experiment.

For conditions of low inoculation, where the majority of exposed surfaces subsequently developed only one or two primary mould spots, the exposure was for 5 min in a laboratory with low mould spore count. Most of the experiments were of this type. For a higher level of inoculation the bread slices were exposed for 3 or 5 min on each side in a bakery while flour was being used. Such exposure to air with high mould spore concentration was used only where specified.

As differences in mould resistance between bread made by the different processes were small, and other variations, such as those between replicate batches of bread, were not negligible, large numbers of replicate samples and of replicate experiments were necessary.

Assessment of susceptibility to mould growth

After controlled natural inoculation the bread was stored in temperature-controlled cabinets. At intervals—twice daily at the higher incubation temperatures—every surface, always in the same sequence, was examined for mould growth without removing the samples from the inner transparent bags. As soon as a minute speck of mould became visible to the naked eye, that surface was regarded as being mouldy.

For each kind of bread the number of surfaces showing some mould growth was plotted against the length of time since exposure to natural inoculation. The curve was occasionally almost linear, but more often showed a short, slow, almost linear stage, followed by another much steeper linear phase and finally a third, parallel to the first, though in a number of experiments the first phase was absent. With such curves, plotting the number of mouldy surfaces on a logarithmic scale was not helpful in deducing the point of onset of mould growth. Similarly, the duration of mould-free shelf life could not be assessed with a great degree of accuracy by extrapolation, on a linear scale, to cut the time axis, on some of the graphs. With very much larger numbers of replicates, or much more frequent inspections, a higher degree of accuracy within each experiment would have been possible, but practical limitations restricted each experiment to a maximum of eighty surfaces of each of five kinds of bread, examined twice a day. It is the moment when the first mould spot becomes visible that is of greatest importance commercially. Estimates of this, by very careful extrapolation, *when*

averaged over many replicate experiments, were considered to give a reasonably reliable guide.

Because it was difficult to determine the time of appearance of the first sign of mould growth in a batch of bread, the time at which half of the surfaces showed some mould growth was also used as a measure of mould susceptibility. This point could be found with much greater certainty on the graphs. The absolute value in days varied considerably from one experiment to another. For instance, times for the normal CBP bread varied from 3·3 to 7·3 days at 70°F. This was due to fluctuations in the concentration of the more rapidly-growing mould spores present in the air at the time of exposure, as well as to day-to-day bread batch variation. However, the time taken by one batch relative to another was more reproducible, so an indirect method was adopted in which the length of time taken by the bulk-fermented bread in an experiment to reach the point at which half the surfaces showed mould was taken as 100 units, and the times taken by the other batches were calculated relatively to the bulk-fermented bread. This was the more useful measure of mould susceptibility for comparing the batches of bread used within an individual experiment.

Bread analysis methods

Ascorbic acid was determined according to the method of Moor (1957).

Estimations of equilibrium relative humidity were based on the weight change of replicate crumbled samples placed overnight at 70°F in chambers, the humidity of each chamber being controlled by water or a water–salt sludge. For relative humidity of 93%, the salt used was potassium nitrate and for 97%, potassium sulphate, water alone giving 100%. The curve of weight change per gram of sample plotted against chamber humidity crossed the zero weight change line at the equilibrium relative humidity. The accuracy was ± 1%.

For the determination of ethyl alcohol about 0·5 g sample was weighed into a dish and supported, in a jar, above 10·0 ml of a reagent made up as 4·903 g potassium dichromate dissolved in, and made up to 1 litre with, 1 : 1 (v/v) sulphuric acid. Directly on to the sample was added 1 ml of a freshly mixed reagent consisting of equal volumes of 36% (w/v) sodium hydroxide solution and 5% (w/v) mercuric chloride solution. The sealed jar was immersed in a water bath at 113°F ± 2°F for 2 hr. The liquid in the jar was then diluted to 100 ml with water and the extinction was measured in a 1-cm cell at 440 mμ. The alcohol content was found by reference to a calibration graph. The method was accurate to 0·1 mg.

Moisture content was found by determining the weight loss from a 5-g sample dried at 221°F for 5 hr, and subtracting from the percentage of volatile substances thus obtained the ethyl alcohol content of the bread as separately determined.

The pH of a mixture of 50 g bread and 20 g water was measured on an EIL meter.

Propionic acid was determined according to the method of Walker, Green & Fenn (1964).

Results and discussion

Aspergillus spray technique

This was used a few times. Although replicate surfaces were very much alike in the form and rate of mould growth, *Aspergillus niger* produced largely black growth on plain CBP bread and mainly yellow growth on bulk-fermented bread, with the CBP bread containing brew showing a larger proportion of yellow to black the stronger the brew with which it was made. Brew filtrate with new compressed yeast added at dough mixing produced the same effect as whole brew. Alcohol also produced an effect similar to that given by brews. It was, therefore, impossible to compare the amounts of mould growth on different types of bread. While these experiments must be regarded as unsuccessful in achieving the desired end, they demonstrated strikingly that the different types of bread used in this investigation influenced the physiology of one mould species in different ways.

Mould-free shelf life after controlled natural inoculation

Mould-free shelf life means and ranges are shown in Table 1. At 55°F and at 80°F the bulk-fermented bread had a mean mould-free shelf life that was, respectively, 0·7 and 0·6 days longer than that of the normal CBP bread. The difference at 70°F was 0·8 under similar inoculation conditions, but was reduced to 0·2 day with heavy inoculation in a hot atmosphere, an effect which is discussed below. Adding brews of increasing strength to the CBP dough tended to extend the mould-free shelf life progressively to near that of the bulk-fermented bread.

TABLE 1. Estimated mould-free shelf life, in days from inoculation, of the various bread types

Bread type	Low,* 80°F†		High,* 70°F†		Low,* 70°F†		Low,* 55°F†	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Bulk-fermented	2·4	2·1–2·6	2·9	2·7–3·2	3·5	2·9–3·8	6·5	5·8–6·9
Normal CBP	1·8	1·3–2·3	2·7	2·5–2·9	2·7	2·2–3·2	5·8	4·8–6·7
CBP+brew (initially 6 lb/sack sugar)	—	—	2·5	2·2 and 2·8	3·2	2·8 and 3·6	—	—
CBP+brew (initially 8 lb/sack sugar)	2·3	2·2 and 2·4	2·7	2·5 and 2·9	3·4	3·1–3·7	6·4	5·8–6·7
CBP+brew (initially 10 lb/sack sugar)	2·3	2·0–2·7	3·0	2·9–3·0	3·5	3·0–3·9	6·1	5·0–6·8

*Mould spore concentration. †Incubation temperature.

Half surfaces mouldy after controlled natural inoculation

Incubated at 70°F, normal CBP bread showed mould growth on half of its cut surfaces in about 86% of the time taken by bulk-fermented bread. A fermented-out sugar brew started with 10 lb of sugar per sack improved the mould resistance of the

CBP bread to the extent that it took, on average, 96% of the bulk-fermented bread time. Weaker brews gave shorter extensions. All the results are summarized in Table 2.

TABLE 2. Times taken for half the bread surfaces to show some mould growth, expressed as percentages of time taken by bulk-fermented bread

Bread type	Low,* 80°F†		High,* 70°F†		Low,* 70°F†		Low,* 55°F†	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Normal CBP	85	69–100	91	84–96	86	79–95	97	87–110
CBP + brew (initially 6 lb/sack sugar	—	—	93	93–94	90	83–97	—	—
CBP + brew (initially 8 lb/sack sugar	111	104 and 118	95	92–98	95	89–104	103	93 and 114
CBP + brew (initially 10 lb/sack sugar)	99	93–102	98	96–101	96	91–99	102	91–114

*Mould spore concentration. †Incubation temperature.

At an incubation temperature of 80°F, the average results for normal CBP and 10 lb/sack brew bread, relative to bulk-fermented bread, were similar to those obtained at 70°F. The 6 lb/sack brew was only tested at 70°F. The 8 lb/sack brew was used in only two experiments with an incubation temperature of 80°F, and on both occasions the brew bread took longer than the bulk-fermented bread to show mould on half the surfaces.

At 55°F the normal CBP bread at 97% was hardly worse than the bulk-fermented, and brew bread was marginally better. This indicated that the lower the temperature and slower the absolute growth rate, the less effect the inhibitory factor had relatively.

Under the conditions of these experiments, species of *Penicillium* constituted the principal mould type at all three temperatures. The next most frequent species was different in different experiments, and exact analysis of the species present was made in only one experiment, in which some slices from each exposure were incubated at each of the three temperatures. The proportion represented by *Cladosporium* fell with each increase in temperature and the other main species, *Aspergillus flavus*, was more plentiful the higher the temperature. Different kinds of bread showed no clear differences in proportions of different species, but a more detailed study might have proved profitable.

Exposing to air with high mould spore concentration in a hot atmosphere and incubating at 70°F, the times taken for the three types to become half mouldy were, on average, closer together than at the lower inoculation level, normal CBP bread taking 91% of the bulk-fermented time, and brew bread lying between 91 and 100%.

CONTENTS

*

Bread analysis and mould resistance

Analyses were made on slices from the loaves that were used in the natural-inoculation mould tests. These analyses relate to the composition at the end of the exposure of surfaces, at the start of incubation. It would be expected that some at least of the properties examined would have a profound effect on mould growth when varied over a wide range. These results refer only to the ranges encountered in the bread used. Both estimated mould-free time and relative time taken for 50% of surfaces to become mouldy were used to assess mould resistance.

Propionic acid is a minor product of yeast fermentation and, together with its salts, is well known as an anti-mould agent. There was no correlation between the concentration of propionic acid (including propionate) in the bread and its mould resistance in the four experiments in which analyses were made for propionic acid. The differences in level were very small, not exceeding 10 ppm between bread types within any experiment, and the absolute levels were low, the highest value determined being 25 ppm and the mean of all determinations being 15 ppm.

Altering the pH appreciably would be expected to affect the rate of mould growth, and lower concentrations of fermentation acids might be expected in plain CBP bread than in the other types. However, the calcium carbonate in the brews gave a good buffering effect, and flour also acts as a buffer. Determinations of the pH of the bread were included in most of the experiments, but again there was no correlation with the rate of appearance of mould growth, either regarding the experiments individually, or when the mean pH of each bread type over all the experiments was considered. All the pH values measured were from 5·7 to 6·4, and the greatest difference between bread types in any experiment was 0·4. The mean value for bulk-fermented bread was 5·9, and the mean values over all experiments for each type were in the range 5·9 ± 0·1 (Table 3). With such small differences a correlation with mould growth would have been surprising.

TABLE 3. Mean pH and alcohol content of bread after exposure of surfaces for natural inoculation in the laboratory

Bread type	pH	% alcohol
Bulk fermented	5·9	0·31
Normal CBP	6·0	0·15
CBP + brew 6*	5·9	0·25
CBP + brew 8†	5·9	0·28
CBP + alcohol equivalent to brew 8†	5·8	0·30
CBP + brew 10‡	5·9	0·34
CBP + alcohol equivalent to brew 10‡	5·9	0·34

*Fermented-out brew started with 6 lb of sugar/sack.
†Fermented-out brew started with 8 lb of sugar/sack.
‡Fermented-out brew started with 10 lb of sugar/sack.

No relationship could be traced between the mould resistance and either the moisture content of the bread or its equilibrium relative humidity. Differences in the latter were small, almost always within experimental error and usually not exceeding ± 0·5% in the experiments in which this was determined. Mean values were all close to 98%. The percentage of moisture in the bread, determined in most of the experiments, appeared to be unrelated to mould susceptibility within individual experiments and also when taken as an average property of the different bread types. These means differed by only ± 0·4%, and the normal CBP bread had a mean moisture content which was only 0·1% greater than that of the bulk-fermented bread in these tests.

The percentage of ethyl alcohol in the bread at the end of the exposure time did suggest some correlation, though not a close one, with resistance to mould growth. The alcohol concentration was determined in most of the experiments and the means for the different kinds of bread are shown in Table 3. Some figures for comparison may be calculated from published values. As a generalization, Johnson (1963) quoted 0·44–0·66% as the range of alcohol contents normally found in American white bread. Bread made from a 3-hr fermentation process contained about 0·5% alcohol (Wiseblatt & Kohn, 1960). Bread in which brews of two different concentrations provided part of the dough liquid, had alcohol contents of approximately 0·17% in experiments of Cole, Hale & Pence (1963).

Comparing Tables 1 and 2 with Table 3 it will be seen that there was a tendency for the more resistant types to have, on average, the higher alcohol contents. However, in only a small number of experiments taken individually was there a clear relationship between the alcohol content of each bread type and its susceptibility to mould growth. It was noticeable that the mould resistance of bulk-fermented bread tended to be better than its alcohol content suggested, and if the bulk-fermented bread was excluded from the comparisons then a moderately good inverse correlation between the alcohol content of the bread and the time taken to show mould on half of its surfaces was apparent within the majority of the experiments considered separately. A correlation with estimated mould-free shelf life was shown in only a few experiments.

The correlation of mould growth with the alcohol content of the bread made by the mechanical-development method could have been due to the effect of some other substance, as other yeast products would probably accumulate in proportion to the alcohol when comparing such brews as these, differing only in concentration. Relative levels of propionic acid, and pH changes due to fermentation acids in general, have already been shown not to explain the observed results.

Direct additions of ethyl alcohol to the dough water gave bread alcohol contents very similar to those given by brews supplying the same amounts of alcohol. As the equivalent amount of alcohol tended to be at least as effective in promoting mould resistance as was a brew, it seems unlikely that any other product of the fermentation of sugar by yeast was involved to any appreciable extent in mould resistance in this mechanically-developed brew bread.

On the other hand, alcohol content alone did not account for the mould resistance of bulk-fermented bread. A possible explanation might be that antibiotic substances were produced by the yeast in the presence of flour, which is a source of nitrogenous substances, but not in the nitrogen-free sugar brew. To test this, in a single experiment 10% of the flour required for the dough was included in the brew, which was compared with the normal brew without flour. No increase in mould resistance was given by allowing the yeast to ferment in the presence of flour under these conditions.

Although more experiments with flour brews would be necessary before proof of this point could be claimed, if it is true that no other fermentation product is important and also that the alcohol exerts an influence in proportion to its concentration, then another possible explanation could be that the mechanically-developed bread contained, in higher concentration than the bulk-fermented bread, some substance which promoted mould growth. The standard dough formulae differ by the 75 ppm of ascorbic acid added in the CBP. This substance and its breakdown and reaction products were considered in one experiment although not known to be required by any mould. In this test ascorbic acid was added at various levels to CBP doughs and doughs for bulk fermentation, and one batch of each was made without any, potassium bromate being used instead in the mechanically-developed one. The concentration was determined in the exposed bread. All the mechanically-developed batches were more susceptible than the bulk-fermented batches, and there was no correlation between susceptibility to mould growth and ascorbic acid content, or the level at which it had been initially added. This test, therefore, left unresolved the question of whether the bulk-fermented bread contained an extra mould inhibitor or the CBP bread a mould stimulant. Knight (1966) found CBP bread to have a reducing sugar content about 25% greater than that of bulk-fermented bread (0·5% greater on the bread weight). The possibility that such a difference is sufficient to promote mould growth was not examined.

In summary, the results of this work show conclusively that fermented-out sugar brews used in the dough can exert a favourable effect on the mould resistance of CBP bread. They leave no room for a trace substance in the inhibitory action of a simple sugar brew, but they do indicate that some other anti-mould agent in addition to ethyl alcohol may be produced during bulk fermentation of the dough.

Apparent effect of inoculation conditions on susceptibility

The more equal mould susceptibility of the different bread types, assessed by either method, after exposure in the bakery as compared with the laboratory has already been referred to. The air of the bakery not only had a higher mould spore concentration but was also at a higher temperature than the air of the laboratory, and presumably this was responsible for the finding that the slices after exposure for 5 min on each side almost always had considerably lower alcohol contents than slices of the same kind of bread exposed in the laboratory. As those with normally higher levels tended to lose

more than the types with less, alcohol contents were more similar and more similar mould resistance in the different types would follow. This would also explain the shorter mould-free shelf life of all types of bread except CBP, as compared with laboratory exposures also incubated at 70°F.

Exposing for 3 min on each side in the bakery resulted in alcohol levels similar to those left after 5 min in the laboratory, and still mould resistance differed less between types than it did after exposure to low mould spore concentration, so that the number of spores per surface was a major factor in these results. It seems likely, however, that this effect was largely an artifact of the method of assessment of mould resistance, which did not distinguish between a surface showing a single mould spot and one with several. A count of mould colonies instead of mouldy surfaces would not necessarily be helpful in adapting the technique to tests using conditions of high inoculation, because secondary colonies arise from spores of primary colonies, and only the latter should be counted. Counting the first colony of each different species on each surface would add only slightly to the precision. Insofar as this effect, of apparent increase in similarity between bread types of mould susceptibility with increasing inoculation level, is an artifact of the method, it would disappear if bread samples whose horizontal surfaces were small enough to receive during the exposure time only about one mould spore each, were used. Based on the number of spores which produced visible colonies over the period of incubation, this would have been in the region of 7 cm^2 in these experiments. This ideal was approached in the low-inoculation tests which formed the principal part of this work. In these, spores settled at something like 1 spore/50 cm^2 in 5 min. Pelshenke (1954) gave a figure for mould spore settling rate in a bakery store room equal to 1 spore/15 cm^2 in 5 min, which is in very good agreement with the present work as such a room would be expected to have a mould spore count between those of a working bakery and a laboratory.

The only way in which actual mould-free shelf life is likely to be genuinely shortened by increasing inoculation level from about one to about seven spores per surface, is if this results in an increase in the chances of a given total area of exposed samples receiving an exceptionally fast-growing spore, and if this were the case then a seven-fold increase in the number or size of surfaces would show the same effect. This was not tested. A reliable determination of mould-free shelf life must have a large enough number of surfaces so that increasing the number does not significantly reduce the measured length of the mould-free shelf life as a result of the lesser number of surfaces not receiving one of the fastest-growing spores that are present in significant numbers.

Another possible factor lay in the distinct possibility that the mould flora of the bakery and that of the laboratory were different. General observation, and a more detailed examination in one test, did not suggest any marked difference. Clearly, if it existed this situation could also lead to measured differences in mould-free shelf life, and perhaps in relative susceptibility of different bread types.

In the absence of evidence to the contrary, it is believed that the only real difference

in mould resistance between bakery and laboratory-exposed bread could be traced to the evaporation of alcohol. In any case, the effect of the higher inoculation level plus the effect of the greater alcohol evaporation caused less reduction in the measured mould-free shelf life than increasing the incubation temperature by 10°F (Table 1).

Commercial importance

There was a difference of approximately 14·5–19 hr in the mean mould-free shelf life of normal CBP and bulk-fermented bread when the inoculation conditions were those of the laboratory. This difference is commercially significant and it was not really affected by incubation temperature. Adding a fermented-out brew at the dough mixing stage nearly always extended the mould-free shelf life of CBP bread, but usually not by as much as 14·5 hr, even when the brew had been started with 10 lb sugar/sack. This work has pin-pointed the primary importance of ethyl alcohol as a substance responsible for mould resistance. It is fairly certain that an even stronger fermented-out sugar brew than those used here would increase the mould resistance of CBP bread to be equal to or better than that of 3-hr bulk-fermented bread, but it is very unlikely that this method would be adopted to solve the problem of mould growth. From the commercial viewpoint this would be an expensive means of increasing mould resistance, especially in view of the fact that the effect varied somewhat from one experiment to another and also perhaps according to the mould-spore concentration in the air. The mould-free shelf life of commercial bread would be expected to be longer than the figures quoted in these experiments because shorter exposure time would be given to the crumb surfaces in commercial slicing than in these tests, so that the alcohol content of the brew bread at the onset of 'incubation' would presumably be higher, and this would also be true of any bulk-fermented bread with which it was being compared. The inoculation level would vary greatly according to bakery hygiene. It is, therefore, extremely difficult to make reliable predictions of mould-free shelf life extensions. Such a strong brew as this would also adversely affect bread odour (Collyer, 1966). For these reasons the use of sugar brews to increase the mould resistance of CBP bread is unlikely to be commercially acceptable unless suitable modifications to such brews can be arrived at.

The use of propionic acid and certain of its salts as mould inhibitors in bread is presently permitted in Britain (Great Britain, Parliament, 1962). Where anti-mould agents are not permitted, the discovery of a sufficiently effective brew which was commercially acceptable could be of more than academic interest. An explanation in chemical terms of the causes of the inhibition of mould growth on bulk-fermented bread could help greatly in the development of such a brew for use in hot weather by bakers who rely on a mechanical development process.

It seems quite safe to assume that alcohol is at least a contributory factor in the resistance of bulk-fermented bread, but there must be at least one other component

which is active also, unless CBP bread contains a mould growth stimulant, which seems rather less likely. A search for the effective substances might lead to the arrival at a modified formula for a sugar brew which would be a source of both alcohol (in moderate concentration) and another mould inhibitor.

The identification of such a substance, whether an inhibitor in bulk-fermented or a stimulant in CBP bread, would be aided if other bread characteristics could be equalized, though this work has indicated that a perfect adjustment of pH, equilibrium relative humidity, moisture content and propionic acid would not help markedly because under these bread-making conditions they were already very similar in the different types of bread. Much more important in giving a clearer picture of the effect of other substances, including other fermentation products, would be the equalization of ethyl alcohol contents. Before subjecting experimental formulae to the more important natural-inoculation test for susceptibility, provided that alcohol was kept equal, the *Aspergillus* spray technique might be a quicker and much less laborious method for the preliminary screening of formulae, for the sporulation of this mould was affected in the same way as was general mould susceptibility by the bread types already tested.

Acknowledgments

This investigation was financed by Messrs Tate & Lyle Ltd and the British Sugar Corporation. I am indebted to Dr N. Chamberlain for criticizing the manuscript and thanks are also due to colleagues in the Bread Bakery for baking the bread and in the Analytical Department for carrying out the chemical analyses, and to Mrs P. J. Walker for assistance in the mould assessment work.

References

CHAMBERLAIN, N., COLLINS, T.H. & ELTON, G.A.H. (1962) *Bakers' Dig.* **36** (5), 52.

CHAMBERLAIN, N., COLLINS, T.H. & ELTON, G.A.H. (1965) *British Baking Industries Research Association*, Report No. 77.

COLE, E.W., HALE, W.S. & PENCE, J.W. (1963) *Cereal Chem.* **40,** 260.

COLLYER, D.M. (1966) *J. Sci. Fd Agric.* **17,** 440.

GREAT BRITAIN, PARLIAMENT (1962) *Preservatives in Food Regulations*, 1962, *Stat. Instrum.* No. 1532.

JOHNSON, J.A. (1963) *Proc. 39th Ann. Mtg Am. Soc. Bakery Engnrs*, 78.

KNIGHT, R.A. (1966) *British Baking Industries Research Association*, Report No. 88.

MOOR, H. (1957) *Fd Mf.* **32,** 119.

PELSHENKE, P.F. (1954) *Brot Gebäck*, **8,** 27.

SEILER, D.A.L. (1962) *British Baking Industries Research Association*, Report No. 64.

WALKER, G.H., GREEN, M.S. & FENN, C.E. (1964) *J. Ass. public Analysts*, **2,** 2.

WISEBLATT, L. & KOHN, F.E. (1960) *Cereal Chem.* **37,** 55.

J. Fd Technol. (1968) **3**, 107–114.

On post-mortem changes in the nucleotides of fresh-water fish muscle

TAINA KUUSI AND MAURI AALTO

Summary. The post-mortem changes in the nucleotides, and their relation to the phases of rigor and changes of quality in fish were investigated. The various compounds of the acid-soluble fraction of the muscle were assayed on the basis of phosphorus content. Further, separate assays were made for adenylic radicals, changes in ultraviolet spectra and hypoxanthine; the latter was estimated as silver salt. At the same time, quality was evaluated in terms of odour, appearance and rigidity. The studies were mainly concerned with rainbow trout (*Salmo irideus*), although pike (*Esox lucius*) was also used in some experiments. The fish samples were kept at 4°C; the length of a complete storage period was 16 days.

The total phosphorus of the acid-soluble fraction displayed an increase which was initially rather steep. Labile phosphorus diminished, reaching a minimum in 25 hr and showing no change subsequently. A corresponding increase occurred in inorganic phosphorus during the same period. A drop in creatine phosphate occurred simultaneously with that of labile phosphorus. Some diminution was also noted in the adenylic radicals. A shift in the peaks of the ultraviolet spectra was noted after 12 hr, the maximum being shifted towards 250 mμ. No appreciable increase took place in hypoxanthine until after 7 days storage.

Introduction

It is well known that fish is a food which deteriorates rapidly during storage. Several methods are in use for evaluating freshness, but as a rule the rise in the values does not occur until the inferior quality of the fish can be noticed already by sensory means. Consequently, methods which would monitor quality changes at earlier storage phases have been the object of a number of recent investigations (including those by Bramstedt, 1962; Jones, 1964; Jones *et al.*, 1964a, b; Spinelli, Eklund & Miyauchi, 1964; Hughes & Jones, 1966). One such class of compounds in which progressive changes take place during storage is that of the nucleotides and their derivatives. Some of these compounds have been shown to act as important taste factors, in particular IMP is a

Authors' address: The State Institute for Technical Research, Laboratory for Food Research and Technology, Otaniemi, Finland.

positive taste factor of fresh fish, but also ATP and AMP possess similar taste properties (cf. Hashimoto, 1964). In contrast hypoxanthine, which occurs in the last part of the decomposition series, seems to act as a negative taste factor, causing bitterness (Jones, 1964; Tarr, 1966). Studies made so far mainly concern marine fish species; fresh-water fish have been the object of comparatively few investigations. Additional information on fresh-water species was, therefore, considered necessary.

Material

The material consisted of pond-reared rainbow trout (*Salmo irideus*); in addition, preliminary experiments were performed with pike (*Esox lucius*). On the average, the former weighed approximately 250 g and were 2 years of age. These experiments were carried out during the summer months and thus the fish had been fed. The fish were transported alive to the laboratory, and allowed time for complete tranquillization before being killed with a blow on the head. The fishes were eviscerated, and the scales and gills removed. After washing with cold water, the fishes were wrapped in waxed pergamine and stored at 4°C. No microbiocidal agents were used. The pike were bought alive in the market, but were not transported to the laboratory in water; otherwise, the treatment applied was similar to that for rainbow trout.

Methods

Samples were taken from the dorso-lateral white muscle; the skin, the bones and the red lateral muscle were removed. Total phosphorus was assayed from separate samples, first wet-ashed by means of sulphuric acid and hydrogen peroxide (cf. Martland & Robison, 1926). The phosphorus determinations were all made by the micro method of Berenblum & Chain (1938). The acid-soluble fraction was extracted with perchloric acid (cf. Jones *et al.*, 1964b). Aliquots were taken from this neutralized extract for direct hypoxanthine assay, with silver-precipitation by the method of Jones *et al.* (1964b). Total acid-soluble phosphorus was determined from other aliquots after wet-ashing. Labile phosphorus was assayed by using the method of Crane & Lipmann (1953), modified by Derache & Lowy (1955) (see also Lowy, Decloitre & Brigant, 1964). Inorganic phosphorus was assayed from a dilution of the neutralized acid-soluble extract directly, without wet-ashing; this fraction also includes the creatine phosphate. A separate assay of creatine phosphate was made by application of the method of Le Page (1951).

The adenylic radicals were measured directly from the neutralized acid-soluble fraction, after suitable dilution, from optical density at 260 mμ. The ultraviolet spectra of these extracts were also taken between 220 and 320 mμ, to check whether there had occurred a shift in the location of the peak; the maximum of adenylic compounds is at 260 mμ, that of IMP at 251 mμ, and hypoxanthine at 248 mμ.

The changes taking place in the fish during storage were observed, with the rigidity,

consistency, appearance and odour, related to rigor phenomena and spoilage, taken into account.

The scheme of analysis illustrates the methods used (Fig. 1).

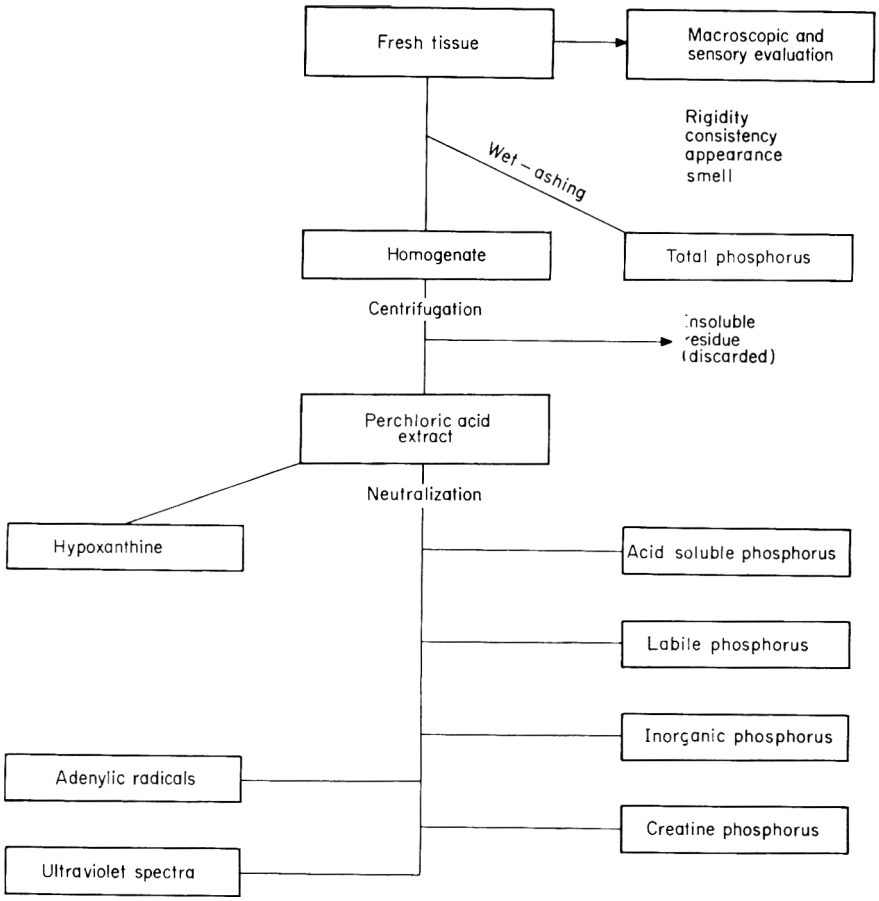

FIG. 1. Scheme of fractionation employed in the analyses of rainbow trout muscle tissues.

Results

Rainbow trout

The results arrived at are indicated in Figs. 2 and 3; the former illustrates changes which occur in total acid-soluble phosphorus, inorganic phosphorus (which also includes phosphorus from creatine phosphate), labile phosphorus, and adenylic radicals. For comparison, the course of rigor mortis under the same conditions, taken from the paper of Partmann (1963), has been drawn in the same diagram. Fig. 3 shows the changes in labile phosphorus and creatine phosphate on a magnified scale, along with

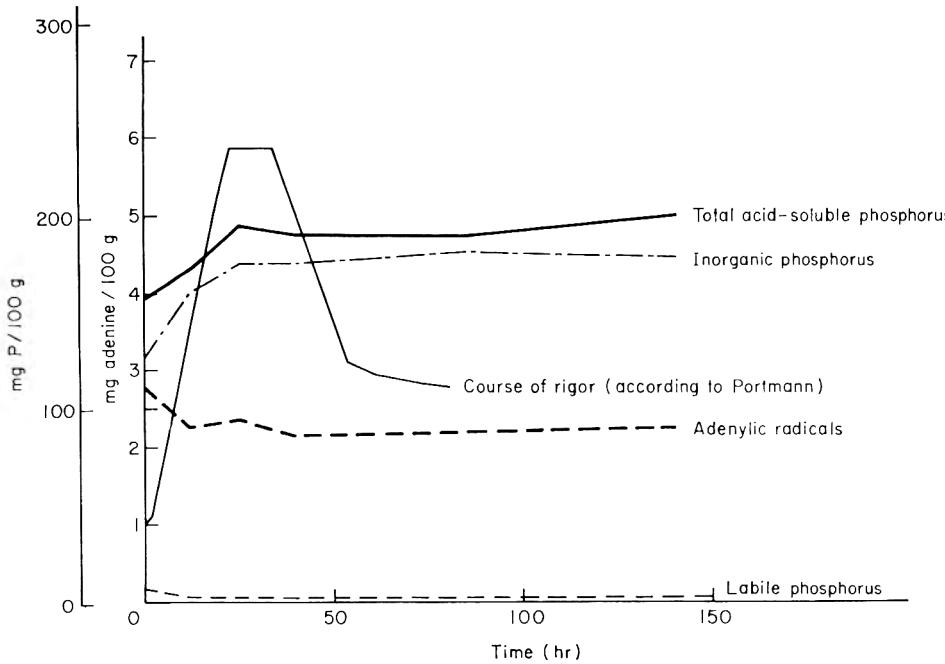

FIG. 2. Course of post-mortem changes in rainbow trout muscle kept at 4°C: total acid soluble phosphorus, inorganic phosphorus, labile phosphorus, adenylic radicals and rigor.

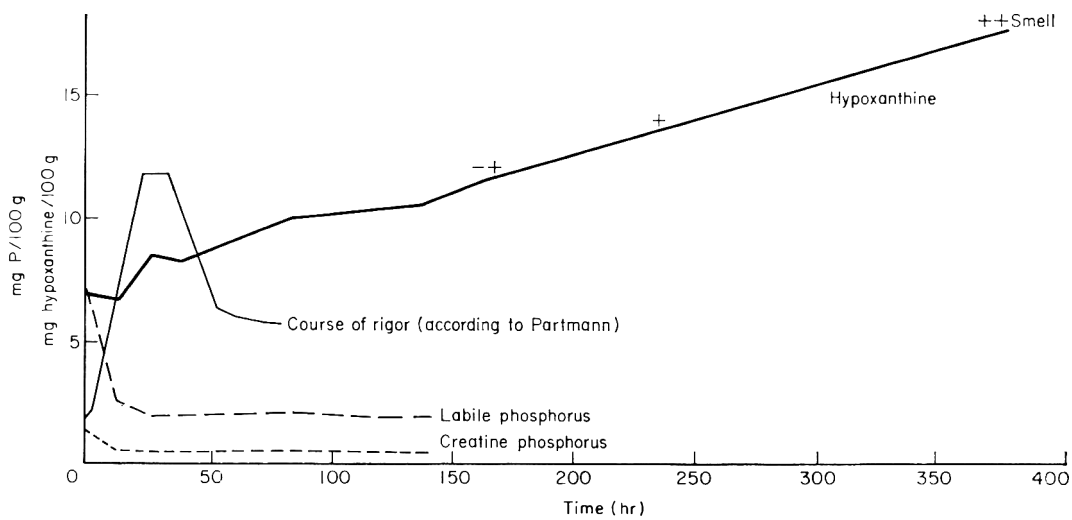

FIG. 3. Course of post-mortem changes in rainbow trout muscle kept at 4°C: labile phosphorus, creatine phosphorus, rigor, hypoxanthine and odour evaluation. Odour: −+ = just detectable; + = weak; ++ = moderate.

the changes in hypoxanthine and corresponding observations relative to the odour of the fish, as well as the course of rigor as above.

The curves illustrate that the compound which undergoes the most rapid change is creatine phosphate; here, the drop takes place during the first 12 hr. The loss of labile phosphate occurs at almost the same speed, although the levelling off is observable somewhat later, coming to an end at 25 hr. Decrease of the adenylic radicals is also most rapid at the beginning, i.e. the first 12 hr, and little further change is apparent after 40 hr. In the total acid-soluble phosphorus and inorganic phosphorus the greatest increase is observable during the first 25 hr and following this the change is only slight. The last change discernible is the increase in the amount of hypoxanthine; this increase continues during the whole period of storage. From the 7th day, an increasingly unpleasant smell is observable; at this point, the amount of hypoxanthine rises to more than 10 mg/100 g of tissue. Simultaneously, an increasing yellow-orange hue of the fish flesh also becomes evident.

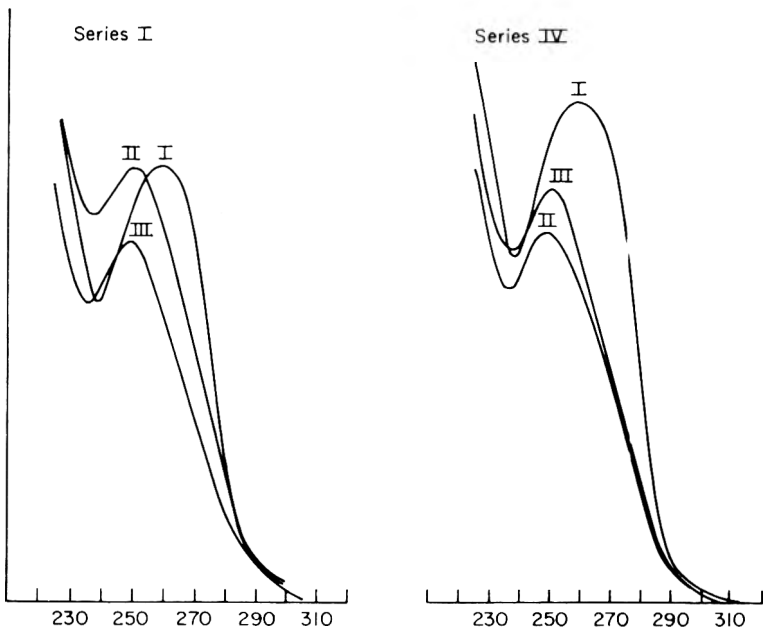

FIG. 4. Ultraviolet absorption spectra of neutralized perchloric acid extracts of rainbow trout muscle after various periods of storage. I, 0 hr; II, 12 hr; III, 140 hr.

Fig. 4 illustrates the change in the ultraviolet spectra of the extracts. Initially, the absorption maximum is at 260 mμ, but already after 12 hr the peak is shifted to the range of 250 mμ, and then remains unchanged until 140 hr. This reflects the loss of adenylic radicals, and the corresponding formation of IMP, inosine and hypoxanthine.

No exact measurement of rigor was made in the present work; according to Partmann the peak occurs approximately at 25 hr. Here the rigor state was definite after 12 and 25 hr, less pronounced after 40 hr, and rigidity was lost at later phases. Some related observations concerning the consistency of the muscle may be mentioned. At the beginning the raw muscle samples were difficult to break, with consequent difficulty in separation of the skin and bones, and removal of the red lateral muscle. After 12 and 25 hr, the coherence of the structure was less evident, and after 40 hr separation of the tissues was an easy matter.

Pike

The results of these experiments, less complete than those concerned with rainbow trout, are illustrated in Fig. 5(a) and (b). As with rainbow trout, the disappearance of creatine phosphate is the first change, followed by the drop of labile phosphorus; the former change is observable during the first 5 hr; the labile phosphorus disappears completely in 50 hr. Inorganic phosphorus continuously increases during the whole period of experiment (75 hr). Decrease in adenylic radicals occurs during the first 25 hr. These results are in close accord with those on rainbow trout.

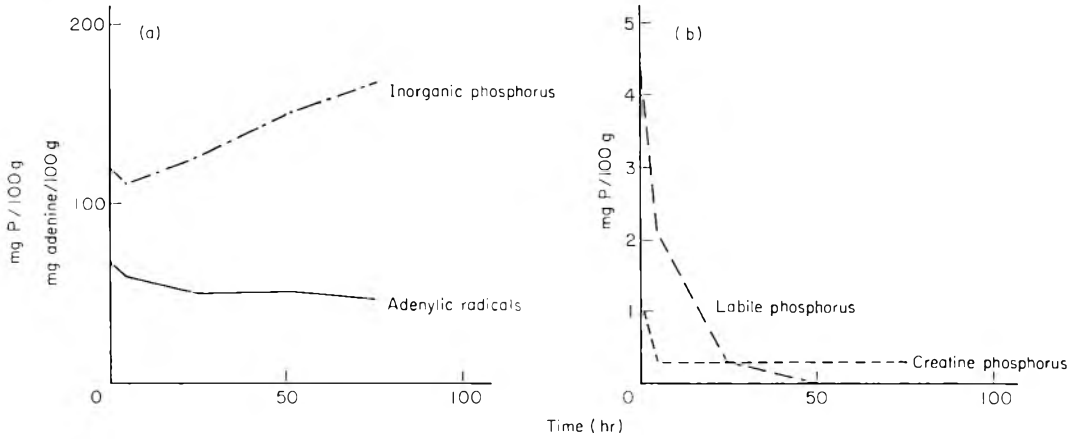

FIG. 5. Course of post-mortem changes in pike muscle kept at 4° C. (a) Inorganic phosphorus and adenylic radicals. (b) Labile phosphorus and creatine phosphorus.

Discussion

The literature contains some data for comparison as regards rainbow trout, but no information has been found on pike.

In principle, pond-reared fish make a favourable experimental object, as they may be obtained in good physiological condition; this means that the early phases in the decomposition series of nucleotides can here be clearly demonstrated, which does not apply for instance to trawled fish. It is known, for example, that the amount of ATP

found depends upon the fishing method: the more the fish has been exhausted the smaller is the amount (cf. Partmann, 1964; Trautner & Bramstedt, 1962). According to Partmann (1964) the first compound to be decomposed is creatine phosphate which cannot be demonstrated unless special precautions have been adopted to avoid factors which speed up the rigor. Here it was possible to show creatine phosphate initially both in rainbow trout and in pike. Nevertheless, the changes which occur in pond-reared fish do not necessarily represent the ordinary conditions which prevail in respect of marketed fish. Here it seems evident that pike more closely represent market fish than do rainbow trout.

If the results concerning the speed of decomposition in rainbow trout and pike are compared, it is observed that in respect of creatine phosphate the decrease in pike occurred in the first 5 hr, whereas the loss in rainbow trout took 25 hr. In regard to the labile phosphorus, in pike the value fell to 0 in 50 hr, the amount having already dropped to 2 mg/100 g in 5 hr. In the rainbow trout, the amount dropped to this level in 25 hr, but no further diminution was observable thereafter. This is in accordance with the results of Partmann (1961): the decomposition of ATP at 5–10°C is complete in rainbow trout in 25 hr, whereas in carp, after the same period, half of the ATP is still left.

The values obtained here for the ATP derivatives may be compared with the corresponding results of Spinelli & Kemp (1966) and Saito, Arai & Yajima (1959). If the differences in methods used are taken into account, a reasonable agreement between the results can be noticed.

Of the post-mortem degradation reactions studied here the first was thus the loss in creatine phosphate, and next the decrease in labile phosphate; also the shift in ultraviolet spectrum took place early. The most interesting as an index of quality would be the change in IMP; unfortunately, no simple and easy method is available to follow this change. One possibility would be to follow the rate of dephosphorylation; for this purpose, relatively rapid and simple methods have been developed recently (Jones & Murray, 1964; Spinelli & Kemp, 1966). Also the change in hypoxanthine has been suggested as useful criterion of post-mortem quality changes in fish, the main advantage being that this change occurs earlier than that of trimethylamine, for example. More research concerning these questions would be valuable.

References

BERENBLUM, I. & CHAIN, E. (1938) *Biochem. J.* **32,** 295.

BRAMSTEDT, F. (1962) *Fette, Seifen, Anstrichsmittel,* **64,** 820.

CRANE, R.K. & LIPMANN, F. (1953) *J. biol. Chem.* **201,** 235.

DERACHE, R. & LOWY, R. (1955) *Bull. Soc. Chim. Biol.* **37,** 1347.

HASHIMOTO, Y. (1964) *FAO Symposium on the Significance of Fundamental Research in the Utilization of Fish,* Husum 26–30 May. Session II, Paper No. WP/II/6.

HUGHES, R.B. & JONES, N.R. (1966) *J. Sci. Food Agric.* **17,** 434.

JONES, N.R. (1964) *FAO Symposium on the Significance of Fundamental Research in the Utilization of Fish,* Husum 26–30 May. Session IV, Paper No. WP/IV/4.

JONES, N.R. & MURRAY, J. (1964) *J. Sci. Food Agric.* **15,** 684.

JONES, N.R., BURT, J.R., MURRAY, J. & STROUD, G.D. (1964a) *FAO Symposium on the Significance of Fundamental Research in the Utilization of Fish,* Husum 26–30 May. Session I, Paper No. WP/I/2.

JONES, N.R., MURRAY, J., LIVINGSTON, E.J. & MURRAY, C.K. (1964b) *J. Sci. Food Agric.* **15,** 763.

LE PAGE, E.A. (1951) *Manometric Techniques and Tissue Metabolism* (Ed. by W.W. Umbreit, R. H. Burris and J.F. Stauffer), p. 185. Burgess, Minneapolis.

LOWY, R., DECLOITRE, F. & BRIGANT, L. (1964) *Enzymol. biol. clin.* **4,** 39.

MARTLAND, M. & ROBISON, R. (1926) *Biochem. J.* **20,** 847. Ref. KING, E. J. (1932) *Biochem. J.* **26,** 292.

PARTMANN, W. (1961) *Z. Ernährungswissensch.* **2,** 70.

PARTMANN, W. (1963) *J. Fd Sci.* **28,** 15.

PARTMANN, W. (1964) *FAO Symposium on the Significance of Fundamental Research in the Utilization of Fish,* Husum 26–30 May. Session I, Paper No. WP/I/3.

SAITO T., ARAI, K. & YAJIMA, T. (1959) *Nature, Lond.,* **184,** 1415.

SPINELLI, J., EKLUND, M. & MIYAUCHI, D. (1964) *J. Fd Sci.* **29,** 710.

SPINELLI, J. & KEMP, B. (1966) *J. Agric. Fd Chem.* **14,** 176.

TARR, H.L.A. (1966) *IFT 26th Ann. Meeting,* Portland, Oregon 22–27 May 1966 (Paper 25). Ref. *Lowry-Cockroft Abstr.* August 1966, **43,** 51.

TRAUTNER, K. & BRAMSTEDT, F. (1962) *Arch. Fischereiwiss.* **13,** 130.

J. Fd Technol. (1968) **3**, 115–122.

Shelf life of canned grapefruit juice — methods for evaluation and factors affecting it

H. C. MANNHEIM, A. BAKAL AND D. REZNIK

Summary. Dissolved tin content was used as a criterion of shelf life for both can and content. Several methods for determining the dissolved tin content are available, but most of them are destructive and involve time-consuming digestion of the samples. A non-destructive In-Can measuring device, which permits the changes in dissolved tin content to be followed in sealed plain tin cans during storage, was found to be highly reliable as a shelf life indicator. Using those methods, the effect of several processing variables on the shelf life of canned grapefruit juice were studied. The detrimental effect of large headspace and slow cooling and the benefit of de-aeration were shown.

Introduction

There are numerous possibilities in selecting the container material for food products. These possibilities include variation of the steel base, tin coating, lacquer, etc. Shelf life is defined as the period during which consumer acceptability is maintained, and its most common criterion is the time required for hydrogen swelling of one-half of the samples of a test-pack. [First failure and vacuum loss are also occasionally used as criteria (Hartwell, 1951).] The use of the dissolved tin content as a can corrosion measure and a quality indicator has also received some attention (Davis, 1958).

In order to obviate the need for prolonged storage tests, a number of rapid corrosivity tests have been developed in recent years, utilizing the electro-mechanical properties of tin plate in food or model solutions (Hoare & Britton, 1960; Kamm *et al.*, 1961). The principal methods are:

(1) Crystal-size etch test (Virgi, 1957).
(2) Porosity (Clark & Britton, 1958; Hoare & Britton, 1960).
(3) Pickle-lag test (Anon., 1960a).
(4) Iron solution value (Anon., 1960b).
(5) Electrical potential of sheet sample (Markridges, 1962).
(6) Alloy tin couple test (Kamm, 1959).

All these methods are based on one or several of the factors affecting corrosion, such as the quality of the steel, the type and thickness of the tin coating, the oxide film and the tin–iron alloy layer. The lack of correlation between them and the test-pack method is attributable to their being based on a small tin plate sample and involving a single factor or a few at most (Kamm & Willey, 1961).

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

While swelling, pitting and vacuum loss are suitable as shelf life criteria for the can, they offer little information about the product. There are many instances in which citrus juice cans still showed vacuum after 6 years, but the product was unacceptable due to sedimentation, off-colour or off-flavour, as well as high tin content. In these circumstances the amount of dissolved tin, which is limited by many food standards to 250 ppm, seems a better criterion. The most important methods used for dissolved-tin determination in food products are the iodometric (Townsend, 1956), polarographic (Goder & Alexander, 1946) and colorimetric (Karvanek, Gunder & Miller, 1966) analysis of acid-digested samples. Atomic absorption methods (Kahn, 1966), which obviate the need for digesting the sample, are being developed. Determination of tin losses from the internal surface of the can was also reported (Hernandez, 1961). An In-Can measuring device was developed by Reznik & Mannheim (1966) for continuous determination of electrochemical parameters in the can-plus-contents system.

Using grapefruit juice as the test medium and dissolved tin as the criterion for both container and contents, certain factors affecting shelf life were investigated.

Materials and methods

Grapefruit juice

Marsh seedless grapefruit were used in all experiments. Except where otherwise specified, cans were uniformly filled with hot (90°C) juice, closed and cooled immediately to 35°C. All cans were stored at 25° and 35°C.

Cans

In all experiments, 307 × 408 uncoated tin cans were used. Three types of tin coatings were evaluated, namely: 50 E = 0·50 lb/base box electrolytic; 100 E = 1·00 lb/base box electrolytic; and 1·5 HD = 1·50 lb/base box hot-dip.

The steel base-plate on all cans was of the MR type.

Processing variables

De-aeration was achieved by heating the grapefruit juice to 43°C and flashing it into a vacuum chamber maintained at 25 in. Hg, followed by pasteurization and filling as outlined above.

Slow cooling was carried out by holding normally-filled cans at 43°C for 24 hr prior to storage.

Headspace (9 mm) was obtained by inverting a plastic plunger into filled cans prior to closing.

Electrochemical measurements

A number of cans (1·5 HD) were fitted, prior to filling, with electrochemical bridges as described by Reznik & Mannheim (1966). The bridges consisted of glass tubes filled with KCl–agar and sealed with asbestos fibre in their tips. These tubes were clamped to the can wall through a Teflon sleeve, using sulphurless 'O' rings. The bridges showed no measurable diffusion, and vacuum was maintained over the period under investi-

gation. With the bridges in place, the cans were filled and sealed as outlined above. Tygon tubes, filled with potassium chloride solution, connected the bridges to a container into which a calomel electrode (SCE) was dipped. The cans to be measured were connected by wires, through a multiswitch, to a 'Doran' potentiometer equipped with a sensitive external galvanometer (Fig. 5).

Dissolved tin determinations were carried out on acid-digested samples, using a modified iodometric titration in a carbon dioxide atmosphere (Townsend, 1956).

Browning was measured on the juice serum in a Bausch & Lomb spectronic colorimeter at 425 mμ after precipitation of all colloidal matter with acetone and centrifugation.

Results and discussion

Can variables

Using a test-pack of grapefruit juice, the corrosion resistance of three types of cans, differing in their tin coating, was evaluated (Figs. 1 and 2). The actual tin coatings on the cans, determined by the fixed-current coulometric method (Kunze & Willey, 1952), are given in Table 1. Figs. 1 and 2 indicate that the rate of tin dissolution was highest in the 50 E cans and lowest in the 1·5 HD cans.

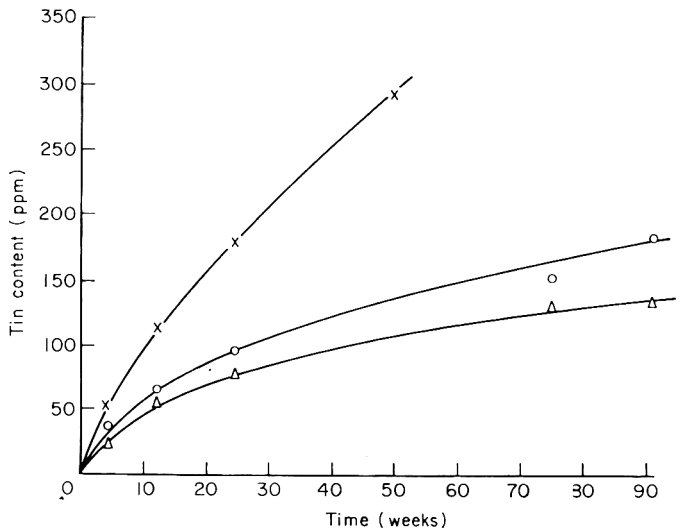

FIG. 1. Tin dissolved in grapefruit juice stored at 25°C. ×, 50 E cans; O, 100 E cans; △, 1·5 HD cans.

TABLE 1. Tin coatings of cans

Designation	Type of coating	Nominal tin coating (lb/base box)	Measured tin coating (lb/base box)
50 E	Electrolytic	0·50	0·45
100 E	Electrolytic	1·00	0·95
1·5 HD	Hot dip	1·50	1·05

Table 2 gives the relationships between dissolved tin content and actual tin coating. It is clearly seen that the ratio of dissolved tin is inversely proportional to the weight of actual tin coating. Furthermore, the ratios of dissolved tin were approximately the same at both 25° and 35°C during the entire storage period. The acceleration effect of temperature on tin dissolution was 20–30% in the initial stages of storage, with a subsequent increase to 100% and more after half a year.

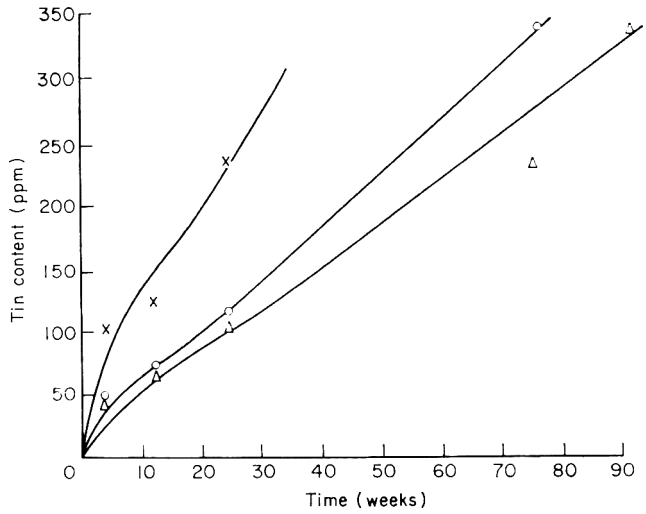

FIG. 2. Tin dissolved in grapefruit juice stored at 35°C. ×, 50 E cans; O, 100 E cans; △, 1·5 HD cans.

TABLE 2. Ratios of tin coatings, tin dissolution and shelf-life

Comparison	Ratio of actual tin coating	Ratio of dissolved tin after 24 weeks at:		Ratio of shelf-life* at 35°C
		25°C	35°C	
100 E *vs.* 1·5 HD	1/1·1	1/1·2	1/1·2	1/1·4
50 E *vs.* 1·5 HD	1/2·3	1/2·3	1/2·3	1/2·9
50 E *vs.* 100 E	1/2·1	1/1·9	1/2·0	1/2·0

*Time required to reach the 250 ppm dissolved tin level.

The accepted view (Hartwell, 1951), so far, has been that shelf life (i.e. the time required for swelling) is directly proportional to actual tin coating weight. The rate of corrosion, i.e. tin dissolution, was considered to be unaffected by tin coating thickness. This does not seem to be the case in our experiments. Differences in the rate of tin dissolution are apparently due to differences in porosity of the tin surfaces or of the tin–iron alloy layer. If porosity has no influence on corrosion, both the 100 E and 50 E

cans should show the same dissolved tin content during storage until swelling sets in, and at the same time should differ in swelling time, which is dependent on steel exposure. The time required to reach the level of 250 ppm dissolved tin should be the same, if the rates of dissolution are identical.

Comparison of the hot-dip and electrolytically-plated cans emphasizes still further the relationship between the rate of corrosion and the inverse of the actual tin coating weight. In this case, an important qualitative as well as quantitative difference in the alloy layer is involved (Kamm *et al.*, 1961). Kamm & Krickle (1965) demonstrated the importance not only of the weight of the alloy layer, but also of its continuity and uniformity, in retarding corrosion.

Processing variables

The effects of three processing variables—namely headspace, de-aeration and slow cooling—on tin dissolution, compared with normal processing, are given in Fig. 3. Results clearly show the beneficial effect of de-aeration in reducing tin dissolution, and the detrimental effect of a large oxygen-containing headspace.

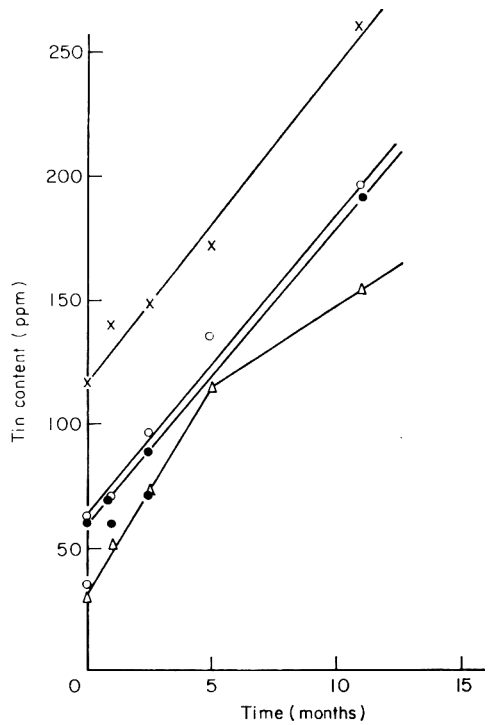

FIG. 3. Tin content in grapefruit juice stored at 35°C. ×, Headspace; O, slow cooling; ●, control; △, de-aeration.

Organoleptic evaluation showed that the sample with headspace was significantly inferior to the others. The flash-de-aerated juice was found to have a less pronounced grapefruit flavour, apparently due to loss of volatile components. Some members of the panel approved of this weaker flavour, while others found it 'watery'. The slowly-cooled juice rated significantly inferior to the control during the entire storage period. All juices containing 250 ppm or more dissolved tin were rejected by the taste panel.

All samples were also subjected to ascorbic acid analysis, vacuum determination and browning measurements. No significant differences either in ascorbic-acid content or in vacuum were found between the different treatments. The vacuum loss in all cans was gradual, and the vacuum dropped sharply to zero shortly before the swelling set in. As regards browning, significant differences were observed between the variants stored at 35°C (Fig. 4). De-aeration was shown to be beneficial, and the harmful effects of slow cooling and large headspace are seen once more.

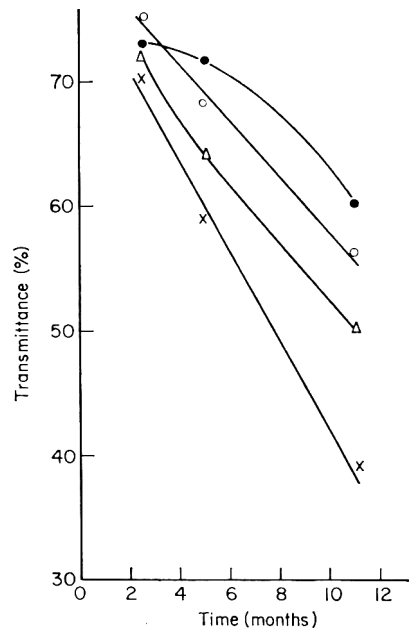

FIG. 4. Effect of various technological treatments on grapefruit juice browning as measured by per cent transmittance at 425 mμ. ×, Headspace; △, slow cooling; ○, control; ●, de-aeration.

Electrochemical measurements

The potential of a series of cans containing grapefruit juice and equipped with electrochemical bridges (Fig. 5) was measured during storage, and cans of the same batch were analysed for dissolved tin content. As shown previously (Reznik & Mannheim,

1966), the two parameters are correlated. The curve in Fig. 6, corresponds to the equation $C = Kt^n$, C being dissolved tin content, t storage time, and K and n constants dependent on product and plate quality.

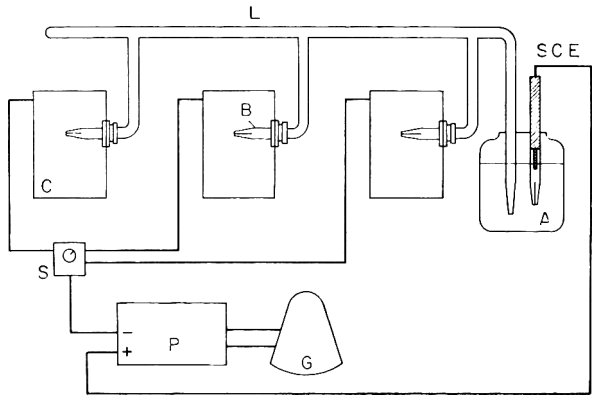

FIG. 5. Schematic diagram of the measuring system. A, Saturated KCl solution; B, electrochemical bridge; C, measured can; L, plastic tube filled with saturated KCl; SCE, standard calomel electrode; S, multiswitch; P, potentiometer; G, external galvanometer.

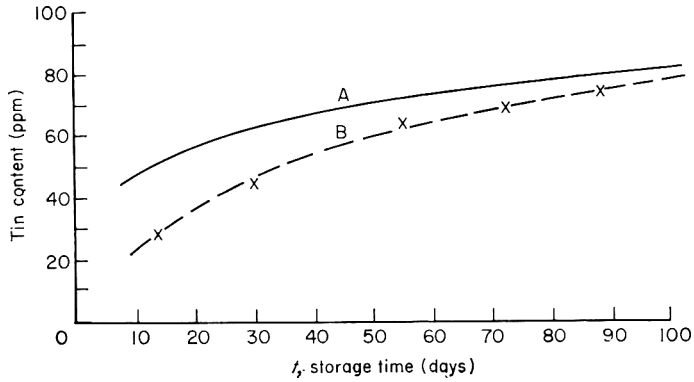

FIG. 6. Dissolved tin in cans containing grapefruit juice during 3 months storage at 25°C A, Calculated from electrochemical data; B, measured by tin determinations during storage.

If an upper limit is set for the dissolved tin content, e.g. 250 ppm, shelf life prediction should be easy, provided it can be shown that the exponent '*n*' in the above equation remains constant throughout the storage period. In the authors' opinion such a limit is preferable as shelf life criterion to first failure or 50% failure, since the limit refers to both product and container, whereas the other two refer to the container only. Further study is called for in this direction.

In conclusion, the study shows that dissolved tin content can serve as a measure of shelf life for both can and product. Several methods for determining the dissolved tin content are available, but most of them are destructive and involve time-consuming digestion of the samples. A non-destructive In-Can measuring device, which permits the changes in dissolved tin content to be followed in sealed plain tin cans during storage, was found to be highly reliable as a shelf life indicator.

References

ANON. (1960a) *Pickle Lag Test.* American Can Co., Res. Dept Bull., Maywood, Illinois.

ANON. (1960b) *The Modified Iron Solution Test.* American Can Co., Res. Dept Bull., Maywood, Illinois.

CLARK, M. & BRITTON, S.C. (1958) *Tin Res. Inst. Bull.* No. 292, Perivale, England.

DAVIS, E.G. (1958) Evaluation of tinplate containers for foods, *Food Preservation Quarterly*, CSIRO, Australia, **18** (No. 4).

GODAR, E.M. & ALEXANDER, O.R. (1946) *Ind. Eng. Chem. Anal.* **18,** 681.

HARTWELL, R.R. (1951) *Advanc. Fd Res.* **3,**328.

HERNANDEZ, H.H. (1961) *Fd Technol., Champaign,* **15,** 543.

HOARE, W.E. & BRITTON, S.C. (1960) *Tinplate Testing.* Tin Research Inst. Bull. Perivale, England.

KAHN, H.L. (1966) *J. chem. Educ.* **43,** 1.

KAMM, G.G. (1959) *The Alloy Tin Couple Test.* American Can Co., Res. Dept. Bull., Barrington, Illinois.

KAMM, G.G. & KRICKLE, J.L. (1965) *K Plate for Heavily Coated Electrolytic Tin-Plate Applications.* American Can Co., Res. Dept Bull., Barrington, Illinois.

KAMM, G.G. & WILLEY, A.R. (1961) *Corrosion,* **17,** 77t.

KAMM, G.G., WILLEY, A.R., BEESE, R.R. & KRICKLE, J.L. (1961) *Corrosion,* **17,** 84t.

KARVANEK, M., GUNDA, D. & MILLER, V. (1966) *J. Sci. Fd Agric.* **14,** i; 310.

KUNZE, C.T. & WILLEY, A.R. (1952) *J. Electrochem. Soc.* **99,** 354.

MARKRIDGES, A. (1962) *Corrosion,* **18,** 338.

REZNIK, D. & MANNHEIM, H.C. (1966) *Mod. Pack.* **39,** 127.

TOWNSEND, C.T. (1956) *A Laboratory Manual for the Canning Industry.* National Canners Association, Washington, D.C.

VIRGI, D.R. (1957) *Crystal Size Etch Test.* American Can Co., Res. Dept Bull., Maywood, Illinois.

J. Fd Technol. (1968) **3**, 123–129.

The effect of doses of gamma radiation up to 16 Mrad on plastic packaging materials for fish

J. N. KEAY

Summary. The effects of doses of gamma radiation of up to 16 Mrad on some plastic packaging materials have been studied in relation to properties considered desirable for the vacuum packing of fish. Nylon 11 appears to be the most suitable in terms of the tests applied.

Introduction

There is an interest in the use of gamma radiation for the sterilization of vacuum-packed fishery products. As a preliminary to microbiological and chemical studies in the field it was considered necessary to determine the resistance of currently available plastic packaging materials to the high dose levels of radiation to be experimented with. The technical literature is replete with references to the effects of ionizing radiation on organic polymers. The amount of information available on the effects of radiation on such materials in relation to their use for food packaging is less prolific. Among earlier articles on this topic that by Tripp (1959) is very lucid and comprehensive without being too lengthy. The excellent review compiled by Payne & Schmiege (1962) for the Continental Can Company summarizes most of the information on the subject up to that time. It also illuminates certain areas in the field in which there is a lack of information. Important among these is the effect that irradiation of the packaging material has on the organoleptic acceptability of the contents, it being all too easy to confuse undesirable odours and flavours arising in the packaging material with those arising in the contents. Wierbicki (1964) has reviewed the past achievements in the United States Army programme in the field and has described the further work that was at that time in progress. The effort was being directed towards examining the physical properties of irradiated packaging materials and to revealing the chemical nature of the substances extracted from such irradiated materials by 'food-simulating' solvents. A very recent paper by Killoran, Breyer & Wierbicki (1967) describes the screening of a number of materials at present commercially available in the United States in relation to their use for the in-package radiation sterilization of bacon, ham and pork.

The present communication describes a study of the effect of doses of gamma radiation ranging from 1 to 16 Mrad on a selection of three materials currently used for food packaging, namely Nylon 11, Polyester–Polyethylene laminate and Poly-

Author's address: Ministry of Technology, Torry Research Station, Abbey Road, Aberdeen.

propylene 'O'–Polyethylene laminate. The properties studied were some which were considered important to the use of the materials for in-package irradiation of fish and which had not been dealt with adequately elsewhere.

Materials and methods

(1) *Packaging materials*

Nylon 11

This material was tested as a pouch measuring 9 × 5 in. made from film 0·0016 in. thick. The plastic is initially extruded as a tube and the open pouches are thus provided with only one (heat) sealed edge during manufacture. The pouches were unprinted.

Polyester–Polyethylene laminate

This material was tested as a pouch measuring 10 × 5 in. The laminate consists of a layer of Medium Density Polyethylene 0·002 in. thick and a layer of Polypropylene 'O' 0·0005 in. thick held together by adhesive. The pouches were constructed in the same way as those in Polyester–Polyethylene, the Polyethylene forming the inner wall of the pouch. These pouches were also printed with a commercial motif.

Polypropylene 'O'–Medium Density Polyethylene laminate

This material was tested as a pouch measuring 10 × 5 in. The laminate consists of a layer of Medium Density Polyethylene 0·002 in. thick and a layer of Polypropylene 'O' 0·0005 in. thick held together by adhesive. The pouches were constructed in the same way as those in Polyester–Polyethylene, the Polyethylene forming the inner wall of the pouch. These pouches were also printed with a commercial motif.

(2) *Irradiation*

Batches of six pouches of each material were wrapped in heavy-gauge aluminium foil and these were placed in paper envelopes, the envelopes being labelled with the name of the materials and the dose level required. Six dose levels were administered, namely 1, 2, 3, 5, 8 and 16 Mrad. Thus eighteen packages in all were despatched for irradiation. Control batches of each material wrapped similarly were kept in the laboratory. The purpose of the aluminium foil wrappings was to obviate the possibility of inter-contamination of batches through the paper envelopes with taint-causing irradiation products.

Irradiation was carried out to the stated dose levels in the Spent Fuel Element Assembly at A.E.R.E., Harwell. Dose levels were measured with a small ferrous dosimeter.

(3) *Tests on irradiated materials*

(a) *Sensory examination of materials*

The irradiated samples and controls were examined as follows:

(i) *The interior of the pouches was smelt and compared with controls.* The character of the odour and any change in intensity throughout the dose range were noted.

(ii) *Development of colour or change in transparency.* A wad of six pouches was held up to the light and compared visually with a similar wad of unirradiated pouches.

(iii) *Development of brittleness, delamination and loss of 'slip'.* These were assessed by handling and visual inspection of the materials. The 'slip' property was tested for by rubbing the two interior walls of the pouches together and comparing the effect with the control.

(b) *Infrared spectral examination*

Each sample was placed in the sample beam of an infrared spectrometer and its absorption spectrum recorded over the range 650–3650 cm^{-1} using a prism-grating monochromator to give high resolution. For a given material, spectra of all the dosage samples were superimposed on one chart in order to reveal changes.

An alternative technique that was tried was to place the control sample in the reference beam and the irradiated sample in the sample beam of the spectrometer, the spectrum thus recorded revealing differences. The absorption spectra of the materials are very intense, however, which leads to low energy conditions and this coupled with differences produced by variations in the thickness of the pieces of film can produce misleading results.

The first method was therefore that which was relied on.

(c) *Gas chromatographic examination*

A 10-cm square was cut from a pouch subjected to irradiation at each dose level. The squares were placed in 15-ml test tubes and the tubes sealed with rubber serum caps. Each tube was placed in a boiling water bath for 5 min and 1 ml of the head-space vapour was then injected into a gas chromatograph fitted with a 5 ft × 4 mm column packed with 10% Polyethylene glycol (molecular weight 400) on 80–100 mesh celite. The column temperature was 100°C, the carrier gas was nitrogen (O_2 free) and a flame ionization detector at high sensitivity was used. The number of peaks on the chromatogram was recorded and an index of the total amount of volatile material was obtained by cutting out the chromatogram profile and weighing. This method was necessary as separation of peaks was incomplete.

Only very weak responses were obtained with head-space samples of material incubated at room temperature for 24 hr.

(d) *Taste panel examination*

Small fillets of cod that had been frozen within 48 hr of catching were vacuum packed in irradiated and control pouches using heat-impulse sealing. The fillets were packed while still in the frozen state and the packages were then individually wrapped in aluminium foil to prevent cross-contamination. They were stored at 2°C for 5 days. The packages were then examined for loss of vacuum and the contents submitted to an expert taste panel of seven members. The fish fillets were removed from the pouches and examined by the panel for raw odour taint. The fish were then cooked by steam heating in casseroles for 35 min and tested for odour and flavour taint.

Discussion of results

(1) *Odour, colour and mechanical properties* (Table 1)

The results of the odour test clearly indicate that both the laminates may after irradiation be capable of conferring taint to their contents and this is borne out by the subsequent taste panel results. The amount of coloration produced in the materials, while

TABLE 1. Examination of irradiated packaging materials: odour, colour and mechanical properties

Material	Odour	Colour	'Slip'	Brittleness	Delamination
Nylon 11	No odour	Very slight yellowing at all doses	No apparent loss	None	–
Polyester–Polyethylene	Acrid, increasing with dose	Very slight yellowing at all doses	No apparent loss	None	None
Polypropylene–Polyethylene	'Paraffin wax' increasing with dose	Distinct yellowing increasing markedly with dose	Marked loss increasing with dose	None	None

quite obvious under the conditions of the test would probably, even at its maximum (16 Mrad Polypropylene–Polyethylene), not be of significance commercially. Similarly, the loss of 'slip' observed in one of the materials while quite marked may not be of such a degree as to effect commercial packaging operations. No brittleness or delamination was observed in any of the materials.

(2) *Infrared spectroscopy and gas chromatographic examination* (Table 2, Fig. 1)

Infrared spectral examination 3 days after irradiation revealed no structural changes in any of the materials even at the 16-Mrad dose level. After a period of 5 months, however, comparison of the spectra of the 16 Mrad and control samples of the three materials showed minor changes in the profiles in the 700–1000 cm^{-1} regions in the case of the Nylon 11 and Polyester–Polyethylene, indicating that in these materials some slow radiation-induced transformation may have occurred.

The gas chromatographic data show a very marked increase in the amount and number of volatile materials present in the Polypropylene–Polyethylene laminate. This correlates with the odour indications described above.

(3) *Packaging properties and taste panel results* (Table 3)

No impairment of barrier or sealing properties was observed in any of the materials.

The raw-fish odour-taint results correlate with the previous observations, both the laminates, after irradiation, being guilty of tainting their contents. Important, however, is the observation that both odour and flavour taint disappear in the cooked fish.

TABLE 2. Examination of irradiated packaging materials: infrared spectroscopy and gas chromatography

Material	Dose (Mrad)	IR spectrum: time after irradiation		Gas chromatography	
		3 days	5 months	Total volatiles*	No. of peaks
Nylon 11	Control		–	7	6
	1		–	3	6
	2	No change	–	4	6
	3	throughout	–	4	6
	5	dosage range	–	4	6
	8		–	6	6
	16		Slight change	6	6
Polyester–Polyethylene	Control		–	3	6
	1		–	4	7
	2	No change	–	5	7
	3	throughout	–	6	8
	5	dosage range	–	9	8
	8		–	12	8
	16		Slight change	14	8
Polypropylene–Polyethylene	Control		–	6	6
	1		–	7	6
	2	No change	–	6	7
	3	throughout	–	14	7
	5	dosage range	–	16	9
	8		–	24	9
	16		No change	33	9

* Index = Weight of profile of chromatogram in g × 100 to nearest whole number.

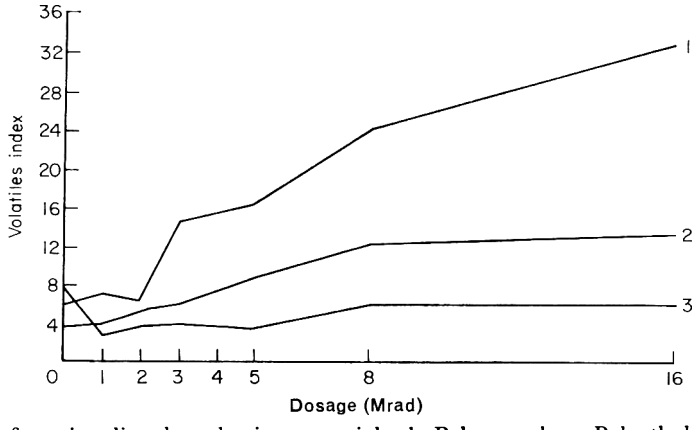

FIG. 1. Volatiles from irradiated packaging materials. 1, Polypropylene–Polyethylene; 2, Polyester–Polyethylene; 3, Nylon 11.

TABLE 3. Examination of irradiated packaging materials: packaging properties and taste panel results

	Dose (Mrad)						
	1	2	3	5	8	16	Control
Nylon 11							
Condition of pack	Good vac.	Good vac.	Good vac.	Good vac.	Good vac.	Good vac.	Good vac.
Odour taint (raw fish)	Absent	Absent	Absent	Absent	Absent	V. slight	Absent
Odour taint (cooked fish)	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Flavour taint (cooked fish)	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Polyester–Polyethylene							
Conditions of pack	Good vac.	Good vac.	Good vac.	Sl. leakage	Good vac.	Good vac.	Good vac.
Odour taint (raw fish)	Slight	Slight	Strong	Moderate	Moderate	Moderate	Absent
Odour taint (cooked fish)	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Flavour taint (cooked fish)	Absent	Absent	Absent	V. slight (one member)	Absent	Absent	Absent
Polypropylene–Polyethylene							
Condition of pack	Good vac.	Good vac.	Good vac.	Good vac.	Good vac.	Good vac.	Good vac.
Odour taint (raw fish)	V. slight	Moderate	Slight	Moderate	Strong	V. strong	Absent
Odour taint (cooked fish)	Absent	Absent	Absent	V. slight (one member)	Absent	Absent	Absent
Flavour taint (cooked fish)	Absent	Absent	Absent	V. slight (one member)	Absent	Absent	Absent

Note: Where flavour or odour taints were present they were invariably described as 'plastic'

(4) *Additional information*

Our research programme on the in-package sterilization of fish is a continuing one and we have very recently examined another material whose composition indicated, on the basis of the data described above, the likelihood of good radiation resistance. This is an extruded laminate of Nylon (a Nylon 6–Nylon 11 co-polymer) and Low Density Polyethylene. The overall thickness of the laminate was 0·0025 in. and the ratio of Nylon to Polyethylene, 1 : 2. The Polyethylene formed the inner wall of the pouches. The method of study was as described in the main text of the paper. The full data are not included here but in general the Nylon–Polyethylene laminate emerged as being slightly superior to the Polyester–Polyethylene laminate but inferior to Nylon 11.

Conclusion

Of the materials studied, Nylon 11 would appear, in terms of the tests applied, to be the most suitable for the in-package irradiation of fish.

Acknowledgments

The author wishes to thank Dr J. M. Shewan for granting the facilities of his expert taste panel, the United Kingdom Atomic Energy Authority (Wantage Laboratory) for irradiation services and Mr J. Robertson for technical assistance.

The work described in this paper was carried out as part of the programme of the Torry Research Station.

References

KILLORAN, J.J., BREYER, J.D. & WIERBICKI, E. (1967) *Fd Technol., Champaign*, **21,** 1125.

PAYNE, G.O. & SCHMIEGE, C.C. (1962) *Survey of Packaging Requirements for Radiation Pasteurized Foods.* Continental Can Company, New York.

TRIPP, G.E. (1959) *Int. J. appl. Radiat. Isotopes*, **6,** 199.

WIERBICKI, E. (1964) *Proceedings of the Eighth Contractors' Meeting, Radiation of Foods Programme, U.S. Army*, p. 139. U.S. Department of Commerce, Office of Technical Services, Washington.

J. Fd Technol. (1968) **3**, 131–142.

Influence of the activity of water on the spoilage of foodstuffs

M. LONCIN,* J. J. BIMBENET* AND J. LENGES†

Summary. An original method for the evaluation of activity of water as a function of water content is described. The effect of temperature on activity, and the effect of the activity of water on drying rate, growth and destruction of micro-organisms, enzyme action, chemical reactions, etc., are determined.

Introduction

The presence of water is normally essential for life to proceed: similarly it is also normally necessary for spoilage of foodstuffs. However, such water must be in an 'available' form and the degree of 'availability' is often expressed in terms of the 'activity' of the water.

Furthermore the water vapour pressure of a moist solid substance is generally different from the vapour pressure of free water at the same temperature. The term 'activity of water' denotes the ratio between the water vapour pressure of a substance and the vapour pressure of free water at the same temperature. This ratio also expresses the relative humidity of a gas in equilibrium with the substance. Whilst vapour pressures vary considerably with temperature, the ratio itself is much less dependent on temperature, as seen below.

Strictly speaking the thermodynamic activity is given by the fugacity ratio and not by the ratio of vapour pressures; however, this difference is slight and belongs rather to the field of theoretical thermodynamics.

The activity described above is sometimes denoted by the symbol a_w standing for the 'activity of water' or by ERH meaning 'equilibrium relative humidity'.

We shall use this symbol a_w in what follows. The curves showing the values of equilibrium relative humidity as a function of the moisture content are almost always obtained by experimental methods.

The reasons why water vapour pressure in a moist substance is often lower than the vapour pressure of free water at the same temperature are numerous. One of the main reasons appears to be the interaction between water and the polar groups, such as –CO–, –NH, –OH, of the natural constituents. Brunauer (Brunauer, Emmet & Tellier, 1938) devised a method of approximately evaluating the activity of water as a function of this interaction. The degree of hydration of the polar groups can be investigated by X-ray spectrography, by infrared spectroscopy, by the study of nuclear magnetic resonance or by long wave spectrography (Guilbot, 1952, 1961). There exist other

†Author's address: Ecole Nationale Superieure des Industries Agricoles et Alimentaires Massy, France.

*Authors' address: Centre d'Enseignement et de Recherche des Industries Alimentaires (IIF-IMC), Bruxelles 7, (Belgium).

important factors, however, which influence the vapour pressure. It is easily shown by physical methods that the vapour pressure of water inside capillaries is lower than the vapour pressure of a plane water surface; this is true for all solvents, the reduction of vapour pressure being proportional to the surface tension of the solvent. Thus, by definition, a porous substance is an adsorbent.

In many practical instances hydrated crystals are formed. For example lactose exists in milk powder at least partially as mono-hydrated crystals, which exert a water vapour pressure considerably lower than the vapour pressure of free water.

When solutes are dissolved in water the vapour pressure is depressed by the well-known phenomenon which is also responsible for elevation of boiling point. This is the main reason for the lowering of the activity of water in dehydrated fruit.

Symbols

a_w = activity of water or equilibrium relative humidity = vapour pressure of water in the product : vapour pressure of free water at the same temperature;
i = relative humidity;
n_a = kg water/kg dry air;
n_s = kg water/kg dry substance;
t = temperature (°C).

Evaluation of the activity of water

The curves indicating the activity of water as a function of the moisture content can be evaluated by the two following main methods:

(a) A quantity of the substance to be examined is brought into contact with a small quantity of gas (usually air) and the relative humidity of this gas is measured when equilibrium is reached (Mossel & Van Kuijk, 1955; Ayerst, 1965).

(b) A sample of the substance is placed in a gas flow at known temperature and relative humidity: the moisture content of the sample at equilibrium is analytically established.

A direct manometric method is also possible which we have applied to vegetable oils (Loncin, 1955); a method of direct measurement of absorption pressure, particularly suitable for very high moisture contents has also been published (Gur-Arieh, Nelson & Steinberg, 1965).

Water content determinations should never be carried out by weight loss but rather by direct methods, such as the Karl Fischer method in the presence of formamide (McComb & Wright, 1954; Thung, 1964).

An apparatus has been developed (Loncin, 1965; Loncin *et al.*, 1965a) to derive these curves based on the second of the above-mentioned methods (b): an air flow of known temperature and relative humidity is brought into a cell containing the sample; the moisture content of this sample (n_s) is determined when a constant weight is reached. This apparatus is original in so far as a relative humidity of the air of 0·75 is obtained by

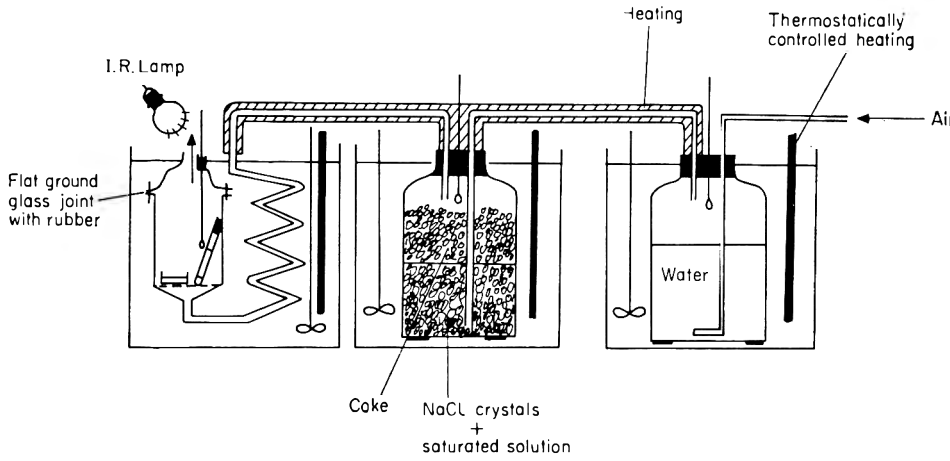

FIG. 1. Apparatus developed to derive curves in method (b).

bubbling it through a saturated NaCl solution containing solid NaCl (Fig. 1). This mixture has an equilibrium relative humidity which is very close to 0·75 at all temperatures between 10° and 90°C.

The advantage of this method over those which saturate the air with water at a given temperature and pressure, lies in the fact that the air leaves the salt solution contactor in an unsaturated state. Thus a slight cooling does not inevitably bring about condensation and it is not necessary to take very elaborate precautions such as special insulation, thermostatic controls and heating to avoid any fortuitous condensation or evaporation.

If it is desired, for example, to produce air having the characteristics of point A on

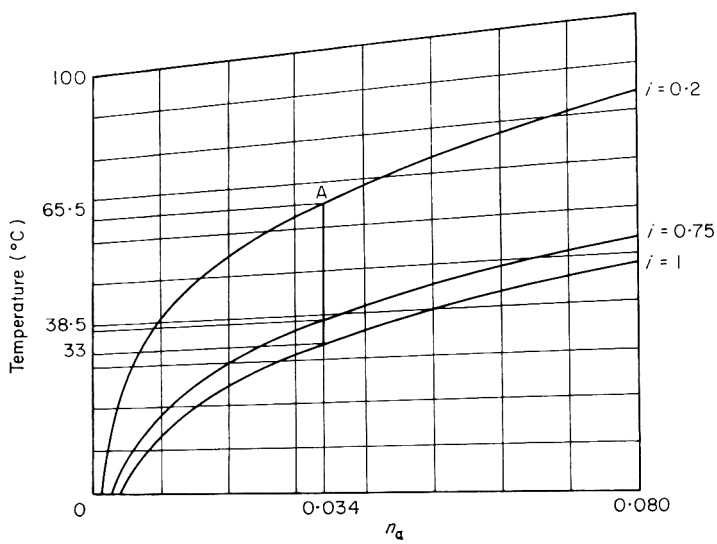

FIG. 2. Enthalpy diagram of wet air.

the enthalpy diagram (Fig. 2), i.e. at a temperature $t_3 = 65{\cdot}5°C$ and a relative humidity $i = 0{\cdot}2$, one needs only to increase the temperature t_2 of the NaCl saturator to $t_2 = 38{\cdot}5°C$, which brings the water content of the air flow coming from this saturator to exactly $n_a = 0{\cdot}034$ kg water/kg dry air. By further increasing the temperature to $t_3 = 65{\cdot}5°C$ in the present case, the air obtained corresponds to point A on the diagram.

Experience has shown the advantage of pre-saturating the air with ordinary water (e.g. at a temperature of approximately 33°C in the preceding example), to avoid excessive evaporation in the NaCl saturator.

For an air throughput of about 2 m^3/hr a water saturator of 3 litres capacity and a NaCl saturator of 2 litres capacity are used. If the air is passed into a measuring cell of 5 cm diameter, the flow rate of 0·3 m/sec results in rapid establishment of equilibrium with the samples. For samples of 200 mg placed in flat glass dishes a contact time of about 1 hr at 60–80°C is quite sufficient to achieve equilibrium conditions, thus allowing thermally sensitive products to be tested. At temperatures of about 30°C the same size samples equilibrate after about 3 hr contact.

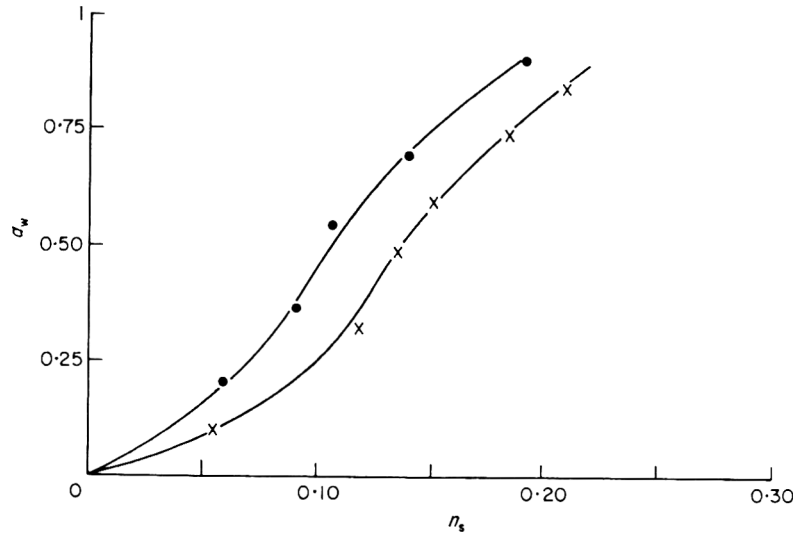

FIG. 3. Activity of water as a function of the moisture content for starch at 80°C (●) and 30°C (×).

The curve in Fig. 3 shows the activity of water as a function of the moisture content for starch, and Fig. 4 shows this activity for casein. It will be seen that hygroscopicity decreases when the temperature rises from 30° to 80°C; the curve is displaced towards the upper left-hand side of the diagram. Formulae have been published enabling this influence of the temperature to be predicted (Smith, 1965).

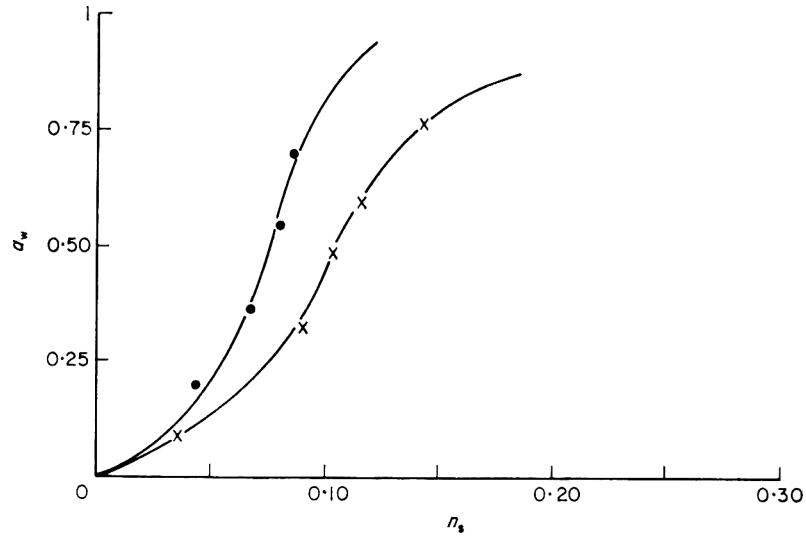

FIG. 4. Activity of water as a function of the moisture content for casein at 80°C (●) and 30°C (×).

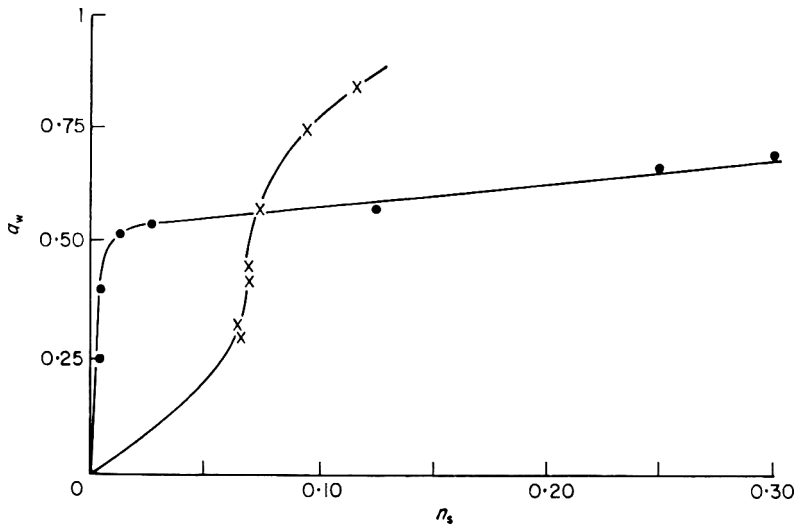

FIG. 5. Activity of water as a function of the moisture content for glucose at 80°C (●) and 30°C (×).

In the case of glucose however, Fig. 5 shows that for low moisture contents hygroscopicity decreases with an increase in temperature, whereas the opposite phenomenon takes place with higher product moisture contents.

The curves in Fig. 6 show the behaviour of different sugars at 80°C. For lactose the abscissa at $a_w = 0$ is exactly equivalent to 1 mole of water, the vapour pressure of which is negligible under such conditions. For glucose and sucrose on the right of the diagram

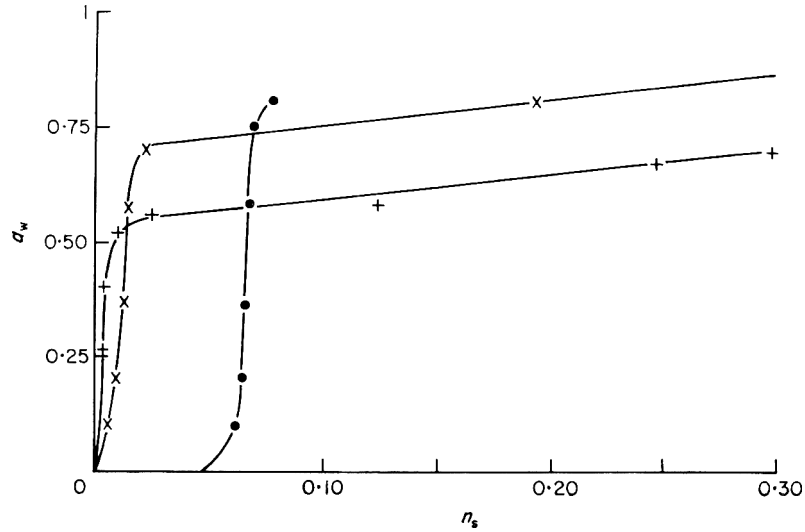

FIG. 6. Activity of water as a function of the moisture content for different sugars at 80°C. ●, Lactose; +, glucose; ×, sucrose.

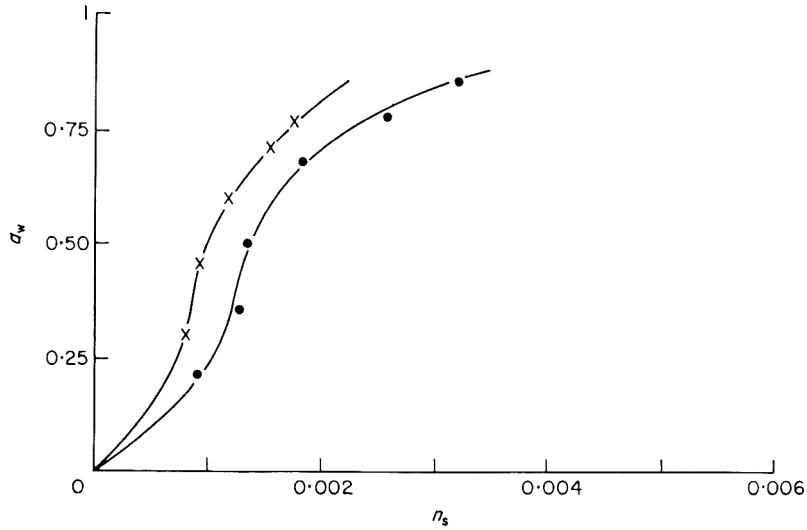

FIG. 7. Activity of water as a function of the moisture content for peanut oil at 80°C (●) and 30°C (×).

the shape of the curve is the result of the dissolution of the sugar in the water; similar curves obtained for sucrose have been published by Roche (1961).

In Fig. 7 it can be seen that hygroscopicity of peanut oil also increases with increase in temperature; the same holds true for oleic acid, as shown in Fig. 8. (It should be noted that Figs. 7 and 8 are drawn with quite different abscissa scales from the preceding figures.) The increase of hygroscopicity of fats with temperature is undoubtedly

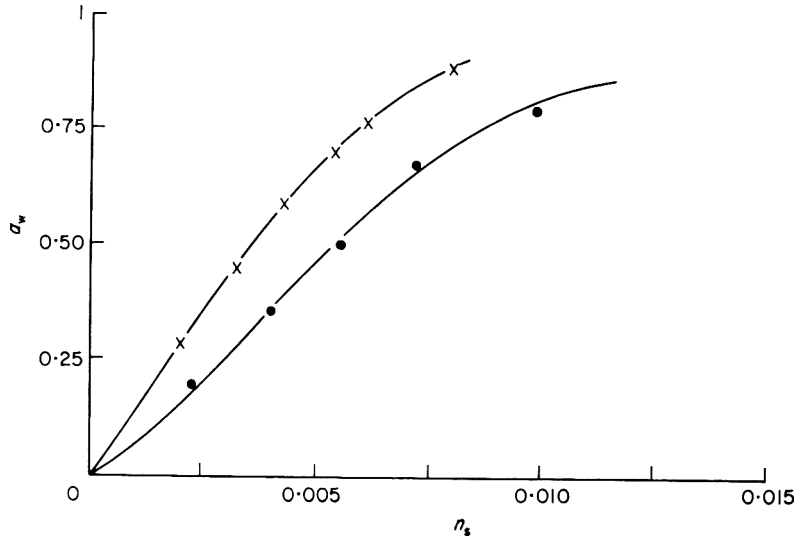

FIG. 8. Activity of water as a function of the moisture content for oleic acid at 80°C (●) and 30°C (×).

due to the increase of the solubility of water in these substances when the temperature increases (Loncin, 1955).

These curves show that the fall in hygroscopicity with rising temperature is far rom being general; the phenomenon is in fact quite the reverse for fats and for sugars such as glucose or sucrose.

Curves for complex substances are always obtained experimentally, the device described above being particularly suitable to determine them.

Practical importance

The activity of water is of special importance in food technology as illustrated by the following examples:

Drying rate

The rate of drying by air at any instant is proportional to the difference between the vapour pressure at the surface of the product and the partial pressure of water in air (Loncin, 1961).

The curves relating a_w and n_s are thus of importance in the prediction of drying rates. It should be mentioned, however, that the moisture content n_s which has to be taken into account is the moisture content on the surface of the product, and this can be quite different from the moisture content existing inside the product.

Undoubtedly the migration of free water by capillary is quicker than the migration of water with an activity below 1 (Van Arsdel, 1947).

The enthalpy of vaporization is also influenced by the activity of water. It increases from 560 to 590 kcal/kg for free water to nearly 1000 kcal for water with an activity of

0·1; at that state it represents an enthalpy of desorption rather than of vaporization.

Growth and destruction of micro organisms

Bacterial growth is generally impossible when a_w is reduced below 0·90. Most mould and yeast strains are inhibited between 0·88 and 0·80, although some osmophile yeast strains can still grow down to 0·6. Reduction of water activity provides a very important means of stabilizing food products. This reduction can be achieved by partial elimination of the water (dehydration) or by the addition of water-soluble substances, such as sugar or salt (as in jams or pickled preserves).

The reduction of water activity by drying or by addition of water-soluble substances has an extremely variable bactericidal effect. Depending on the duration of operation, the a_w reached, the species and even the strain of micro-organism involved as well as its growth phase and the presence of certain substances, the reduction of the population can either be insignificant or, exceptionally, reach values of 10^{-5} or even 10^{-6} (Proon & Hemmons, 1949; Rhodes & Fisher, 1950; Peri, 1965 private communication). Thus dehydrated or freeze-dried products can contain important micro-organic populations and living *Salmonella*, for example, can sometimes be present.

The thermal destruction of micro-organisms becomes more difficult when the water activity is decreased; this fact is responsible for the presence sometimes in spray-dried substances in a warm air flow of very substantial micro-organic population. When the droplets contain large amounts of water, they remain at low temperature; when the temperature increases, the moisture content has already reached a level at which the micro-organisms become much less sensitive.

Enzymic action

Comprehensive studies have been published on the influence of water activity on enzymic reactions (Drapon, 1961; Acker, 1962). The most widespread enzymes such as the amylases, phenoloxidases and peroxidases are completely inactivated when a_w reaches 0·85. On the other hand, lipases remain active at values of a_w below 0·3 or even 0·1 (Acker, 1962; Loncin & Jacobsberg, 1964, 1965; Acker & Beutler, 1965; Purr, 1966); under these conditions, they can pose an extraordinary resistance towards thermal destruction.

Chemical reactions

Amongst the chemical phenomena capable of causing foodstuff to deteriorate the Maillard reactions are of prime importance. It is known that these reactions are strongly influenced by the water activity in solid substances, and that their rate reaches a maximum when a_w reaches 0·6–0·7 (Lea & Harman, 1949). Fig. 9 shows the colour change of milk powder kept at 40°C for 10 days as a function of a_w. The corresponding loss in free lysin is recorded on Fig. 10.

It has been shown, however, that in homogeneous liquid phase, viz. ethylene glycol + water solutions to which 0·01 molar alanine and glucose have been added, the inten-

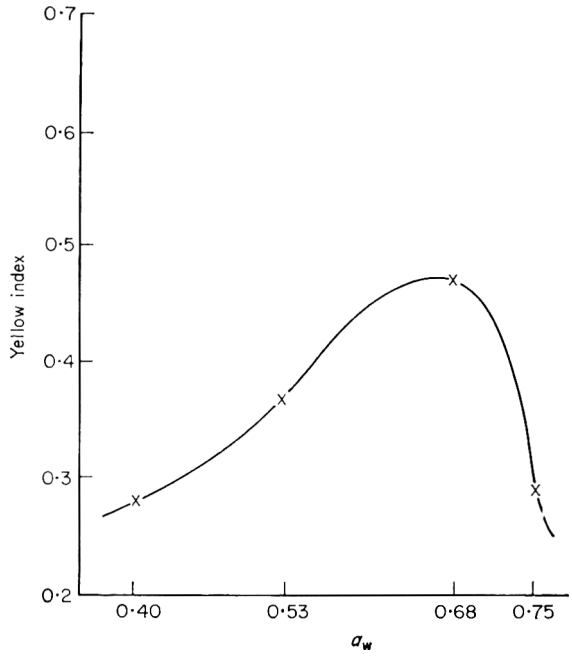

FIG. 9. The colour change of milk powder kept at 40°C for 10 days as a function of a_w.

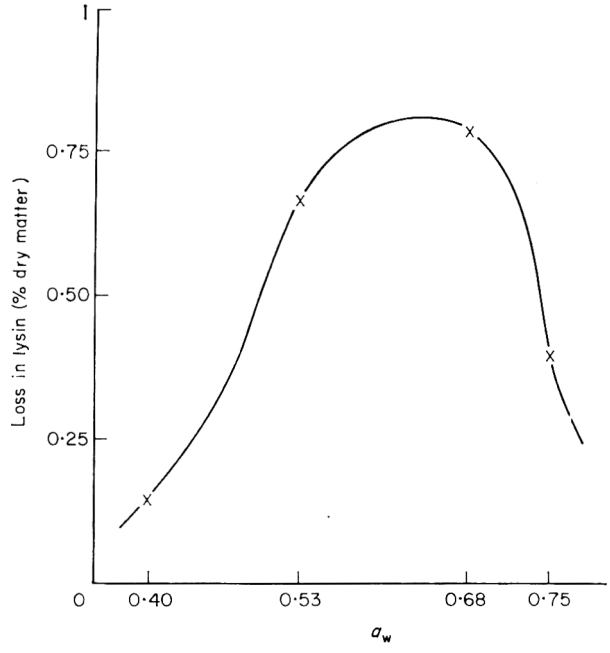

FIG. 10. The loss in free lysin of milk powder kept at 40°C for 10 days as a function of a_w.

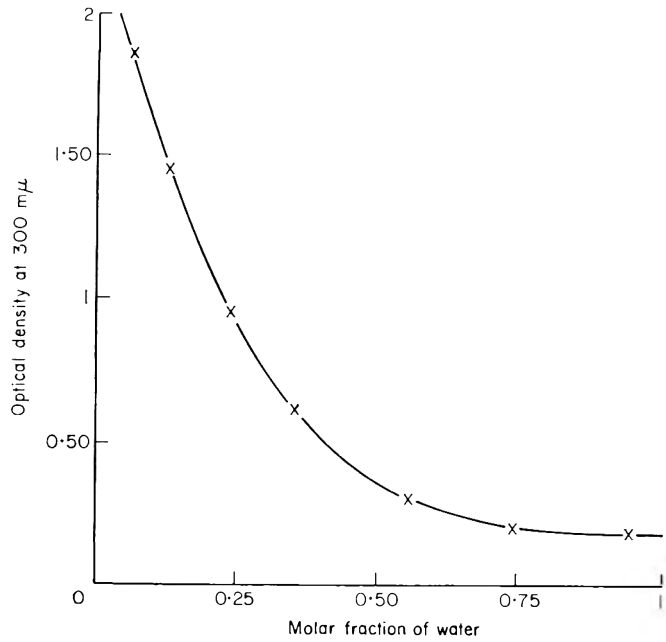

FIG. 11. The intensity of the Maillard reactions.

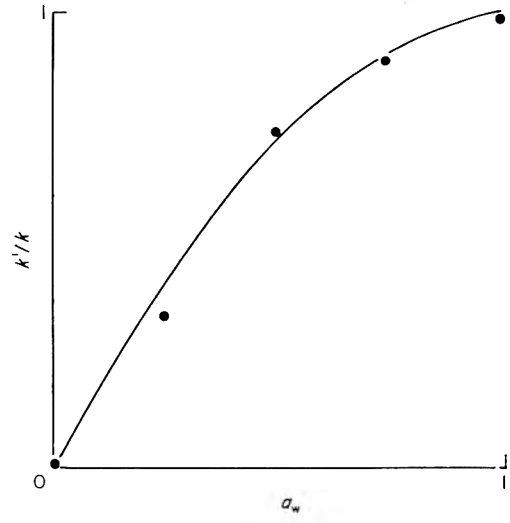

FIG. 12. The ratio of the velocity constants *versus* a_w.

$$\frac{k'}{k} = \frac{\text{Velocity constant of the hydrolosis test sample}}{\text{Velocity constant of water-saturated sample}}.$$

sity of the Maillard reactions, as measured by the optical density at 300 mμ, has a quite different aspect after 2 hr heating at 90°C (Fig. 11). This phenomenon must be attributed to the slowing down action of water which constitutes one of the first products of the Maillard reactions. In solid substances this action is counterbalanced at low moisture contents by an insufficient mobility of the reaction groups. From these two opposing influences results the maximum observed experimentally (Loncin *et al.*, 1965b). The activity of water influences also the transformation of chlorophyll into phaeophytin; this reaction becomes very slow when a_w falls below 0·7.

Hydrolysis of protopectin, and splitting and demethylation of pectin are also influenced by the activity of water (Lenges, 1965 private communication).

The spontaneous autocatalytic hydrolysis of fats (Loncin, 1952, 1953a, b) is strongly influenced by a_w as shown on Fig. 12 which represents the ratio of the velocity constants *versus* a_w. Similar curves have been obtained by plotting the velocity constants ratio against the actual water content.

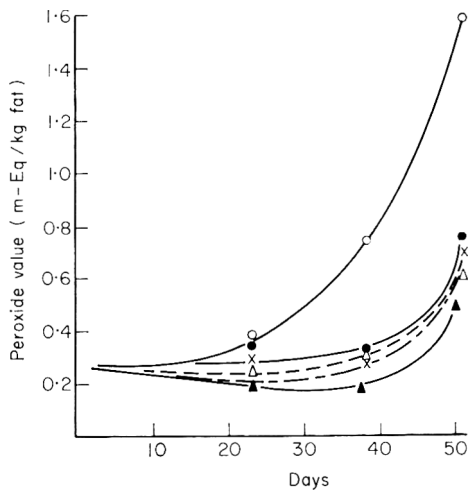

FIG. 13. The oxidation rate of the fat contained in spray-dried milk powder at 37°C recorded for several a_w. ○, 0·00; ●, 0·415; ×, 0·75; △, 0·18; ▲, 0·53.

The influence of the a_w on the oxidation of unsaturated fats can be very important in practice. In Fig. 13 the oxidation rate of the fat contained in spray dried milk powder is recorded for several a_w. The peroxide value was measured on the fat separated following the method by Stine (1954). The stimulation of the oxidation rate is particularly remarkable for a strongly dehydrated product corresponding to a_w below 0·18; no significant difference in the oxidation rate has been observed for a_w between 0·11 and 0·75.

This fact is very important in the choice of the conditions of storage of dehydrated products. An optimum a_w exists at which most of the chemical or biochemical reactions are slowed down, whilst the oxidation rate also remains sufficiently low.

Miscellaneous

During the freeze drying of an aqueous solution of glucose + glycine containing small amounts of acetone, Rey (1962) found that the acetone remained partially adsorbed, notwithstanding a final desorption under high vacuum near 40°C. This fact clearly evidences the influence of the moisture content on the adsorption.

Capacity of solids to retain organic substances. It is probable that essential oils contained in vegetables, for example, are retained partly at least by already dehydrated parts and that the presence of marked moisture gradients in the product must in fact allow a stronger retention by the peripheral part.

References

Acker, L. (1962) *Advanc. Fd Res.* **2,** 263.

Acker, L. & Beutler, H.O. (1965) *Getreide Mehl.* **1,** 4.

Ayerst, G. (1965) *J. Sci. Fd Agric.* **16,** 1.

Brunauer, S., Emmet, P.H. & Tellier, E. (1938) *J. Am. chem. Soc.* **60,** 309.

Drapron, R. (1961) *Thèse Conservatoire des Arts et Mètiers,* pp. 22–11. Paris.

Guilbot, A. (1952) *Inds agric. aliment.* **72,** 7.

Guilbot, A. (1961) *Inds agric. aliment.* **78,** 210.

Gur Arieh, C., Nelson, A.I. & Steinberg, M.P. (1965) *J. Fd Sci.* **30,** 188.

Lea, C.H. & Hannan, R.S. (1949) *Biochem. biophys. Acta,* **3,** 313.

Loncin, M. (1952) *Revue Ferment. Ind. aliment.* **7,** 43–90–199, 159.

Loncin, M. (1953a) *Revue Ferment. Ind. aliment.* **8,** 21–61–111, 156.

Loncin, M. (1953b) *Fette Seifen,* **55,** 7.

Loncin, M. (1955) *Fette Seifen,* **57,** 413.

Loncin, M. (1961) *Les Operations Unitaires du Genie Chimique.* Edit. Dunod Paris, Desoer Liege.

Loncin, M. (1965). *Dechema-Mongr.* Band 56, Nr. 976–993–193.

Loncin, M. & Jacobsberg, B. (1964) *Inds agric. aliment.* **81,** 118.

Loncin, M. & Jacobsberg, B. (1965) *Inds agric. aliment.* **82,** 19.

Loncin, M., Lenges, J.P., Bimbenet, J.J. & Jacqmain, D. (1965a) *Inds agric. aliment.* **82,** 923.

Loncin, M., Jacqmain, D., Tutundjan-Provost, A.M., Lenges, J.P. & Bimbenet, J.J. (1965b) *C.r. Seanc. Acad. Sci. Paris,* **260,** 3208.

McComb, E.A. & Wright, H.M. (1954) *Fd Technol., Champaign,* **8,** 73.

Mossel, D.A.A. & Van Kuijk, H.J.L. (1955) *Fd Res.* **20,** 415.

Proon, H. & Hemmons, L.M. (1949) *J. gen. Microbiol.* **3,** 7.

Purr, A. (1966) *Fette Seifen,* **68,** 145.

Rey, L.R. (1962) *Gefriertrocknungstagung.* Leybold, Köln.

Rhodes, M. & Fisher, P.J. (1950) *J. gen. Microbiol.* **4,** 450.

Roche, R. (1950) *Inds agric. aliment.* **78,** 765.

Smith, S.E. (1965) *J. Amer. chem. Soc.* **69,** 646.

Stine, C.M. (1954) *J. Dairy Sci.* **37,** 202.

Thung, S.B. (1964) *J. Sci. Fd Agric.* **15,** 236.

Van Arsdel, W.B. (1947) *Trans. Am. Inst. chem. Engrs,* **43,** 13.

J. Fd Technol. (1968) **3**, 143–150.

Low temperature injury in yams

D. G. COURSEY

Summary. The tubers of certain species of yams, *Dioscorea alata* and *Dioscorea rotundata*, are susceptible to a form of low temperature injury or 'chilling damage' when stored at temperatures substantially above zero (i.e. 10–12°C). Complete breakdown of the tuber tissue occurs shortly after return to normal tropical ambient temperatures.

Introduction

The yams (*Dioscorea* spp.) are tropical tuber crops, produced mainly in West Africa, South East Asia and the Caribbean, to the extent of about 20 million tons per annum (Coursey, 1967a). As they are usually stored in the producer countries, they are not normally exposed to low temperatures. The provision of adequate ventilation and the avoidance of high temperatures are the major factors in successful storage (Coursey, 1967b). Consequently the phenomenon of low temperature injury or 'chilling damage' has not received the attention that it has in the similar (though botanically unrelated) crop, the sweet potato (*Ipomoea batatas* (L.) Poir.). Low temperature injury is a well-known cause of post-harvest loss in this crop (Lauritzen, 1931; Cooley, Kushman & Smart, 1954; Kushman & Deonier, 1957; Liebermann *et al.*, 1958) and is also known to occur in many other fruit and vegetable materials, especially those of tropical or sub-tropical origin (Desrosier, 1963; Fidler, 1968).

Currently, quantities of the order of 1200 tons of *D. alata* L. and 5000 tons of *D. rotundata* Poir. are imported per annum into Great Britain, together with much smaller quantities of other species. Difficulties have been encountered by shippers, and many consignments have arrived in badly deteriorated condition. Although this has partly been due to poor quality of the material when exported, inadequate packaging, rough handling and stowage under unsuitable conditions, it is considered that chilling occurring during transport, or after arrival in Great Britain is also a causative factor.

A few reports in the literature indicate that yams are susceptible to low temperature injury. The growth of *D. hispida* Dennst. was shown (Copeland, 1916) to be impaired by chilling the tubers. Referring to the cultivation of *D. alata* in Florida, Young (1923) suggests that the tubers should be stored in frostproof buildings, and that 50–60°F is the

Author's address: Tropical Products Institute, Ministry of Overseas Development, 56/62 Gray's Inn Road, London, W.C.1.

optimum storage temperature. Experiments in Puerto Rico on the storage of 'Guinea Yams' (*D. rotundata*) at 34°F (Anon., 1937) were a complete failure: after 10 days at this temperature, the tubers decayed and disintegrated into an inedible slimy mass. Storage experiments in Venezuela (Czyrhinciw & Jaffe, 1951) on *D. alata* at 3°C, 12°C and ambient temperature showed that the tubers suddenly lost weight after 3 and 4 weeks storage, respectively, at the two reduced temperatures, and showed complete breakdown shortly after being returned to the ambient temperature. During investigations on the fumigation of vegetables being imported into the U.S.A., *Dioscorea* tubers were damaged by fumigation with methyl bromide at 55°F, but not at higher temperatures (Roth & Richardson, 1966). Irreversible damage to the respiratory metabolism of tuber tissue of *D. rotundata* caused by holding the tissue at 5°C, but not at 10°C has been reported (Coursey, Fellows & Coulson, 1966).

The present paper is intended to define more closely the temperatures at which low temperature injury occurs in yams, and also to describe and illustrate the nature of the effects of such injury in greater detail than earlier publications on the subject.

Materials and methods

Two species of yam, *D. alata* and *D. rotundata*, which are important in international trade were used. Material of the former, cultivar 'White Lisbon', was provided (two consignments) by the Ministry of Agriculture and Labour, Barbados, West Indies. Samples of the latter were derived from commercial sources in Brazil. The experimental material was consigned to Great Britain by air shortly after being harvested, in stowage where exposure to low temperatures did not occur. Samples were conditioned for 1 week at 25°C (i.e. near tropical ambient) on arrival.

Groups, each of five whole, undamaged yam tubers, were stored at temperatures between 5° and 25°C for various periods. Temperatures were controlled to $\pm 0{\cdot}5$°C: humidity was not controlled.

The tubers were weighed and inspected for visible deterioration individually twice weekly throughout the storage period. At the termination of the storage periods, specimen tubers were cut open and the internal tissue examined.

Results

Changes in weight of groups of yam tubers during storage at various temperatures are shown graphically in Figs. 1–3. Fig. 4 illustrates the individual behaviour of *D. alata* tubers. Table 1 records the onset of softening in the tubers at different temperatures.

The symptoms of low temperature injury are as follows:

D. alata

The tubers, after various storage periods, became soft to the touch, initially in patches, and later all over. When specimen tubers were cut open (after 6 weeks) the flesh was

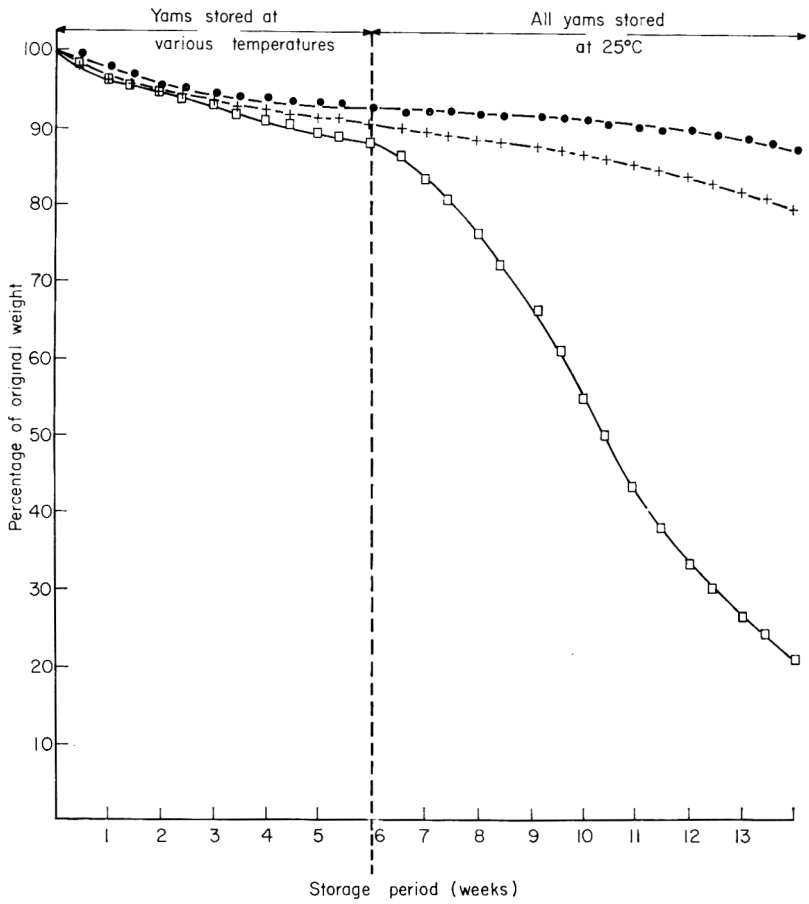

FIG. 1. Changes in weight of groups of *D. alata* tubers stored at various temperatures (first consignment). ●, 25°C; +, 15°C; □, 5°C.

found to have a slightly mottled, greyish appearance (instead of the normal clear creamy white), and to be flecked with reddish-brown. The immediate subcutaneous layer was darkened to brown, the depth of penetration of the discolouration being variable, from 0 to 5 mm. The whole tuber was of a spongy, waterlogged texture. On subsequent storage at 25°C, some tubers softened further, and within a few days became putrescent, supporting a profuse and varied growth of fungi and yeasts, while others dried and shrivelled up.

D. rotundata

Softening of the tubers, similar to that observed in the other species, but more rapid, occurred. When specimens were cut open, the flesh was found to be discoloured to a dirty buff colour: the tissue had a soggy, waterlogged consistency, and was considerably

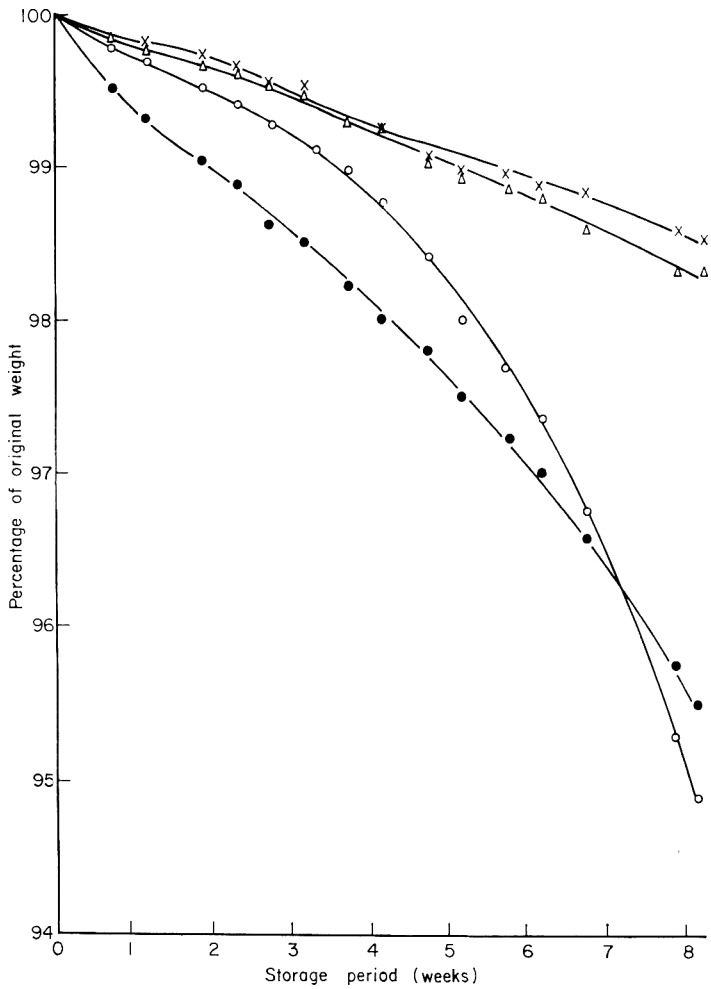

FIG. 2. Changes in weight of groups of *D. alata* tubers stored at various temperatures second consignment). ●, 25°C; ×, 12·5°C; △, 7·5°C; ○, 5°C.

softer than in the case of *D. alata*. On return to 25°C, further softening took place, the skin separated, and within 2 weeks the tubers had virtually disintegrated, and were decaying and malodorous: one (from storage at 5°C) had started to liquefy. None dried and shrivelled, as did some of the former species.

Discussion

As the two species of yam investigated behaved somewhat differently they may conveniently be discussed separately.

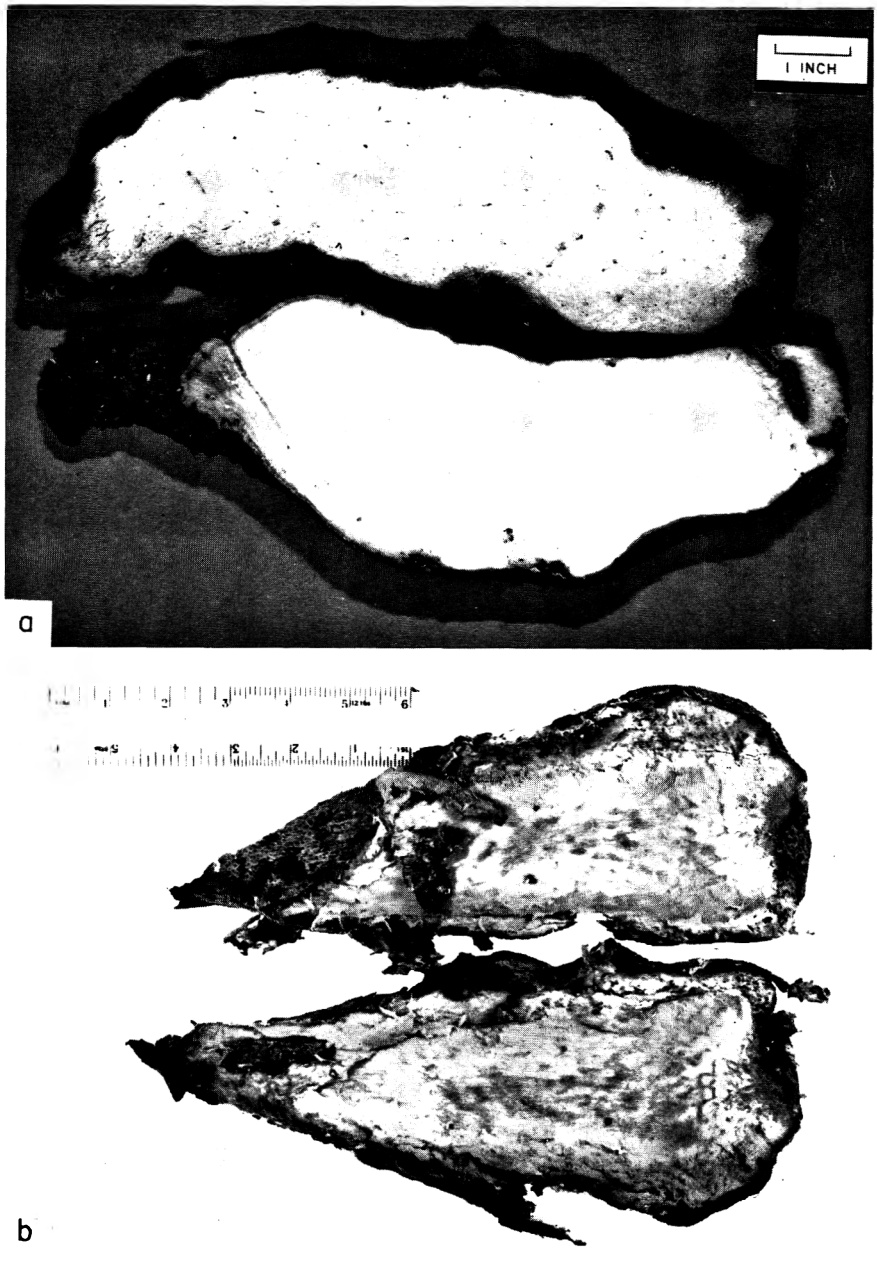

PLATE 1. (a) Longitudinal sections of *D. alata* tubers. Bottom, tuber stored at 15 C for 6 weeks, showing no injury (the dark area near the base is the result of mechanical damage, probably at harvesting). Top, tuber stored at 5°C for 6 weeks, showing mottling, flecking and subcutaneous browning produced by the low temperature.

(b) Longitudinal section of a *D. rotundata* tuber, after storage for 6 weeks at 5°C, followed by 2 weeks at 25°C. The flesh is discoloured and the whole tuber is decayed and disintegrating.

(*Facing p.* 146)

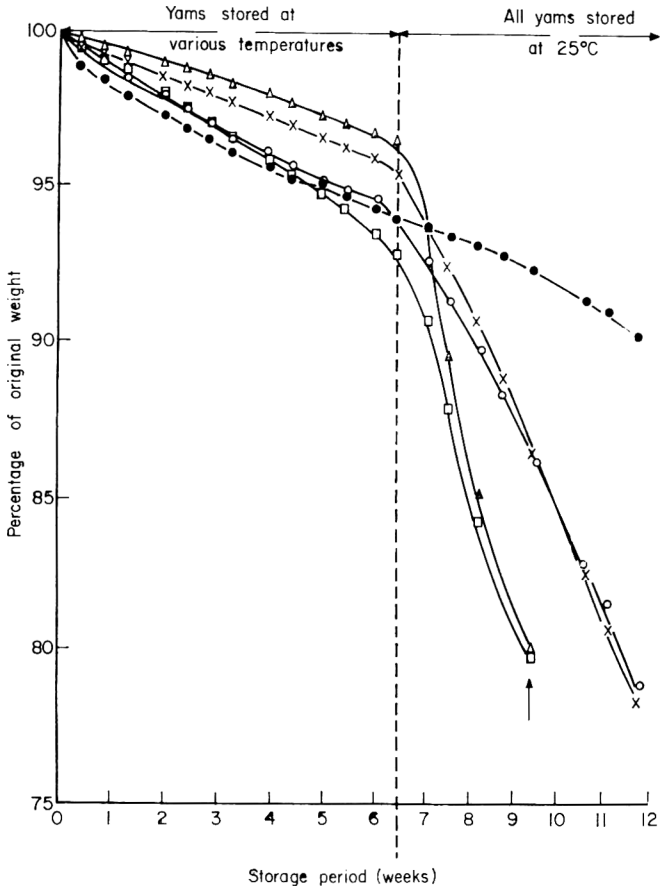

FIG. 3. Changes in weight of groups of *D. rotundata* tubers stored at various temperatures (arrow indicates where part of the experiment had to be abandoned, owing to massive deterioration of the tubers). ●, 25°C; ×, 12·5°C; ○, 10°C; △, 7·5°C; □, 5°C.

D. alata

A form of low-temperature injury was found to occur in all tubers stored at 5°C within 5 weeks but not in any of those stored at 15° or 25°C. This damage is manifested as a softening of the tissue, followed by a rapid loss of weight and either decay or shrivelling on return to normal temperature. It can be seen from Fig. 1 that tubers stored at 15°C lost weight rather more rapidly than the controls (stored at 25°C throughout), and continued to do so after return to 25°C. Those stored at 5°C lost weight rather faster again, but when returned to 25°C, the loss of weight became catastrophically rapid. Of the tubers stored at intermediate temperatures (which were derived from the second consignment, and were more fully mature), only a proportion of those at any one temperature showed breakdown: weight losses for the groups are

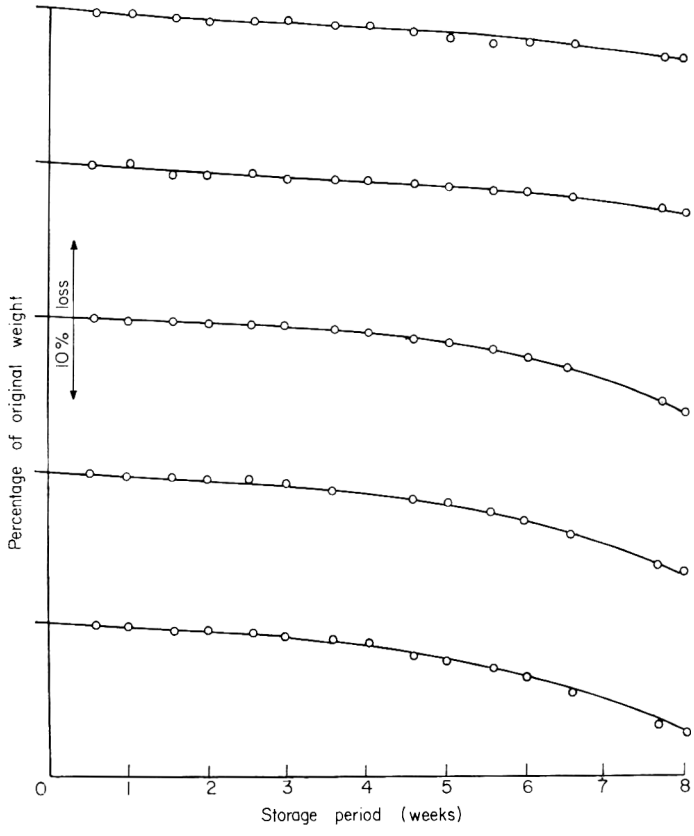

FIG. 4. Changes in weight of individual *D. alata* tubers stored at 10°C, illustrating variation in behaviour. The tubers represented by the two top-most curves showed no visible symptoms of low temperature injury: those represented by the three lower curves softened during storage (the individual curves are separated from each other, by 10% on the vertical scale, for clarity).

shown in Fig. 2. It may be noted, however, that those tubers which remained sound lost weight more slowly than those which softened, as is shown in Fig. 4, where the behaviour of individual tubers stored at 10°C is illustrated. Tubers stored at 12·5°C, in particular, which showed no breakdown, also showed considerably less weight loss than the controls.

These observations suggest that temperatures of 10°C or below are not suitable for the storage or shipment of this species of yam [it may be noted, however, that fewer tubers of those stored at 7·5°C exhibited injury than those stored at either 5° or 10°C (see Table 1)]. This is in fair agreement with the suggestions of Young (1923) and the report of Czyrhinciw & Jaffe (1951). The breakdown referred to by the latter as occurring at 12°C suggests that there may be differences in the critical temperature for chilling to occur between different forms or cultivars of the same species.

TABLE 1. Incidence (%) of visible deterioration in yam tubers after various storage periods at different temperatures

Temperature (°C)	Period of storage (weeks)					
	1	2	3	4	5	6
(a) *D. alata*						
5·0	–	–	–	100	100	100
7·5	–	–	–	–	20	20
10·0	–	–	–	–	40	60
12·5	–	–	–	–	–	–
15·0	–	–	–	–	–	–
25·0	–	–	–	–	–	–
(b) *D. rotundata*						
5·0	–	–	–	–	–	20
7·5	–	–	–	60	60	100
10·0	–	–	–	–	–	60
12·5	–	–	–	–	60	60
25·0	–	–	–	–	–	–

D. rotundata

Low temperature injury was also observed in this species of yam, all the tubers stored at 5° or 7·5°C exhibiting the effect after return to 25°C. It is interesting to note, however, that the rate of development of chilling injury was faster at the higher of these two temperatures, and in fact was not obvious in some of the 5°C specimens until after their return to the control temperature. Similarly anomalous behaviour has been reported in the chilling of the sweet potato (Lauritzen, 1931), where it was found that chilling damage occurred more rapidly at 4·5°C than at 0·1°C, and also in various soft fruit (Fidler, 1968). Breakdown was, however, more rapid in the 5°C samples after return to 25°C. Tubers stored at the intermediate temperatures of 10° and 12·5°C also broke down in some instances, but the effect was not general, and deterioration was less rapid than in the yams chilled at the lower temperatures (Fig. 3).

It would appear that this species of yam is rather more susceptible to low temperature injury than is *D. alata* and temperatures higher than 12·5°C are necessary for its safe storage and transport. The softening and breakdown that occur in chilled tissue also appear to proceed more rapidly in this species. The behaviour reported here is similar to that observed in the same species chilled to near the freezing point (Anon., 1937).

General

No attempt has so far been made to determine the biochemical basis for the low temperature injury observed, but the spongy and waterlogged condition of the chilled

tissue suggests that changes in membrane permeability may be a factor, as is the case with sweet potato (Lieberman *et al.*, 1958). Interference with one or more stages of the citric acid respiratory cycle may also be involved, as is known to occur in apples (Hulme, Smith & Wooltorton, 1964) and in sweet potatoes (Minamikawa, Akazawa & Uritani, 1961). It may be noted that the respiration of slices of *D. rotundata* tuber tissue is disturbed when chilled to 5°C (Coursey *et al.*, 1966): the differences in the temperature at which chilling effect occurred may be due to varietal, cultural or climatic factors. Work on the location of specific biochemical lesions is in progress, together with studies on the occurrence of low temperature injury in other *Dioscorea* spp.; in different cultivars; and in material grown under different conditions.

Acknowledgments

Much of the routine work involved in these experiments was carried out by Miss H. C. Russellsmith.

Experimental material of *D. alata* was provided by the Ministry of Agriculture and Labour, Barbados, through the good offices of Dr J. Nabney, while specimens of *D. rotundata* were made available through the courtesy of Mr K. N. Maharaj of Chris Foreign Foods (Wholesalers) Ltd.

The paper is published by permission of the Director, Tropical Products Institute, Ministry of Overseas Development.

References

Anon. (1937) *Agric. expt. Station, Puerto Rico*, Ann. Rep., 1937, p. 42.
Cooley, J.S., Kushman, L.J. & Smart, H.F. (1954) *Econ. Bot.* **8,** 21.
Copeland, E.B. (1916) *Philipp. J. Sci. C,* **11,** 227.
Coursey, D.G. (1967a) *Yams.* Longmans, Green, London.
Coursey, D.G. (1967b) *J. Stored Prod. Res.* **2,** 229.
Coursey, D.G., Fellows, L.E. & Coulson, C.B. (1966) *Nature, Lond.* **210,** 1292.
Czyhrinciw, N. & Jaffe, W. (1951) *Archos venez. Nutr.* **2,** 49.
Desrosier, N.W. (1963) *The Technology of Food Preservation.* Avi, Westport, Connecticut.
Fidler, J.C. (1968) Low temperature injury to fruit and vegetables. *The Low Temperature Biology of Foods* (Ed. by J. Hawthorn and E. Rolfe). Pergamon Press, Oxford.
Hulme, A.C., Smith, W.H. & Wooltorton, L.S.C. (1964) *J. Sci. Fd Agric.* **15,** 303.
Kushman, L.J. & Deonier, M.T. (1957) *Proc. Am. Soc. hort. Sci.* **70,** 425.
Lauritzen, J.I. (1931) *J. agric. Res.* **42,** 617.
Lieberman, M., Craft, C.C., Audia, W.V. & Wilcox, M.S. (1958) *Pl. Physiol., Lancaster,* **33,** 307.
Minamikawa, T., Akazawa, T. & Uritani, I. (1961) *Pl. Cell Physiol., Tokyo,* **2,** 301.
Roth, H. & Richardson, H.H. (1966) *J. econ. Ent.* **58,** 1086.
Young, R.A. (1923) *The Cultivation of the True Yams in the Gulf Region.* U.S.D.A. Bull. No. 1167.

J. Fd Technol. (1968) **3**, 151–158.

The use of sea-water ice for storage of cod

K. O. KELLY AND W. T. LITTLE

Summary. The use of sea-water ice as a replacement for fresh-water ice in 'wet' storage of cod has been investigated for periods of up to 10 days. Quality deterioration as measured by texture, flavour, moisture, ash or soluble nitrogen contents was not significantly altered with the use of sea-water ice. Its usefulness is suggested where there is a shortage of fresh water for ice production, particularly on board ship where the ice could be manufactured and used directly for storing fish.

Introduction

The use of sea-water ice as a replacement for fresh-water ice in the iced storage of sea fish has been the subject of earlier investigations, Taylor (1953), Field (1953), Hansen (1956) and Peters & Slavin (1958). Both Field and Hansen reported slight advantages with the use of sea-water ice. Hansen found that average scores from texture and flavour were in favour of cod stored in sea-water ice for up to 17 days. He concluded that overall quality of sea-water iced fish gained more from delay in spoilage than it lost with partial freezing and salt uptake. Peters & Slavin (1958) reported no significant differences in quality between haddock stored with sea-water ice and those stored with fresh-water ice. In Hansen's work only did it appear that the flesh of the fish were frozen during storage. Eddie (1961), in a review article, came to the conclusion that any marginal gain in quality was offset by the fact that sea-water ice could not be carried to the fishing grounds without being cooled to sub-zero temperature, otherwise the concentrated brine contained in the ice would quickly drain away, leaving more or less fresh-water ice behind. This is a serious objection to the use of sea-water ice unless it could be made on board. Eddie concluded that this could never be as economical as the cost of production of fresh-water ice in efficient block ice plants such as those at Grimsby or Hull.

However, there are many fishing areas where fresh-water ice is scarce or unavailable. In some cases the alternatives are either complete freezing or chilling with ice made from sea-water. This paper describes the use of sea-water ice and comments on the quality of cod stored in it and its use for processing.

Authors' address: Unilever Research Laboratory (Colworth), Greyhope Road, Torry, Aberdeen.

Methods and materials

Ice manufacture

The sea-water ice was manufactured on an Atlas slice-ice machine, Type V, stationary model (loaned by A/V Atlas, Copenhagen). The machine has a vertical, slowly rotating drum which is cooled internally and on to which is sprayed sea-water. The ice, frozen on to the surface of the drum is removed by a vertically mounted scraping knife. The machine is capable of making $\frac{1}{4}$–$\frac{1}{3}$ ton/hr. It is possible to vary the temperature of the ice made between − 12·5° and − 6·5°C by varying: (i) the amount of water sprayed on the drum, and/or (ii) the speed of rotation of the drum.

Coastal sea-water, ultraviolet sterilized, was used for ice production. The sodium chloride content of the sea water was between 3260 and 3400 mg/100 ml. The ice was manufactured just before it was used to ice down fish, and fresh-water flake ice was used for control experiments.

Fish selection

The cod (*Gadus morrhua*) used in these experiments were caught inshore and landed locally at Aberdeen. They were purchased, gutted (with head on), 1 day or less after catching.

Storage

In an initial experiment fish were stored in conventional boxes, later a bulk storage experiment was carried out.

(a) *Small-scale experiment:* Groups of six cod were stored in fish boxes which had free drainage. The fish were iced on board ship immediately after catching. On arrival at the laboratory this was changed for sea-water ice. The ice/fish ratios were 1·0/1·0. The temperature of the ice as manufactured was − 6·5°C. The boxes were held in a low temperature room (2–4°C) during the storage period of 10 days. One box of fish (six fish) was used for quality assessment on each of the following storage days: 1, 2, 3, 6, 7, 8, 9 and 10.

(b) *Bulk storage experiment:* A stainless steel box measuring 6 ft long, $4\frac{1}{2}$ ft wide and $3\frac{1}{2}$ ft deep was used for storing the cod. The box was insulated on its sides and top with a 2-in. thick layer of expanded polystyrene and held in an unheated processing area (8–12°C) during the experiment. The melting ice was able to drain freely from the bottom of the storage box. One ton of gutted cod was mixed with $\frac{1}{2}$ ton of sea-water ice (at − 6·5°C) in the storage box, and the whole was topped up with a further $\frac{1}{4}$ ton of sea-water ice. The fish was assessed for quality at the beginning of storage and after 9 days on ice.

Results

Measurements made during the storage period

(a) *Temperature of the fish was monitored during storage.* The fish were at a temperature

of 1·0°C just before storage with sea-water ice. Within 2 hr fish temperature had dropped to just below 0°C. During storage the fish temperature remained between − 1·0 and − 2·5°C in the bulk storage experiment. As a result of this the fish were partially frozen at the end of the storage period and had to be thawed before they could be filleted. In the small-scale storage experiment the fish temperature was − 1·0°C after 3 days storage (Table 1). Thereafter, the fish temperature slowly rose as the ice became depleted until it was + 0·6°C after 9 days storage.

(b) *Loss of ice due to melting was measured in the small-scale experiment by collecting the drip from the box.* The results are shown in Table 1.

TABLE 1. Fish temperature and losses of ice due to melting (small-scale experiment)

Days storage	Sea-water ice (% loss)	Fish temperature (°C)	Fresh-water ice (% loss)	Fish temperature (° C)
1	48	−1·1	4·2	+0·1
2	62·5	−1·1	4·7	+0·1
3	67·5	−1·0	5·0	+0·1
6	73·5	−0·6	7·0	+0·1
7	77·0	−0·4	8·5	+0·1
8	80·0	−0·4	10·0	+0·1
9	87·5	+0·6	15·5	+0·1
10	92·5	+0·6	20·0	+0·1

These results show that sea-water ice melts much more rapidly than fresh-water ice. This is only to be expected due to the depression of the melting point of sea-water ice by the presence of salts.

It was not possible to make similar measurements of losses from the bulk storage tank. However, after 9 days storage in the large tank, which was well insulated, the fish still had an excellent ice covering. The low fish temperature reported in the above section confirmed that the ice loss in the large storage tank had been very much less than from the small boxes.

(c) *Sodium chloride content of the melted ice.* At intervals, samples of the melting ice coming from the bulk storage container were taken for estimation of chloride content (A.O.A.C., 1960). In the small-scale experiment the entire volume of melted ice was collected daily (except for days 4 and 5) from a box of fish stored with sea-water ice and from one stored with fresh-water ice. The results are shown in Table 2 below.

TABLE 2. Sodium chloride content of melted ice (mg/100 ml)

Day of sampling	Bulk storage tank	Small storage fish boxes	
	Sea-water ice (mg NaCl/100 ml)	Sea-water ice (mg NaCl/100 ml)	Fresh-water ice (mg NaCl/100 ml)
1	2870	4750	940
2	2638	3042	134
3	2398	2580	–
6	1752	1930	117
7	1742	1375	526
8	–	1199	420
9	1521	877	280
10	–	589	175

The results show that in the early stages of storage in sea-water ice the fish are in contact with a strong salt solution. The low sodium chloride level at the end of storage in the small-scale experiment is due to the much greater rate of melting than in the bulk storage experiment.

Assessment of fish after storage

(a) *Weight change of fish stored in the bulk storage tank was measured,* the fish were weighed before and after storage and the weight loss was 1·6% over the 9 days storage period.

(b) *The appearance of the fish and fillets was assessed visually.* As previously mentioned the fish were partially frozen after storage. Fish from the small boxes were quite normal for 9 days storage. Those from the large storage tank, particularly from the bottom, were rather distorted. After thawing the appearance was quite normal. The fillets were acceptable after cutting, and did not appear any worse than normally expected after 9 days storage.

(c) *Filleting yields:* filleting was done by experienced hand filleters. The yield of skin-on fillets stored in bulk with sea-water ice for 9 days was 42% (minus belly flap) and the yield after skinning was 39%.

(d) *Trimethylamine (TMA) content of the muscle was determined throughout the storage period.* The method used was that of Dyer (1945). Six fish were filleted at each storage time investigated. One fillet from each pair was minced, thoroughly mixed and samples taken for the test. An increase in TMA value is associated with the development of off-odours and flavours in fish which had undergone bacteriological deterioration. The results are shown in Table 3. Due to the small changes found, only figures obtained on the first and final days of storage are given.

TABLE 3. TMA values (mg TMA/100 g muscle) of sea-water and fresh-water ice stored fish

Days storage	Small-scale storage experiment		Bulk storage experiment	
	Fresh-water ice	Sea-water ice	Sea-water ice	
0	0·4	0·4	0·3	
9	1·0	1·0	0·6	Sample from top of tank
			1·8	Sample from bottom of tank

The slight increase in TMA values are insignificant, and the values are well below levels which would make the fish unacceptable from a flavour point of view. Sea-water ice is as satisfactory as fresh-water ice for delaying bacteriological deterioration.

(e) *Moisture and ash contents of the fish muscle were determined at the beginning of, and throughout storage.* Samples of the minced muscle from six fish were taken. Moisture contents averaged 81·0% (± 0·5) before storage and remained unchanged during storage with fresh-water and with sea-water ice. Similarly ash contents averaged 0·988% (± 0·1) and did not change with storage.

(f) *Estimations of muscle soluble nitrogen were made* (Kelly, 1968) *on fish stored in fresh-water ice and on fish stored in sea-water ice for* 9 *days.* Three fish from each group were sampled. Sea-water ice stored fish averaged slightly lower (82·1%) than fresh-water ice stored fish (85·9%). This is within the range normally expected for fresh fish, viz. 80–90%. It indicates that no significant denaturation of the muscle proteins occurred during storage with sea-water ice as compared with fresh-water ice.

(g) *Texture and flavour of the fish was assessed by a trained taste panel.* Six fillets were steamed for 30 min for assessment and a five-point scoring system was used, where: 5 = very good; 4 = good; 3 = acceptable; = 2 poor; 1 = very poor. Toughness was also scored on a five-point scale where: 2 = optimum; 3–4 = tough; 0–1 = soft. Each result is the average of six samples.

Table 4 shows the texture and flavour scores at the beginning and end of storage. The results show that there was no significantly greater deterioration with sea-water ice storage than with conventional fresh-water ice storage. All fish had acceptable flavour and texture scores after 9 days storage. Toughness scores showed that softening occurred to a slightly greater extent with sea-water ice stored fish than with fresh-water ice stored fish.

Table 4. Taste-panel assessment of sea-water and fresh-water ice stored cod

	0 days ice storage			9 days ice storage		
	Texture	Toughness	Flavour	Texture	Toughness	Flavour
Small-scale experiment						
Sea-water ice	4·0	2·1	4·2	3·5	1·7	3·4
Fresh-water ice	4·0	2·1	4·2	3·6	1·9	3·2
Bulk-scale experiment						
Sea-water ice	3·8	2·1	3·8	3·6	1·6	3·6

(h) *Quality of fish after freezing.* After filleting, cod, stored in bulk with sea-water ice, were frozen into blocks and their quality further assessed after 1 week's storage at − 18°C. Methods of assessment were: (1) taste panel, and (2) liquor loss at 20°C (Little & Smithies, 1964). The results are shown in Table 5, and represent the average values for six fillets sampled.

Table 5. Taste-panel and liquor loss assessment of cod fillets frozen after sea-water ice storage

Texture	Toughness	Flavour	% liquor loss at 20°C
3·2	1·6	3·2	11·5

Textures and flavour scores are slightly lower than those for the same lot of fish before freezing (Table 4) but are still of an acceptable level.

The average liquor loss at 20°C was 11·5%. Normally, with fish stored 9 days on fresh-water ice and then frozen, one would expect the loss to be 5–8% (Little, unpublished). Sea-water ice has, therefore, increased the potential drip loss of the cod.

Discussion

This project was undertaken to investigate the possibility that the use of sea-water ice for storage would lower the eating quality of fish by:

(a) *Flavour deterioration.* It has been observed, Roach *et al.* (1961), that the flavour of cod held in chilled sea-water had detectable salt-fish flavour after 5 days and reached an objectionable level after 9 days. One explanation for this has been the absorption of salts from the sea-water by the muscle.

(b) *Partial freezing.* Temperatures just below 0°C were thought to produce maximum rates of protein denaturation hence the eating texture would be toughened and the drip losses on processing considerably increased.

When the sea-water ice was made it had a temperature of − 6·5°C. At this temperature the ice consists of a large amount of fresh-water ice crystals and a small amount of unfrozen salt solution. The latter has a concentration of approximately 9% (calculated as sodium chloride). As the ice melts the fish are initially bathed in this strong salt solution. As melting proceeds the salt concentration of the melting solution gradually decreases. Ash and moisture analysis of the muscle showed that there was no net change in salt or water content of the muscle, and taste-panel members did not detect any 'salty' flavours in fish which had been stored with sea-water ice. It has been shown by Cowie (1962 unpublished) that build up in salt in cod in chilled sea water (0°C) was considerable after 3 days. It can be concluded from results in the present work that as the fish were partially frozen, the absorption of salt was inhibited.

One might expect a flavour advantage in the sea-water ice stored fish as the rate of bacterial spoilage should have been reduced at the lower temperature of storage. Power, Morton & Sinclair (1968), recently showed that bacterial counts of cod stored with fresh-water ice at a super-chill temperature of − 3°C were approximately one-third of those found at − 0·5°C after 30 days storage. This is not apparent from our results as storage was only for 9 days, but might well explain the better taste-panel scores reported by Hansen for sea-water ice stored cod as compared with fresh-water ice stored cod after 17 days storage. No significant difference was noted in the rate of TMA production.

The fish after storage were in a 'soft' frozen condition and had to be thawed before being filleted. Thawing was carried out in cold running water and the thawed fish were filleted producing a normal yield and no unusual drip problems. The protein solubility figure did not change significantly during the storage period and this together with the lack of drip would suggest that no undue protein denaturation occurred. This is surprising and on previous knowledge would be very difficult to explain. However, Love (1968) has recently observed that fish muscle cooled to − 1·6°C did not deteriorate as quickly as fish muscle brought up to the same temperature from − 9°C.

Taste-panels found that the sea-water ice stored fish was in fact softer than the fresh-water controls. Further processing and freezing produced a product with acceptable flavour and texture scores though with a drip loss slightly higher than would be expected with fish of similar history stored in fresh-water ice.

Rapid melting of sea-water ice should not be a problem provided there is adequate insulation and no air circulation in the storage area. This was shown in our bulk storage experiment where the losses due to melting were kept to a minimum.

The size of the bulk storage tank used approximated to the size of a trawler storage pound. Pressure effects were no worse than those normally expected in a pound of this size and improvements could be obtained with the use of shelving.

In conclusion, the use of sea-water ice for storing 'wet' fish over a limited period has proved satisfactory and does not significantly alter fish quality after further processing.

Its usefulness is suggested where there is a shortage of fresh-water for ice production, particularly on board ship where the ice could be manufactured and used directly for storing fish.

References

A.O.A.C. (1960) *Methods of Analysis* (Ed. by W. Horwitz), p. 235.

DYER, W.J. (1945) *J. Fish Res. Bd, Canada*, **6,** 351.

EDDIE, G.C. (1961) *Wld Fish.* **10,** 46.

FIELD, C. (1953) *Fishing Gazette*, **70,** 54.

HANSEN, P. (1956) Chilling of fish. *Fish Process Technologists Meeting, Rotterdam* (Ed. by E. Hess and G.N. Subba Roa). F.A.O.

KELLY, K.O. (1968) *F.A.O. Technical Conference on the Freezing and Irradiation of Fish, Madrid.* (In press).

LITTLE, W.T. & SMITHIES, R.H. (1964) *Chem. Ind.* p. 1294.

LOVE, R.H. (1968) *F.A.O. Technical Conference on the Freezing and Irradiation of Fish, Madrid.* (In press).

PETERS, J.A. & SLAVIN, J.W. (1958) *Comm. Fish. Rev.*, **20,** (1), 6.

POWER, H. E. , MORTON, M.L. & SINCLAIR, R.E. (1968) *F.A.O. Technical Conference on the Freezing and Irradiation of Fish, Madrid.* (In press).

ROACH, S.W., HARRISON, J.S.M., TARR, H.L.A., MCCALLUM, W.A., CHAN, M.S. & LANTZ, A.W. (1961) *Fish. Res. Bd, Canada*, Bull. No. 126, Ottawa.

TAYLOR, F. (1953) *Sthn Fisherman*, **13,** 94.

J. Fd Technol. (1968) **3**, 159–163.

The characterization of acid-hydrolysed corn syrups: A theoretical appraisal

G. G. BIRCH

Summary. The value of DE (dextrose equivalent) as a means of characterizing corn syrups is questioned and a relationship between DE and degree of hydrolysis of the starch molecule is attempted. The difference between the amounts of the saccharide components in corn syrups and those demanded by theory are ascribed to lack of randomness in the conversion process.

Introduction

Although commercial corn syrups are usually characterized by DE (dextrose equivalent) this index has in many cases (for example enzyme-converted syrups) been shown to be unsatisfactory in describing their technological properties. Obviously in the case of enzyme-converted syrups the purity and specificity properties of the enzymes employed will govern the distribution of the component sugars in the product and hence DE. Thus it is possible to have two syrups of the same DE but differing in the composition of their sugars. Even in the case of acid hydrolysed syrups it has not yet been made clear whether or not DE is a linear function of the degree of hydrolysis of the starch molecule.

Hydrolysis of the polymer

Acid hydrolysis of starch will approximate more closely to random degradation of the polymer than enzyme or dual conversion processes and on this basis it is generally agreed that DE (total reducing sugars expressed as dextrose and calculated as a percentage of the total dry substance) is proportional to the degree of hydrolysis or scission of the polysaccharide, s (where s = No. of glycosidic bonds broken/Total No. of glycosidic bonds). However, DE by definition is not a function of the number of scissions produced in the polysaccharide, and thus the number of aldehydo groups produced, but is a *chemical property* of the molecules to which the aldehydo groups are attached.

If acid degradation of the polysaccharide is assumed to be random despite process fluctuations in temperature, pH and concentration, it can be assumed that the relative concentrations of the saccharide components of a particular DE syrup is constant and

Author's address: National College of Food Technology, University of Reading, Weybridge, Surrey.

thus DE varies with the degree of scission, although it is not necessarily a linear function of it. A relationship between DE and extent of the hydrolysis may be demonstrated by plotting the former parameter against the yield (Y_n) of each saccharide fraction consisting of n glucose units. (Such fractions are mixtures of 1,4- and 1,6-linked oligomers due to branching in the polysaccharide.) Graphs of this type are available (Corson, 1957) for n = 1–7 (Fig. 1).

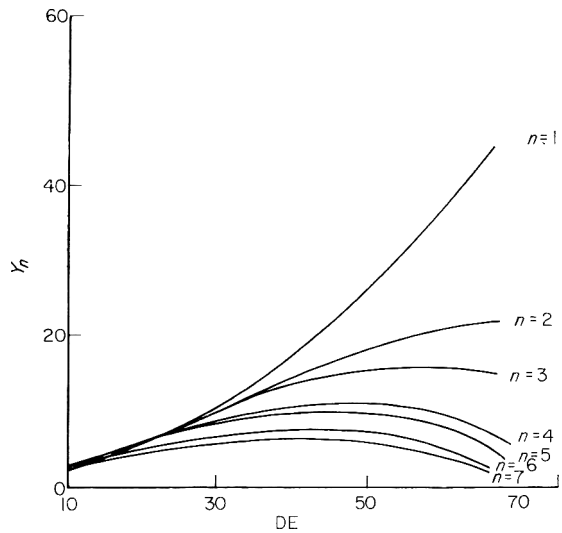

FIG. 1. Relationship between yield of n-mer (Y_n, %) and DE.

Because DE is not necessarily linearly related to degree of scission it follows that it is not necessarily a linear index of the chemical changes which have occurred during hydrolysis. A better index would be degree of scission which must, by definition, be directly proportional to the number of bonds broken in the polymer. As far as the author is aware no convenient method for determining the degree of scission in glucose syrups has yet been described. However, Painter (1963), by a mathematical extension of Kuhn's (1930) treatment of random hydrolysis, has related degree of scission (s) to yield of n-mer (Y_n) by the following equation:

$$Y_n = ns(1 - s)^{n-1}\left[r + (1 - 2nr)\, s + \frac{nr}{2}\,(n + 1)\, s^2\right],$$

where r = degree of branching.

By assuming 4–5% of branching in amylopectin and 72–80% of amylopectin in starch it is possible to allocate values to r in the range 0–0·04. Y_n can then be related to

s for particular values of n. Solving these equations up to $n = 4$ gives the set of curves shown in Fig. 2.

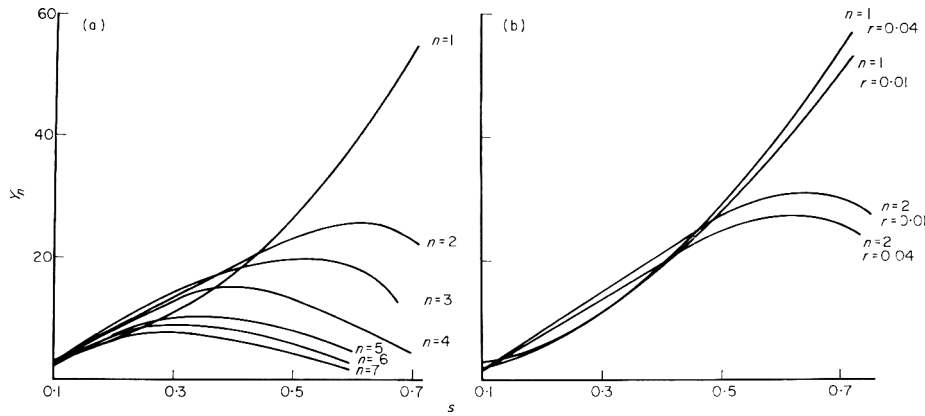

FIG. 2(a) Relationship between yield of n-mer (Y_n, %) and degree of scission (s) (s has values between 0 and 1·0 in the range 0–100% hydrolysis). $r = 0·04$. (b) Dependence of the relationship between Y_n and s on degree of branching, r ($r = 0·01 = 1\%$ branching in corn starch; $r = 0·04 = 4\%$ branching in corn starch). For all higher values of n different values of r produce only one curve.

It is immediately apparent that these curves are of the same general shape as those in Fig. 1, thus indicating the close relationship between DE and s. The actual relationship was elucidated and is as shown in Fig. 3.

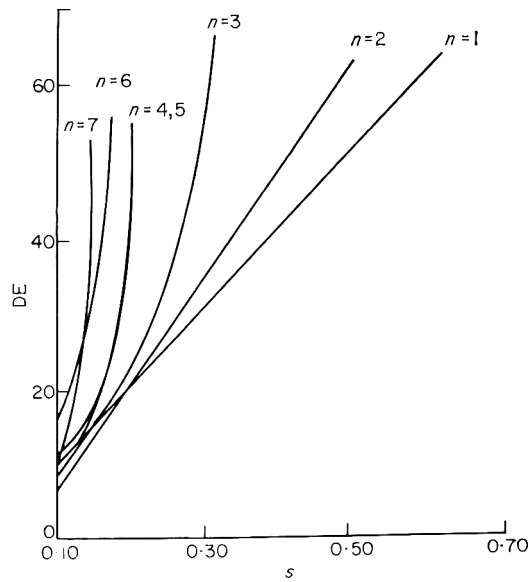

FIG. 3. Relationship between DE and s.

There are, however, major differences between the curves in Figs. 1 and 2 in respect of the maxima for corresponding values of n. These are in most cases significantly higher in Fig. 2, despite the fact that theory would demand equal value. Thus each oligosaccharide is formed and subsequently hydrolysed during the conversion process, passing through a maximum at a certain stage; and Painter's equation provides a theoretical method for predicting the position and height of the peak values of the percentages of the saccharide components, under random conditions of hydrolysis. The curves in Figs. 1 and 2 both represent the formation and breakdown of oligosaccharides. Thus, under random conditions the peak heights should be the same. Either Painter's equation must, therefore, be inapplicable to the present problem or the data issued by the Corn Industries Research Foundation (Corson, 1957) must be questioned. The former hypothesis appears to be correct because the dextrose equivalent corresponding to the amounts of the saccharide components composing the curves in Fig. 1 tally with those calculated by adding the reducing powers of the components. This technique can be applied by assuming each fraction to be more than 90% linear maltodextrin, and utilizing the reducing power of maltodextrins calculated on a molar stoicheiometric basis (Whelan, 1966).

Thus DE is a theoretically predictable parameter in acid hydrolysed corn syrups, in which the percentages of the component saccharides from $n = 1$ to $n = 7$ are known. Hoover *et al.* (1965) have found disagreement between the reducing power, based on ferricyanide number, of maltodextrins and DE presumably calculated on the same basis. It should be pointed out, however, that reduction of the ferricyanide ion by sugars is not only non-stoicheiometric with respect to copper salts but highly complex in mechanism. There is, therefore, little reason to believe that DEs may be theoretically predicted from ferricyanide numbers.

Several of the values for Y_n contained in Fig. 1 have been independently checked in the author's laboratory by a thin-layer chromatographic technique and similar results were obtained.

Results for Y_n in the range $n = 1$ to $n = 5$ differed from those in Fig. 1 by no more than 2% in any of the cases studied. The results were obtained on 250 μ thicknesses of Kieselgel G, using the solvent system n-butanol–ethanol–water (2 : 1 : 1) for development.

Fig. 3 confirms that Painter's equation, in its present form, is inapplicable to the commercial hydrolysis of starch, because the relationship between DE and s is dependent on n, whereas it would be expected that at any particular stage of the conversion process represented by one value of s there would be only one corresponding value of DE under random conditions. The curves appear to bunch as n increases, suggesting possible coincidence for high values of n, whereas for low values of n the greater divergence of the curves (maximum for $n = 1$) can be attributed to lack of randomness in the hydrolysis process. Low molecular weight oligomers will be more accessible to attacking protons than high molecular weight analogues, and will therefore tend to

hydrolyse more readily. This is exemplified, for example, by the ease of hydrolysis of maltose and hence the greater rate of hydrolysis of the terminal glycosidic bond at the non-reducing ends of branched polymers (Wolfrom, Lassetre & O'Neill, 1951; Griffin, Erlander & Senti, 1967). Thus the differences in maxima in Figs. 1 and 2 become more apparent as the degree of scission increases.

Since it is reasonable to assume that only one value of DE exists for each value of s below Y_n (max) in Fig. 1 the divergent curves in Fig. 3 must represent unreal relationships between DE and s, resulting from the inapplicability of Painter's equation.

Acknowledgment

The author wishes to thank his colleagues Messrs R. H. Skinner and N. D. Cowell for assistance with the mathematical theory of these problems and Beecham's Food and Drink Division Ltd, for their interest in this work and financial assistance.

References

Corson, G.E. (1957) *Critical Data Tables* compiled for the Corn Industries Research Foundation.
Griffin, H.L., Erlander, S.R. & Senti, F.R. (1967) *Die Stärke*, **1**, 8.
Hoover, W.J., Nelson, A.I., Milner, R.T. & Wei, L.S. (1965) *J. Fd Sci.* **30** (2), 253.
Kuhn, W. (1930) *Ber. dt. chem. Ges.* **63**, 1503.
Painter, T.J. (1963) *J. Chem. Soc.* 779.
Whelan, W.J. (1966) Personal communication to author.
Wolfrom, M.L., Lassetre, E.N. & O'Neill, A.N. (1951) *J. Am. chem. Soc.* **73** (2), 595.

J. Fd Technol. (1968) **3**, 165–170.

An improved automated analysis of hypoxanthine

J. R. BURT, J. MURRAY AND G. D. STROUD

Summary. The level of hypoxanthine in fish muscle is a good indicator of fish quality. Its routine determination can be carried out reliably and quickly with the AutoAnalyzer using a redox indicator dye. Interference from formaldehyde and acetaldehyde in extracts of fish muscle, at concentrations greater than would be expected there, is not significant. Results obtained using this method correlate very well with those from the standard manual enzymatic assay procedure.

Introduction

The hypoxanthine content of fish muscle is now recognized as affording a good indication of deterioration in many species of chill-stored fish (Burt, Stroud & Jones, 1968; Jones, 1960, 1965; Jones & Murray, 1962; Jones *et al.*, 1964; Kassemsarn *et al.*, 1963; Spinelli, 1967; Spinelli, Eklund & Miyauchi, 1964) of pre-canning quality in canned herring (Hughes & Jones, 1966) of pre-freezing quality in frozen cod (Connell, 1968) and of quality in irradiated fish (Spinelli, Pelroy & Miyauchi, 1968). The currently available methods of measuring hypoxanthine concentrations in deproteinized extracts differ considerably in their accuracy, speed of execution and the amount of instrumentation required. The automated enzymatic procedure (Jones, Murray & Burt, 1965) which uses xanthine oxidase and which monitors hypoxanthine as its oxidation product, uric acid, is reasonably rapid and accurate but requires complex equipment, including a recording ultraviolet spectrophotometer, for its execution. A separate blank determination has also to be carried out on each extract in order to compensate for varying background ultraviolet absorptions at the monitoring wavelength. The present paper describes the automation of an enzymatic method in which the reaction mixtures incorporate an oxidation–reduction indicator dye (2,6-dichlorophenol indophenol) (Burt *et al.*, 1968); the advantages being that extract blanks do not need to be determined, a simple colorimeter may be used and the reaction time is much reduced. Additionally, the dye method is more appropriate to those cases where the extracts being processed have high ultraviolet absorptions, which makes this procedure potentially very valuable for use with extracts of Crustacea for instance. These contain homarine at high and varying levels of concentration.

Authors' address: Torry Research Station (Ministry of Technology), 135 Abbey Road, Aberdeen, AB9 8DG.

The visual xanthine oxidase assay procedure (Burt *et al.*, 1968) upon which this method is based, consists of mixing neutralized perchloric acid extracts with a series of phosphate buffer–dye mixtures and with a diluted enzyme solution. After incubation, the tubes are examined by eye for the presence or absence of colour and the result recorded as negative or positive, respectively; this indicates whether hypoxanthine is present in concentrations greater or less than the level equivalent to the particular concentration of dye used in each tube. The automated procedure takes advantage of this fact, that the amount of dye decolourized is proportional to the amount of hypoxanthine present. However, whereas the instability of the decolourized dye precludes its convenient use for manual assays, other than in a stepwise screening test such as the one described, it is not too serious a drawback in the AutoAnalyzer system where, by the nature of the instrument, the optical densities of all samples and standards are read after exactly the same times of incubation.

Before this method could be adopted as a standard, routine assay procedure for determining hypoxanthine concentrations in extracts of fish muscle for research or quality control purposes, the effects of the reported (Booth, 1938) broad specificity of xanthine oxidase had to be determined. While the oxidation of compounds such as formaldehyde and acetaldehyde (known substrates of this enzyme and components of fish muscle) would not lead to increases in optical density at 290 mμ, the monitoring wavelength for the earlier (Jones *et al.*, 1965) assay procedure, it could conceivably interfere with a redox dye system.

Procedure

Apparatus

An AutoAnalyzer (Technicon Instruments Co., Chertsey, Surrey) consisting of sampler, proportioning pump, colorimeter (618 mμ filter) and recorder modules is assembled as shown in Fig. 1 which also gives details of the flow rates required in each line. Incubation of the reaction mixture proceeds during its passage through two standard 6-in. mixing coils which are immersed in a water bath at 37°C: this gives an incubation time of about 2½ min.

Reagents

Phosphate buffer solution (0·17 M, pH 7·6) containing 2,6-dichlorophenol indophenol (20 μg/ml).

Xanthine oxidase solution (0·018 i.u./ml) kept in a chilled reservoir throughout the assay period.

Neutralized perchloric acid extracts of fish muscle prepared so that 8 ml of extract is equivalent to 1 g of fish muscle.

Standard solutions of hypoxanthine containing up to 100 μg/ml.

Operation

Water is passed through all the lines except the air line to establish an initial baseline and to ensure that the debubbler is functioning correctly. The buffer–dye and

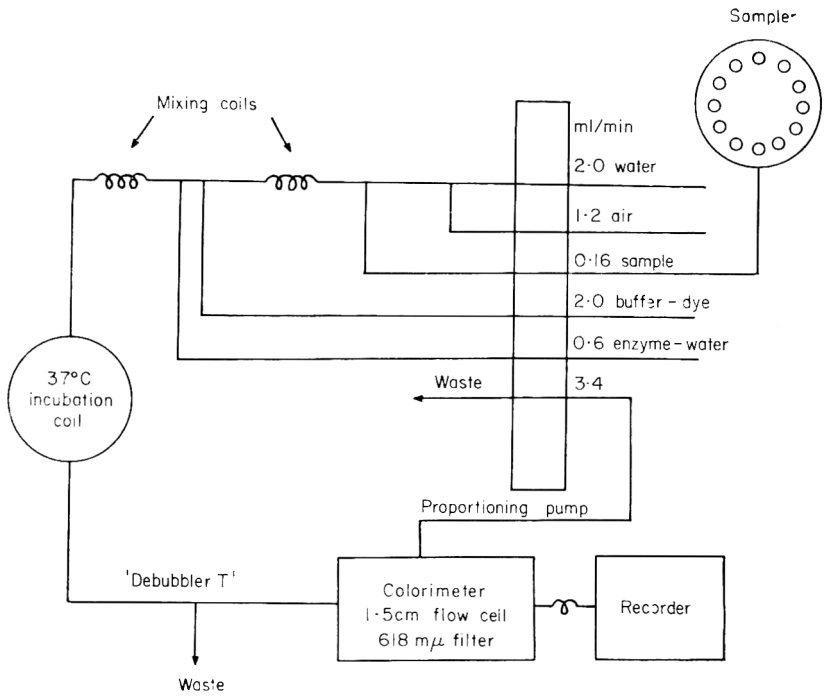

FIG. 1. Schematic flow diagram for the improved automated analysis of hypoxanthine. Further details are given in the text under 'Procedure'.

enzyme solutions are then introduced through their respective lines to record the 'blank' optical density levels in the region of 0·55. Extracts, standards and water blanks are then sampled at a rate of 40/hr. (This can be increased to 60/hr with only a slight reduction in accuracy.) If all the extracts are expected to have similar hypoxanthine concentrations, single determinations are adequate; but, if large variations are expected, samples should be arranged in duplicate in order to minimize errors due to sudden transitions from high to low concentrations or *vice versa*. In the latter cases, the value obtained from the second of the duplicate samples run is used. Standards are always processed in duplicate, the optical density change obtained with the second of each pair being used to prepare a calibration curve.

Results and discussion

The effects of formaldehyde and acetaldehyde on the determination of hypoxanthine using this automated procedure were first of all studied. The aldehydes were added separately in varying amounts to different standard solutions of hypoxanthine and the amount of hypoxanthine determined was expressed as a percentage of the amount found in the absence of added aldehyde. The results are given in Table 1. The four

TABLE 1. Effect of aldehydes on the determination of hypoxanthine

Hypoxanthine concentration (μmoles/ml)	Formaldehyde		Acetaldehyde	
	Range of concentrations added (μmoles/ml)	Hypoxanthine found (%)	Range of concentrations added (μmoles/ml)	Hypoxanthine found (%)
0·735	0·613–1·47 (4)*	100–101 (mean 100·4)	0·735 (1)*	101
0·551	0·460–1·65 (5)*	98·2–100 (mean 99·1)	0·551–1·10 (2)*	97·8–98·5 (mean 98·2)
0·367	0·306–1·84 (8)*	94·8–100·6 (mean 97·4)	0·367–1·47 (3)*	98·5–102 (mean 100·7)
0·184	0·153–1·84 (10)*	95·8–104 (mean 99·6)	0·184–1·47 (6)*	98·0–101 (mean 99·7)

*No. of concentration levels tested within the range given.

hypoxanthine concentrations chosen were selected so as to cover the range of usefulness of the test while up to ten times as much aldehyde (on a molecular basis) was added. Trends were not detectable and it is concluded that at these concentrations formaldehyde and acetaldehyde do not interfere with the determination of hypoxanthine. The greater variability of response at the lower concentrations is solely a reflection of the innate imprecision of a determination based on the difference between two comparatively large optical density values. However, in the absence of hypoxanthine, formaldehyde at concentrations of 1·38 and 2·76 μmoles/ml gave responses equivalent to approximately only one-fortieth these concentrations of hypoxanthine. It is not felt that these observations have any practical implication in the field of fish freshness testing. In any case, the amounts of formaldehyde and of acetaldehyde found naturally in extracts of fish muscle are not expected to be higher than 1 μmole/ml. The maximum level of formaldehyde reported in the muscle of gadoid fish is 5 μmoles/g for a sample of frozen Pacific cod (Amano, Yamada & Bito, 1963; Amano & Yamada, 1964). Much lower concentrations were found in iced fish. Concentrations of the same order of magnitude have been found in the muscle of Atlantic cod (Howgate, 1968). Of the three species (non-gadoid) in the muscle of which Ota (1958) determined acetaldehyde concentrations, 0·5 μmoles/g was the highest recorded value (in mackerel). Quantitative data are not available for acetaldehyde in the flesh of gadoid species although it is reported to be present there (Mendelsohn & Steinberg, 1962; Wong, Damico & Salwin, 1967).

The accuracy of this procedure was checked by determining the hypoxanthine concentrations of over eighty extracts of cod muscle using it and also the manual enzym-

atic assay of Jones *et al.* (1964). Fig. 2 illustrates the excellent agreement obtained between the two sets of answers; the solid line indicating perfect correspondence and the broken line the computed best fit. The coefficient of correlation between the two sets of data was computed and found to be 0·991 which is very highly significant and the regression equation is $y = 1{\cdot}065x - 0{\cdot}135$, where y and x are the manual and automated results, respectively.

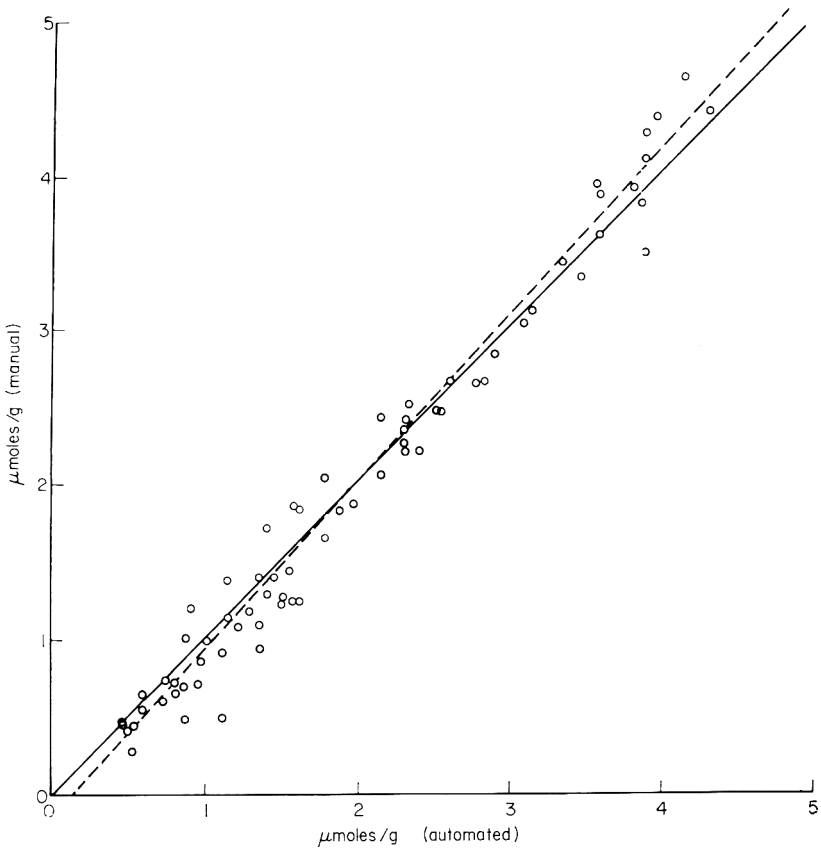

FIG. 2. Hypoxanthine concentrations in neutralized perchloric acid extracts of fish muscle. Comparison of results obtained with the automated dye procedure and the standard, manual enzymatic one.

The expected inhibition of xanthine oxidase action at high substrate concentrations (cf. Jones *et al.*, 1965) was also observed in this system. Maximum responses were obtained with hypoxanthine standards of about 125 μg/ml. Higher levels than this gave deflections that, with increasing concentration, decreased to the point that 500 μg/ml solutions would give the same response as 100 μg/ml ones. However, the possibility of

confusion in practice is remote, if this procedure is strictly adhered to, since extracts containing hypoxanthine at concentrations over 100 μg/ml would be rare and those over 150 μg/ml most unlikely to occur. A further safeguard would be present in the existence on the recorder trace of 'blips', similar to those reported earlier by Jones *et al.* (1965), where severe substrate inhibition occurs thus enabling easy identification of it.

In conclusion, this new procedure has proved so much more rapid and convenient to operate than others that it is currently being used as the method of choice for processing routinely all hypoxanthine assay samples in this laboratory. We believe it is now at a stage where the fish processing industry could adopt it for quality control purposes. Furthermore, the procedure described above has additional advantages over the automated procedure published earlier: it is cheaper to instal as an ultraviolet double beam spectrophotometer is not required, cheaper to run as less xanthine oxidase can be used per assay and is less time consuming to operate as 'blank' runs need not be made.

Acknowledgments

Mr P. F. Howgate carried out the statistical analysis.

The work described in this paper was carried out as part of the programme of the Torry Research Station. Crown Copyright Reserved.

References

Amano, K. & Yamada, K. (1964) *Bull. Jap. Soc. sci. Fish.* **30,** 430.

Amano, K., Yamada, K. & Bito, M. (1963) *Bull. Jap. Soc. sci. Fish.* **29,** 695; 860.

Booth, V.H. (1938) *Biochem. J.* **32,** 494.

Burt, J.R., Stroud, G.D. & Jones, N.R. (1968) *FAO Symposium on Freezing and Irradiation of Fish, Madrid.* (In press).

Connell, J.J. (1968) *FAO Symposium on Freezing and Irradiation of Fish, Madrid.* (In press).

Howgate, P.F. (1968) Unpublished data, Torry Research Station.

Hughes, R.B. & Jones, N.R. (1966) *J. Sci. Fd Agric.* **17,** 434.

Jones, N.R. (1960) *Analyst,* **85,** 111.

Jones, N.R. (1965) *The Technology of Fish Utilization* (Ed. by R. Kreuzer), p. 179. Fishing News (Books), London.

Jones, N.R. & Murray, J. (1962) *J. Sci. Fd Agric.* **13,** 475.

Jones, N.R., Murray, J. & Burt, J.R. (1965) *J. Fd Sci.* **30,** 791.

Jones, N.R., Murray, J., Livingston, E.I. & Murray, C.K. (1964) *J. Sci. Fd Agric.* **15,** 763.

Kassemsarn, B.-O., Sanz Perez, B., Murray, J. & Jones, N.R. (1963) *J. Fd Sci.* **28,** 28.

Mendelsohn, J.M. & Steinberg, M.A. (1962) *Fd Technol., Champaign,* **16,** 113.

Ota, F. (1958) *Bull. Jap. Soc. sci. Fish.* **24,** 334.

Spinelli, J. (1967) *Comm. Fish. Rev.* **29** (11), 69.

Spinelli, J., Eklund, M. & Miyauchi, D. (1964) *J. Fd Sci.* **29,** 710.

Spinelli, J., Pelroy, G. & Miyauchi, D. (1968) *FAO Symposium on Freezing and Irradiation of Fish, Madrid.* (In press).

Wong, N.P., Damico, J.N. & Salwin, H. (1967) *J. Ass. off. agr. Chem.* **50,** 8.

J. Fd Technol. (1968) **3**, 171–173.

Post-mortem changes in myofibrillar protein solubility

C. VALIN

Summary. During the ageing of bovine muscle there is an increase in the solubility of the myofibrillar protein as tropomyosin and associated myofibrillar proteins become extractable with the Weber–Edsall solution. This fact agrees with the concomitant structural changes believed to occur in the sarcomere.

The progressive increase of myofibrillar protein solubility is a well-known effect of ageing which occurs as tenderization of meat proceeds.

During storage of rabbit muscle, Fujimaki *et al.* (1965) showed that the approximate content of myosin A and actin in the extracted actomyosin varied with time of storage. We observed (Valin, 1967) a similar result during the ripening of beef skeletal muscle and found a decrease in the percentage of actin during this period; concurrently, an investigation of the properties of actomyosin, especially in solutions of low ionic strength (0·15 M-KCl and 0·06 M-KCl) showed widely different rates of superprecipitation between actomyosin extracted in rigor and that extracted at 8 days post mortem. These differences might be interpreted as the consequence of a progressive increase of extracted tropomyosin, during ageing.

Szent-Gyorgyi & Kaminer (1963) suggested that actomyosin is a ternary complex of a metachromatic component, actin and myosin A. They reported methods for preparing the metachromatic component, and named it metin. Later, Azuma & Watanabe (1965a, b) found that tropomyosin was the major component of metin prepared from rabbit skeletal muscle and bovine cardiac muscle and that the minor component was identical with Ebashi's protein troponin (Ebashi & Ebashi, 1964).

In our experiments, beef carcasses were stored at 4°C. We prepared metin directly from beef skeletal muscle by the isoelectric precipitation method of Szent-Gyorgyi & Kaminer (1963), except that the washing of muscle brei was done with 30 mM-$NaHCO_3$. We also prepared metin indirectly from the actomyosin complex extracted: (a) at 1 day post mortem, and (b) after 8 days of storage at 4°C.

For this purpose, the actomyosin was prepared by extracting ground beef muscle (longissimus dorsi) with Weber–Edsall solution (0·6 M-KCl–0·01 M–Na_2CO_3–0·04

Author's address: Laboratoire de Recherches sur la Viande, Institut national de la Recherche agronomique, Jouy-en-Josas (Yvelines), France.

M-$NaHCO_3$). This was purified twice by dilution and precipitation. Preparation was carried out at a temperature below 4°C. The actomyosin in suspension in 0·1 M-KCl, 30 mM-$NaHCO_3$ was centrifuged, the precipitated actomyosin suspended in 0·05 M-NaCl and then rapidly heated to 60°C while being stirred vigorously. The suspension was cooled in ice and after filtration, the clear filtrate was dialysed overnight against deionized water. The metin was precipitated isoelectrically at pH 5. The precipitate was then separated by centrifugation and redissolved by neutralizing the suspension with 0·1 N-KOH.

TABLE 1. Preparation of metin from the actomyosin complex, extracted from beef muscle (temperature — 4°C)

Animals	% metin in actomyosin	
	1 day post mortem	8 days post mortem
1	0	5·1
2	2·77*	4·3
3	0	2·86
4	0	4·29
5	0	4·33
6	0	3·12
7	0	5

*For animal No. 2, 1 day post mortem, the extraction of actomyosin was inadvertently performed at 10°C.

The average yield of metin by direct extraction of the muscle was 0·3% of the wet weight, whether from muscle just in rigor at 1 day post mortem, or after 8 days of storage at 4°C. However, as Table 1 shows, very different results were obtained when metin was prepared by the indirect method from extracted actomyosin. Now almost no metin is extracted along with actomyosin from the meat stored only 1 day, whereas after 8 days the extracted actomyosin complex contains about 4% metin. This increase in extracted metin at 8 days is accompanied by an increase of about 25% in the amount of extracted actomyosin complex, as reported by Khan & van Den Berg (1964) and McIntosh (1967). It should be noted that the extracted metin is contaminated with an equal amount of unknown protein. We have also studied the effect of storing meat for 15 days, but were unable to detect an increase either in the total actomyosin complex extracted or in metin, above the values for 8 days storage.

As shown in Table 1, if actomyosin is extracted from muscles in rigor at temperatures above 4°C, some metin is solubilized. In our previous study, we found that actomyosin extracted in rigor at room temperature exhibited properties similar to actomyosin extracted at below 4°C after 8 days of ripening. This last result may be useful in understanding the actual mechanisms involved in the progressive loosening of the sarcomere

structure, and in explaining the increase of myofibrillar protein solubility during meat ripening.

That the metin complex is extracted along with actomyosin from aged meat is of great interest, because it is now clear that the tropomyosin and troponin components of metin are closely associated with the actin filaments and the Z-disc, and probably serve, along with other proteins in trace amounts, to bind the whole structure together. Thus the progressive increase in their extractibility on ageing meat agrees well with the idea of Davey & Gilbert (1967) that one of the main effects of ageing is the disruption and disappearance of the Z-discs.

Acknowledgment

We are greatly indebted to Dr J. R. Bendall for his suggestions.

References

Azuma, N. & Watanabe, S.K. (1965a) *J. biol. Chem.* **23,** 3847.
Azuma, N. & Watanabe, S.K. (1965b) *J. biol. Chem.* **23,** 3852.
Davey, C.L. & Gilbert, K.V. (1967) *J. Fd Technol.* **2,** 57.
Ebashi, S. & Ebashi, F. (1964) *J. Biochem.* **55,** 604.
Fujimaki, M., Arakawa, N., Okitani, A, & Takagi, O. (1965) *J. Fd Sci.* **30,** 937.
Khan, A.W. & van den Berg, L. (1964) *J. Fd Sci.* **29,** 597.
McIntosh, E.N. (1967) *J. Fd Sci.* **32,** 208.
Szent-Gyorgy A. & Kaminer, B. (1963) *Proc. nat. Acad. Sci. U.S.A.* **55,** 1033.
Valin, C. (1967) *XIIIth European Meeting of Meat Research Workers,* Rotterdam.

J. Fd Technol. (1968) **3**, 175–182.

Book Reviews

Odour Preferences. By R. W. MONCRIEFF.

London: Grampian Press, 1966. Pp. 357. 90s.

The amount of information in this book makes it an almost essential book of reference for food scientists and technologists interested in the flavours of food. It is not a work to be treated superficially, attention must be concentrated. To ensure that nothing has been missed this reviewer advises that it be read a second and even a third time before using it as a work of reference.

The book is in three main sections, viz.: (I) The search for trends: a study of the odour preferences of a few people towards a wide range of odours; (II) Development of the trends: a study of the preferences of many people towards ten dissimilar odours; and (III) Comparison of the findings with the prior art. If the title of Part I has not been fully appreciated this part will, at first sight, appear disappointing in that conclusions are drawn regarding the preferences of as few as twelve people for 132 substances. It is quickly realized that this part should be regarded as a sorting test which is used to decide the conditions for Part II where results from people of all ages, both sexes and varying temperaments are collected and analysed. The important conclusions for the food scientist, development chemist and marketing executive lie in the careful analysis of preferences by age and sex. In this connection the second may well be of paramount importance to the test marketeer for, although the overall preferences will ultimately decide the market, it is the women who make the greater part of food purchases. As the author says: 'The known dependence of selling on pleasant olfactory associations should encourage wider and deeper investigation of sex differences in preferences'.

In Part III, where the relation of the findings to existing practice, knowledge and conjecture is made, there is much useful material. The author points out how little work has been done to confirm or refute some firmly held ideas. One difficulty which is mentioned frequently is the fact that odour always has some emotional effect, even if it is only that of pleasantness or the reverse.

For the reader concerned with food, Chapter 19, 'Olfactory preferences in foods', will probably be of greatest interest and here considerable attention is given to practical matters including investigation of flavour composition. In this part of the book Moncrieff expresses some opinions with which most food scientists will agree, for example:

> '. . . are the flavour preferences of the working classes the same as those of the board? . . . Here is wonderful opportunity and one so burgeoning with *results of practical value* that the tycoons of the food industry might be tempted to spend a little more on research and sponsor some of the work. *At present the food*

industry is one which spends rather less than its share on research (italics reviewers).

On the other hand while this reviewer agrees with the following there may be many who do not:

> 'But there is a danger in modern research of equipment becoming too complicated, so that its maintenance takes too much attention; the research direction must not become the slave of its own equipment, must not lose sight of the main issues . . .' '. . . cannot fail to be impressed by the analytical installation and the hard work that is put into its application, but he may perhaps feel that more use could be made of organoleptic appraisal and testing and of a more sustained effort to link it (flavour) with the analytical results'.

The author sums up many of his conclusions in 124 rules. With the majority of these one must agree but there are a few for which the evidence is somewhat thin. There are a few things with which one could disagree, e.g. the form in which some of the odours were presented but small criticisms like this do not detract from the very real value of this work. This is an extremely useful book both for the flavour chemist and the perfumer.

S. H. CAKEBREAD

The Inhibition of Fat Oxidation Processes. By N. M. EMANUEL and YU. N. LYASKORSKAYA.

Oxford: Pergamon Press, 1967. Pp. 389. £6 6s. 0d.

In recent years the scientific literature of the Soviet countries has become more and more easily available, even to non-Russian readers, and the latest advances in Russian science are fairly regularly presented at international conferences. The appearance of an English translation of a Russian textbook, therefore, no longer opens a window for us on the world beyond the wall and its worth is to be judged more on the degree to which it magnifies or clarifies our view.

The present volume is a translation of an original published in 1961 with eleven pages summarizing trends in the development of the subject in the following five years, thus the original Russian papers listed have long been available to Western readers at least in abstract form. The book lives up to its Preface in giving 'a world-wide collection of the results of theoretical and experimental work' and only one-sixth of the references are to journals of Eastern origin. The text deals, in general, at greater length with the Western literature, for example, no less than forty-four pages (of 389) are devoted to Tables of antioxidant efficiency reproduced from the *Journal of the American Oil Chemists Society*. Russian contributions to the theory of the kinetics of the autoxidation process are presented in detail but this discussion is based more on the high temperature oxidation of hydrocarbons than on processes of immediate application to the problems of food fats.

From the food technologist's point of view the last chapter presenting the theory and application of antioxidants is the most valuable. The material is not comprehensive and is presented in a completely uncritical fashion; a more serious fault arises in the table of antioxidants and synergists legally permitted for use in various countries; although accurate at the time of the original publication this has not been revised to take into account more recent changes.

D. N. RHODES

Toxicants Occurring Naturally in Foods.

Publication 1354. Washington: National Academy of Science, National Research Council, 1966. Pp. 301. $6.

This monograph was prepared by a number of specialists in their particular fields at the invitation of the U.S.A. Food Protection Committee of the Food and Nutrition Board with the avowed intention of presenting current information, however derived, concerning the presence of naturally occurring toxic agents in man's foods.

It is an excellent compilation dealing with naturally occurring goitrogens, oestrogens, carcinogens and tumorigens, lathyrogens, haemagglutins, glycosides, depressants and stimulants, pressor amines, allergens, enzyme inhibitors, fungal toxins together with specific chapters on hypervitaminoses, nitrates and nitrites, oxalates, mineral and dietary salt effects, toxic substances in food fats, seafood toxins and gossypol.

Each contributor of a chapter has compiled a list of references which to any student of this subject must prove of immense value and the reviewer can personally testify to this. In addition to the chapters mentioned there is one on natural radioactivity in the biosphere and foodstuffs concisely written with informative tables and an objective discussion concludes the book.

In all this volume contains a wealth of information which cannot wholly be found elsewhere and provides data to explode the fallacy that because foods are natural they are safe. It is no exaggeration to say that one cannot commence reading this book without being unable to put it down until completed. It is as exciting as a novel yet is a work to which one will continually refer for exact information on Nature's Toxic Tricks. No one who either is or pretends to be an authority on food should be excused from digesting the information so clearly and calmly presented.

J. B. M. COPPOCK

Strontium Metabolism. Ed. by J. M. A. LENIHAN, J. F. LOUTIT and J. H. MARTIN. London: Academic Press, 1967. Pp. 354. 84s.

'For a century or more, Strontium . . . had made a mildly spectacular contribution to the manufacture of fireworks. A great and terrible firework has made the name of the once obscure element an epitome of mankind's anxiety over the impact of nuclear science on military technology'.

This book, from the preface of which the above quotation is taken, contains together with a brief historical introduction, thirty-five original papers which were presented to an International Symposium on 'Aspects of Strontium Metabolism' held in 1966 at Chapelcross, Glasgow, and at Strontian (a village in Argyll where strontium ores were formerly mined and after which the metal was named).

It has been known for many years that ordinary, stable strontium is present in small amounts in most, if not all animal and plant tissues; in mammals the adult bones contain more than other tissues. In the average adult human diet the ratio stable Sr–Ca is about 1·6 : 1000 and, like calcium, strontium is excreted both in the urine and the faeces. In former years the close similarity between the chemical properties of calcium and strontium rendered the accurate determination of minute amounts of the latter in presence of relatively large amounts of the former a matter of great analytical difficulty. Modern methods such as emission spectroscopy and neutron activation have largely solved the analytical problem. The advent of ^{90}Sr—with which radioactive isotope much of the experimentation described in this book deals—has also facilitated Sr determinations, in animal tissues, in plants and in soils; the movement of ^{90}Sr from soils to plants, to grazing animals, to products such as milk and into other constituents of human diets can now be followed with a comforting, or perhaps a discomforting degree of certainty.

None of the earlier experimental findings has shown that stable Sr is an essential micronutrient for any plant or animal, but as with other trace metals, some plants are capable of accumulating surprisingly large quantities of this element from soils containing more than the usual low percentage of Sr. As regards radioactive-^{90}Sr, the bone-seeking character of strontium and the long half-life (28 years) of this isotope make it a dangerous (probably the most dangerous) product of the long-lived fission products formed in nuclear explosions. The irresponsible orgy of aerial atomic explosions in the 1950s and early 1960s liberated (amongst many other nuclides) several megacuries of radio-active strontium into the stratosphere, to be carried all over the world, first in the upper atmosphere and later to be deposited, or washed down by rain, on to plants and soils and into the oceans. Especially in those land regions with a high rainfall, easily measurable quantities of ^{90}Sr were taken up by plants and by grazing animals.

From the results of the investigations described in this book, the metabolic similarities between radioactive strontium and calcium (either stable or the radioactive ^{45}Ca) emerge clearly. Thus the changes in the movement of radioactive strontium, both within the body and into the excreta, which take place after the administration of hormones and other physiologically active substances to mammals previously contaminated with ^{90}Sr or ^{85}Sr, parallel fairly closely those found in the movements of calcium after such administration, though some Sr–Ca discrimination is said to occur both in the kidney and the intestine.

In experiments with human volunteers who ingested, for 21 days or more, the less dangerous, but still radioactive and bone-seeking ^{85}Sr (added to milk), a mathe-

matical analysis of the rate of excretion of the strontium following the end of the exposure period indicates that there are three 'pools' of strontium in the adult human body, one, believed to consist of blood plasma, extra cellular fluid and bone surfaces, with an average 'half-life' in the body of 2·7 days, another, attributed to a closer binding of Sr with the apatite crystals in the bone, with an average half-life of 19 days, and a third, probably resulting from the incorporation of Sr into the structural crystal lattice of the bone apatite, with a long half-life of about 750 days. With the long-lived ^{90}Sr it is these latter sites in the bone, in some of which the rate of Sr turnover is particularly low, that the risk, and in ^{90}Sr-treated rabbits the actual incidence, of bone carcinoma is highest. In an endeavour to increase the mobilization and excretion of radioactive strontium from such deposits and thus prevent pathological developments, a large number of potentially Sr- and Ca-mobilizing substances have been tested both singly and two or more together, with a certain degree of success. In this connection it has been found that levels of ^{90}Sr in human bones 'naturally' contaminated with that isotope are lower in towns with a relatively high content of fluoride in the drinking water.

No less than sixty-nine research workers have contributed to the series of papers published in this book. Even if this represents the majority of those engaged in research in this field it seems, to the present reviewer, a surprisingly large number.

The layout, printing and binding of the book are very satisfactory; in view of this quality and today's trends, the price must be regarded as not unduly high.

H. D. KAY

Aroma- und Geschmackstoffe in Lebensmitteln. Ed. by J. SOLMS and H. NEUKOMM.

Zürich: Forster-Verlag AG, 1967. S.F. 59.0 DM 59.0.

This volume contains the lectures delivered at a post-graduate course on 4 and 5 April 1967 at the Agricultural Chemistry Institute in Zürich. There is a sensible balance between the three sections of the report which consists of: (a) New methods, (b) Chemistry and Biochemistry, and (c) Technological and Practical Problems. The introduction by Professor Neukomm stresses the importance of flavour as a property of foods.

The three lectures in the first section: 'Basis and possibilities of gas chromatography' by Professor Simon; 'Application of gas chromatography to the study of food odours' by Weurman & van Lunteren; and 'Application of thin layer chromatography to the investigation and analysis of aroma and flavouring substances' by Schmidt; are very definitely practical in approach. While by no means elementary they are so written as to be clear to the layman (provided he can read German). It is of interest that Weuman *et al.* (this article is in English) draw attention to the fact that while analysis separates the components the aroma is the mixture itself; a point frequently overlooked.

The middle section, occupying the greater part of the volume, is the most important. While it is a review of material already published its value is in bringing scattered information together. 'Aroma compounds in fruits' by Gierschner & Baumann attempts to cover much ground including definitions of terms, attempts at classification and theory, possible routes of formation and methods of analysis. Its last section giving the compounds which have been found in twelve characteristic fruits is probably the most valuable. One effect on this reviewer is to remind him of the enormous number of aromatics common to quite different fruit flavours. The tables of compounds occupy eleven close printed pages and the literature references number 254. van Duin's paper on milk and milk products deals with difficulties in investigation and then with the flavour components found in milk itself and most of the milk derived products, there are fifty-six references.

Neukomm's chapter on 'Sulphur containing flavours and their formation' brings together a lot of information on this very important class. In particular the discussion on the routes of formation is very useful (forty references).

The two papers: 'Roasting aromas' by Streuli and 'Volatile aroma compounds of coffee' by Winter, Stoll *et al.* should be taken together. Streuli deals with different types of foods in which flavour is developed by roasting and in some instances lists the potential aroma compounds. There is then a section dealing with some specific compounds followed by a discussion on the formation of the volatile products and 172 references. The paper by Winter *et al.* deals first with the earlier work and includes an interesting comparison of methods of analysis available in 1920 and 1960 and a chart of the number of compounds identified in the period 1920–67. There follows the up-to-date results and the sobering remark 'Trotz der Indentifizierung von mehr als 300 Substanzen enthält das Kaffeearoma eine grosse Anzahl noch unbekannter Komponenten' (fifty-one references).

Solms in 'Flavour enhancing compounds', lists the known substances involved and gives some idea of their effects in different types of food (eighty-two references).

In the last section the paper by Morgan on 'Evaporation and aroma recovery' is disappointing being too strongly biased towards methods developed in the U.S.A. (nine references). On the other hand Emch on 'Aroma concentration and extraction' casts his net wider and considers methods based on practical experience in various parts of the world (nineteen references).

Egli's paper 'Formation and alteration of flavours . . . in manufactured foods' first describes the probable chemical reactions involved and then their effects in specific foods. A useful contribution including forty references. Finally Riklin has a practical paper on 'Application of aroma concentrates in manufactured foods' which contains a classification of types of flavouring material based on origin, gives some idea of usage and of regulations in some countries (nine references).

This is an interesting, useful book and of great value for reference. What is most

important is that the references in the majority of the papers go up to 1967. To be recommended.

S. H. CAKEBREAD

Yams. By D. G. COURSEY.
London: Longmans, 1967. Pp. xiv + 230. 63s.

One of the greatest needs at the present time is the conservation of the produce of tropical countries between harvesting and retail sale. A few of the commodities—those such as bananas and cacao beans which have secured an established position in world markets—have been intensively studied, but there are many which have not enjoyed this advantage, even though they provide the staple foodstuffs of large populations. Among these are the yams, the tubers of several kinds of tropical plants, but usually signifying those of *Dioscorea* species. It is with these that the present book is mainly concerned.

It is an unusual book because it covers with equal expertise so many areas of specialist knowledge. The author is as much at home with the general and taxonomic botany of the Dioscoreaceae as he is with cultural and storage practices and the economic uses, including pharmaceutical, of the yams. Partly, of course, this is because of the limited amount of literature under all these various headings. What, one might ask oneself, would be the size—and cost—of an equally detailed account of apples or potatoes? But it is just this sort of all-rounder who is so much needed to grapple with the problems of all manner of crops over the whole under-developed tropical belt—that fantastically prolific area that is at present producing, relatively, so fantastically little.

The author has, in fact, contributed usefully to the literature on the storage and transport of yams in a paper published in the present issue. Although this is especially relevant to the marketing of yams in colder areas than those in which they are usually consumed, the kind of study it represents will become increasingly needed as refrigerated storage becomes available in the countries where they are grown.

E. C. BATE-SMITH

Evaluation of the World Food Literature. Results of an International Survey.
Commonwealth Agricultural Bureaux, Farnham Royal, Bucks, 1967. Pp. 207. $15.00.

During the last 5 years or so, there has been a growing demand for the establishment of a comprehensive food abstracting and documentation service covering the world literature on food science and technology.

As a preliminary step to the setting up of such a service, it was considered essential to carry out a detailed study of the world's scientific and technological periodicals for their 'food literature' content in order to be able to assess more accurately the size of the world food literature as well as the subject and language distribution of papers on food in the primary scientific and technical literature. Although several lists of primary

journals claiming to cover the world's food literature have been prepared during the last few years, no contents analysis of these journals had been made and statements on the likely yield of food papers from these journals were based largely on guess-work.

The survey—the results of which are in this publication—involved analysing one year's issues of over 2000 of the world's scientific and technical periodicals for their food literature contents.

Food papers picked up in the survey were coded according to subject by a specially designed classification system indicating both the commodity and discipline area. In addition, papers were coded according to language, and much additional information on journals (e.g. publication frequency, editorial address, language of summaries, etc.) was recorded.

This book presents an alphabetical list of all journals surveyed with details of the number of food papers found, frequency of publication, language of original and language of summaries, and editorial or publishing address. In addition to giving the actual number of food papers found in each journal, the journals are given a letter category (A–E) according to the number of food papers—allowing readers to extract for themselves quickly and conveniently various lists of journals according to the depth of food coverage.

The book should prove an invaluable reference work to all persons and organizations with an interest in the documentation of food science and technology and related fields. Little has been known hitherto of either the quantitative or the qualitative nature of the food science and technology literature in the world's scientific and technical periodicals. This book will go a long way toward answering these questions.

GEORGE F. STEWART*

*Reproduced by kind permission of the author and the Institute of Food Technologists from: *Fd Technol., Champaign*, 1967, **21**, 1612.

Book received

Nahrungsmittelbestrahlung. Ed. by J. C. SOMOGYI.
Zürich: Forester-verlag AG, 1967. Pp. 84.

Ministry of Overseas Development

Tropical Stored Products Centre

A member of the staff is recuired for general investigations of overseas food storage problems and initially to assist with consultancy work carried out on behalf of the World Food Programme in the field of food packaging, transportation and storage under tropical conditions. In the initial phase the officer appointed would be expected to participate in the training of World Food Programme officials in the London area and with the preparation of a Manual on food storage for use by officers in the field, as well as undertaking advisory work on fooc packaging and storage. Travel to overseas countries will be involved.

The post will be graded Senior Scientific Officer/ Scientific Officer or Experimental Officer/Assistant Experimental Officer.

Salary will be according to age and experience within the following scales:

S.S.O.	—	£1,744	-	£2,155
S.O.	—	£ 926	-	£1,574
E.O.	—	£1,365	-	£1,734
A.E.O.	—	£ 568	-	£1,243

Qualifications: A University degree or a diploma in technology with first or second class honours in a scientific subject is required and experience in food handling, storage, packaging and stored products entomology together with experience of working in overseas countries would be advantageous.

Applications in writing should be addressed to:

Dr D. W. Hall,
Ministry of Overseas Development,
Tropical Stored Products Centre,
London Road,
Slough, Bucks.

INSTITUTE OF FOOD SCIENCE AND TECHNOLOGY

TENTATIVE PROGRAMME, 1968–9

Date	*Time and Place*	*Subject*	*Speakers*
Thursday, September	Findus, Croydon	Ladies evening	
Thursday, October	N.C.F.T. Weybridge Joint with Students Society	Drying	Mr Tucker, Irish Sugar Co.
17 October	Symposium all day Joint with R.S.H. and F.G.	New Sources of Proteins for Human and Animal Nutrition	Dr Bender, Dr Spicer, Dr Shaklady, Dr Head
13 November	14 Belgrave Square, S.W.1 Joint with F.G.	Flavour research	Dr Scott, Colworth House
3 December	Royal Society, Welcome Lecture Hall. 5.30 p.m.	A.G.M.	
	Charing Cross Hotel. 8.00 p.m.	A.G.M. Dinner	
23 January	Falstaff Hotel, 6.30 p.m. for Eastcheap 7.00 p.m. Buffet 8.15 p.m.	Film evening	
February	Reading Joint with Students Society	Animal Feeding Stuffs in the Dairy Industry	
6–13 March	Borough, London	N.R.D. Corporation	
26 March	Half-day Symposium Joint with F.G.	With Danes	
10 and 11 April	Symposium (2 days)	Automatic Systems in the Food Industry	
	Falstaff Hotel, 6.30 p.m. for Eastcheap 7.00 p.m.	Symposium dinner	
1 or 8 May	Borough, London Joint with Students Society	Two papers by students Two papers by young members	

JOURNAL OF FOOD TECHNOLOGY: NOTICE TO CONTRIBUTORS

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

Typescripts (two complete copies) should be sent to the Editor, Dr E. C. Bate-Smith, A.R.C. Institute of Animal Physiology, Babraham, Cambs. Papers should be typewritten on one side of the paper only, with a 1½ inch margin, and the lines should be double-spaced. In addition to the title of the paper there should be a 'running title' (for page headings) of not more than 45 letters (including spaces). The paper should bear the name of the author(s) and of the laboratory or research institute where the work has been carried out. The full postal address of the principal author should be given as a footnote. (The proofs will be sent to this author and address unless otherwise indicated.) The Editor reserves the right to make literary corrections.

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

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

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

Abbreviations. Abbreviations for some of the commoner units are given below. The abbreviation for the plural of a unit is the same as that for the singular unless confusion is likely to arise.

gram(s)	g	second(s)	sec
kilogram(s)	kg	cubic millimetre(s)	mm^3
milligram(s)		millimetre(s)	mm
(10^{-3} g)	mg	centimetre(s)	cm
microgram(s)		litre(s)	l
(10^{-6} g)	μg	millilitre(s)	ml
nanogram(s)		pound(s)	lb
(10^{-9} g)	ng	gallon(s)	gal
hour(s)	hr	milliequivalent	mEq
minute(s)	min	R_F values	R_F

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. Letters and numbers must be written lightly in pencil. 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 capitals with an Arabic number, e.g. TABLE 2. Each table must have a caption in small letters. Vertical lines should not be used.

Page proofs will be submitted to the contributors for minor corrections and should be returned to the Editor within 3 days. Major alterations to the text cannot be accepted.

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

JOURNAL OF FOOD TECHNOLOGY
Volume 3, Number 2, June 1968

Contents

Printed by The Sidney Press Ltd, Bedford